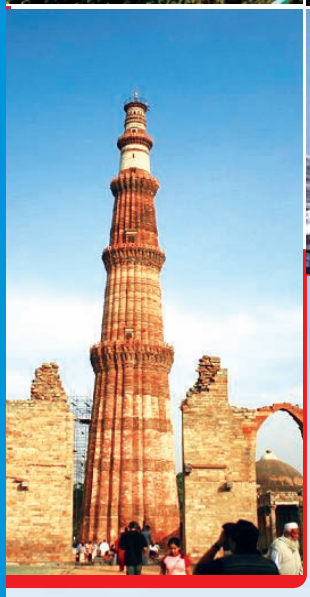

ENVIRONMENTAL HEALTH
MANAGEMENT SERIES: EHMS/01/2012

Epidemiological Study on Effect of Air Pollution on Human Health (Adults) in Delhi

CENTRAL POLLUTION CONTROL BOARD
MINISTRY OF ENVIRONMENT & FORESTS
Website: http://www.cpcb.nic.in
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Central Pollution Control Board
(A Govt. of India Organisation)
Ministry of Environment & Forests
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FOREWORD

Rapid urbanization and economic growth has led to growth of vehicles in India, which has serious implication on the air quality. The substantial increase in number of vehicles has resulted in increased emission of air pollutants, specially the particulate matter which exceeds the prescribed standards in many cities. Particulate matter is associated with mortality and morbidity. Fine particles on their own or in combination with other air pollutants are linked to number of health problems. For rational planning of pollution control strategies, scientific information is needed on nature, magnitude and adverse health effects of air pollution.

To assess the impact of air pollution on human health (adults), CPCB initiated an Epidemiological study in Delhi in 2002 with the help of Chittaranjan National Cancer Institute, Kolkata. The study was carried out over a period of four years during which several health camps were organized in different seasons, covering different parts of the city. The study included questionnaire survey as well as clinical examination.

The findings of the study were Peer reviewed by Indian Council for Medical Research (ICMR), New Delhi and report was updated based on Peer review comments. Dr. Sanghita Roychoudhury, Research Associate, Sh. Tarun Darbari, Scientist B, Dr. Sanjeev Agrawal, Scientist D, have finalized the report under the supervision of Dr. D.D. Basu, Scientist E, and guidance Sh. J.S. Kamyotra, Member Secretary.

I hope the findings of the Report would be useful to all concerned.

(Mira Mehrishi)

3rd July, 2012

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ABBREVIATIONS USED

AM	:	Alveolar macrophage
ALT	:	Alanine aminotransferase
AST	:	Aspartate aminotransferase
ATS	:	American Thoracic Society
BAL	:	Broncho-alveolar lavage
B(a)P	:	Benzo(a)pyrene
BMI	:	Body mass index
CD	:	Cluster determinant
CNG	:	Compressed Natural Gas
COPD	:	Chronic obstructive pulmonary disease
CO	:	Carbon monoxide
CPCB	:	Central Pollution Control Board
95%CI	:	95% confidence interval
CNCI	:	Chittaranjan National Cancer Institute
CRP	:	C-reactive protein
CSE	:	Centre for Science and Environment
CVD	:	Cardiovascular disease
DBP	:	Diastolic blood pressure
DEP	:	Diesel Exhaust Particles
FVC	:	Forced vital capacity
FEV_1	:	Forced expiratory volume in one second
$FEF_{25-75\%}$	:	Forced expiratory flow, 25-75%
GOLD	:	Global Initiative for Chronic Obstructive Lung Diseases
IAQ	:	Indoor air quality
LDL	:	Low density lipoprotein
LRS	:	Lower respiratory symptoms
MMEF	:	Mid maximum expiratory force
NAAQS	:	National ambient air quality standards
NEERI	:	National environment engineering institute
NHLI	:	National Heart, Lung institute
NK	:	Natural killer
NOx	:	Oxides of nitrogen
O_3	:	Ozone
OR	:	Odds ratio
PAH	:	Polycyclic aromatic hydrocarbon
PEFR	:	Peak expiratory flow rate
PFT	:	Pulmonary function test
PM	:	Particulate matter
PM_{10}	:	Particulate matter with less than 10 µm diameter

$PM_{2.5}$	:	Particulate matter with less than 2.5 µm diameter
Pap	:	Papanicolau
RBC	:	Red blood corpuscles
RSPM	:	Respiratory suspended particulate matter
SBP	:	Systolic blood pressure
SD	:	Standard deviation of mean
SES	:	Socio-economic status
SOD	:	Superoxide dismutase
SOX	:	Oxides of sulfur
TERI	:	Tata Energy Research Institute
t,t-MA	:	Trans,trans muconic acid, a benzene metabolite
UFP	:	Ultrafine particle with diameter of leas than 0.1µm
UNEP	:	United Nations Environment Program
URS	:	Upper respiratory symptom
US EPA	:	United States environment protection agency
VOC	:	Volatile organic compound
WBC	:	White blood corpuscles
WHO	:	World health organization

EXECUTIVE SUMMARY

Central Pollution Control Board had sponsored the epidemiological studiy '**Epidemiological Study on Effect of Air Pollution on Human Health (adults) in Delhi**' carried out during September 2002–2005 and conducted by Chittranjan National Cancer Institute, Kolkata. The findings of these studies are as follows:

A. Objectives

- To assess air pollution related respiratory symptoms among the residents of Delhi.
- To assess the degree of lung function impairment in persons chronically exposed to city's air.
- To explore the underlying mechanism of air pollution related pulmonary dysfunction at the cellular and subcellular level.

B. Study details

- 6005 apparently healthy adults (4467 men and 1538 women) who were residents of Delhi for the past 10 years or more, aged between 21 – 66 years have been surveyed through questionnaires
- 1438 individuals have been clinically examined in Health Camps.
- Control group: 1046 apparently healthy subjects (775 men and 271 women), aged between 21 – 67 years from the rural areas of North and South 24-Parganas, Hooghly, Nadia, West and East Medinipur districts of West Bengal were enrolled
- Study carried out during November 2002 and August 2005 in various parts of Delhi and in different seasons.

C. Study protocol

- Evaluation of respiratory symptoms through questionnaire survey and clinical examination
- Assessment of lung function by spirometry
- Assessment of cellular lung response to air pollution by sputum cytology and cytochemistry
- Assessment of hematological, immunological, metabolic and cardiovascular changes associated with air pollution
- Changes in liver and kidney function
- Assessment of genotoxic effects
- Assessment of neurobehavioral problems
- Correlation between health effect and air quality

D. Findings

4. Respiratory symptoms

➢ A total of 33.2% residents of Delhi had one or more respiratory symptoms compared to 19.6% of control subjects indicating that respiratory symptoms were 1.7 times more prevalent in Delhi indicating breathing problem.

- Upper respiratory symptoms (URS such as sinusitis, runny or stuffy nose, sneezing, sore throat and common cold with fever) was present in 21.5% residents of Delhi compared with 14.7% control subjects indicating 1.5-times greater prevalence of URS indicating increased viral infection. Lower Respiratory Symptoms (LRS include chronic dry cough, recurrent sputum-producing cough, wheezing breath, breathlessness on exertion, and chest pain or tightness) was 1.8 times higher among the residents of Delhi. RSPM level was positively associated with LRS.

5. Lung function

- Lung function was reduced in 40.3% individuals of Delhi compared with 20.1% in control group. Residents of Delhi showed statistically significant ($p<0.05$) increased prevalence of restrictive (22.5% vs. 11.4% in control), obstructive (10.7% vs. 6.6% in control), as well as combined (both obstructive and restrictive) type of lung functions deficits (7.1% vs. 2.0% control).
- Lung function reduction was more prevalent in women than in men both in rural and urban settings. Besides gender, smoking habit, body mass index, socio economic status and particulate air pollution was positively associated with lung function deficits.
- Chronic obstructive pulmonary disease (COPD) was detected in 3.9% residents of Delhi against 0.8% of controls indicating lung obstruction.
- Greatest prevalence of reduced lung function was recorded in obese subjects as 46.4% of obese and 43.4% of overweight had increased prevalence of obstructive type of lung function deficits. The higher level of obesity may be due to the higher level of nutrition as well as the higher availability of junk food compared to control areas.

6. Cellular lung reaction to air pollution

- Sputum of Delhi's citizens contained 12.9 ±2.6 Alveolar Macrophages per hpf in contrast to 6.9±1.6 AM/hpf in controls, which were heavily loaded with particles resulting in increase of cell size indicating high particulate exposure..
- The citizens of Delhi had greater prevalence of several cytological changes in sputum compared with rural controls. The changes in sputum cytology were positively correlated with ambient PM_{10} level.
- Metaplasia (15.9% of non-smokers) and dysplasia (3.0% individuals) of airway epithelial cells were more frequent in Delhi's residents which maybe risk factors for cancer in the exposed tissues. Both metaplasia and dysplasia were more prevalent among residents of northern, western and central Delhi compared with residents of southern and eastern parts of the city.
- The number of iron-laden macrophages (siderophages) was significantly increased in sputum of the citizens of Delhi suggesting covert pulmonary hemorrhage in the lungs.

- A considerable rise in elastase activity in both alveolar macrophages and neutrophil was found among residents of Delhi which emphasizes greater risk of damage to the bronchial and alveolar walls that may lead to emphysema.

7. Hematological and immunological changes associated with air pollution

- Overall, 36.1% residents of Delhi had hypertension which was nearly 4-times higher than controls. It is a risk factor for cardiovascular diseases. RSPM (PM_{10}) level positively correlated with both systolic and diastolic blood.
- Platelet P-selectin was remarkably unregulated (2.8-times more activated platelets in circulation than controls) in residents of Delhi, suggesting hyper activation of circulating platelets and cardiovascular risk.
- Greater prevalence of several hematological abnormalities like target cells, toxic granulation, anisocytosis, poikilocytosis, hypochromic RBC, immature neutrophils, metamyelocytes and giant platelets were found in the individuals from Delhi in comparison to the control population indicate altered liver function, increased bacterial infection and cardiovascular risk.
- A significant reduction in the percentage of CD4+ T-helper cells and CD19+ B cells and concomitant increase in the percentage of CD8+ T-cytotoxic cells and CD56+ natural killer (NK) cells was found among the residents of Delhi indicating altered immunity.
- 30% depletion of erythrocyte superoxide dismutase level and 76% reduction in total antioxidant status compared with rural controls imply down-regulation of body's antioxidant defense

8. Genotoxicty

- High concentration of trans trans muconic acid (t,t-MA; a biomarker of benzene exposure) was found among the subjects of Delhi indicating benzene exposure (326 µg/g creatinine in auto rickshaw and taxi drivers, 218 µg/g creatinine in Delhi's office employees vs 102µg/g creatinine of t,t-MA in control subjects)
- Delhi's non-smokers had 2.3-times more micronucleus than control non-smokers which may indicate chromosomal damage. RSPM and benzene level of the city positively correlated with MN formation.

9. Neurobehavioral symptoms

- About 16% citizens of Delhi showed severe depression compared with 2.4% control subjects.
- Delhi's residents had increased prevalence of other neurobehavioral symptoms like anxiety, burning sensation in extremities, inability to concentrate, transient loss of memory, and palpitation. PM_{10} level was found to be positively associated with increased prevalence of transient loss of memory, burning sensation in extremities, and depression and benzene exposure was positively associated with transient loss of memory, inability to concentrate and anxiety

- Delhi residents had elevated epinephrine (E), norepinephrine (NE) and a decline in plasma dopamine (DA) levels in blood plasma than their rural counterparts indicating a stress effect. Elevated t,t-MA excretion was found to be associated with rise in plasma E and NE. Stress of urban living could have played a role in eliciting neurobehavioral problems as observed among the participants from Delhi

CHAPTER-1.0

BACKGROUND AND OBJECTIVE OF THE STUDY

1.1 BACKGROUND

(a) Health effects of air pollution

Air pollution is recognized as a major threat to human health. The United Nations Environment Programme has estimated that globally 1.1 billion people breathe unhealthy air (UNEP, 2002). Epidemiological studies have shown that concentrations of ambient air particles are associated with a wide range of effects on human health, especially on the cardio-respiratory system (Bates, 1992; Dockery and Pope, 1994). A growing body of evidence indicates that particulate pollution increases daily deaths and hospital admissions throughout the world (Pope et al., 1995; Zanobetti et al., 2001). Gaseous co-pollutants, seasonal patterns or weather did not confound the association between particulate pollution and cardiopulmonary mortality (Schwartz, 1994; Samet et al., 1998, 2000). Similarly, it was not modified significantly by race, sex and socioeconomic status (Zanobetti and Schwartz, 2000c). Thus, the association between particulate air pollution exposures and cardio-pulmonary mortality appeared causal.

(b) The historical perspective

Our concern about air pollution and its effect on human health stemmed primarily from three major air pollution episodes- Meuse Valley of Belgium in 1930, Donora in Pennsylvania of USA in 1948, and London smog episode in 1952. These episodes prompted many countries in Europe and North America to initiate legislative and regulatory measures to control outdoor air pollution. From the 1960s through 1980s several population-based studies were taken up in the industrialized countries and these investigations confirmed the adverse effects of air pollution on human health (Pope, 2000; Lave and Seskin, 1970). A series of epidemiological studies that followed in a short period of six years from 1989 to 1995 unveiled the role of particulate matter as the chief mediator of toxic effects of air pollution (Pope et al., 1995; Dockery et al., 1993; Schwartz and Dockery, 1992). This finding opened a floodgate of epidemiological and toxicological investigations on fine and ultrafine particulate air pollution. It became later obvious that besides respiratory diseases, air pollution adversely affect the cardiovascular system of the body (Pope et al., 2004; Brook et al., 2004). It was found that long-term, repeated exposures to air pollution increases the cumulative risk of chronic pulmonary and cardiovascular disease and even death (Pope et al., 2004; Brook et al., 2004; Pope et al., 2002; Clancy et al., 2002; Hoek et al., 2002). In fact, more deaths from air pollution occur due to cardiovascular causes rather than pulmonary diseases (Pope et al., 2004).

The World Health Organization (WHO) has estimated that urban air pollution is responsible for approximately 800,000 deaths and 4.6 million lost life-years each year around the globe (WHO, 2002). The burden of ill-health is not equally distributed as approximately two-thirds of the deaths and lost life-years occur in developing countries of Asia. The estimated health impact of urban air pollution is based largely on the results of epidemiological studies conducted in industrialized countries of Europe and North America that had been extrapolated to other countries of the world. Admittedly, the constituents of air pollution in different parts of the world are largely similar, but the magnitude of exposure, general health status of the people, nutritional and other disparities and the level of health care facilities are different across the globe. These inherent differences make extrapolation of findings from developed to developing countries questionable.

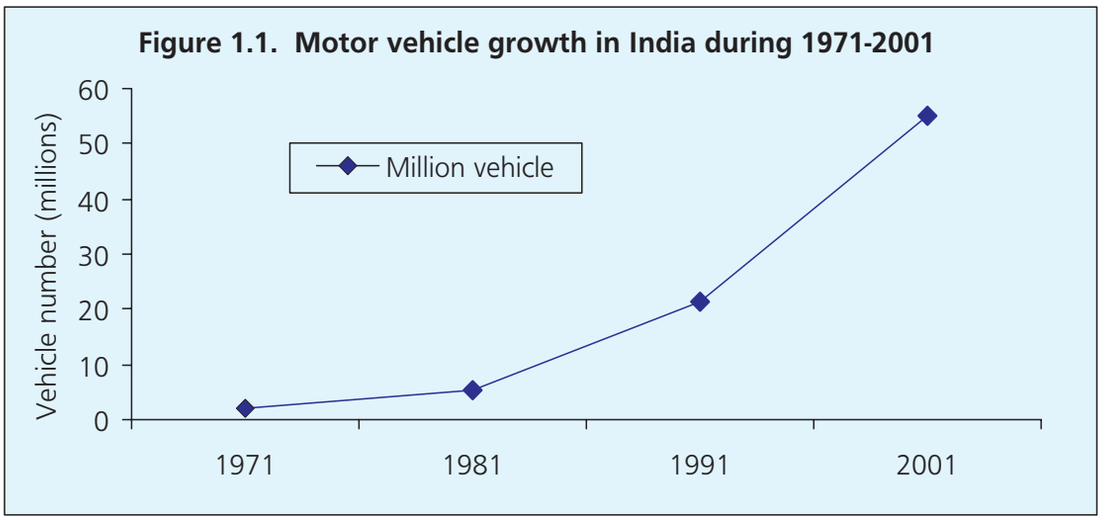
1.2 URBANIZATION AND AIR POLLUTION IN INDIA

Air pollution in Asian cities is closely tied to levels and trends in economic and social development. Besides, rapidly increasing industrialization, urbanization, population growth and demand for transportation along with meteorologic conditions influence air pollution in many Indian cities. In recent time, India is experiencing a rapid growth and economic development reflected by industrialization, urbanization, rise in income and motor vehicle use. Currently about two-third of Indians live in rural areas. But the pattern is changing rapidly as more people are moving to the cities in search of livelihood.

Vehicular sources of urban air pollution in India

In general, combustion is the chief contributor to outdoor air pollution. In most cities, the major source of combustion is fuel use, which tends to increase along with population size and economic activity. In the last three decades, the number of motorized vehicles in India has increased 29-times, from 1.9 million in 1971 to 55.0 million in 2001 (Badami, 2005; Figure 1.1). The increase was not uniform for all vehicle types: it was 7-fold for buses, 9-fold for trucks, 10-fold for car, Jeeps and taxis, but a remarkable 67-fold for two-wheelers (Badami, 2005).

Figure 1.1. Motor vehicle growth in India during 1971-2001



Motor vehicles have internal combustion engine which burns a mixture of air and fuel to produce energy that propels the vehicle. The type and quantity of the pollutants released during this combustion is influenced by more than a dozen factors. The kind of fuel- petrol, diesel or compressed natural gas (CNG) is just one of them. However, fuel type is an useful indicator of potential emissions. Coal and biomass are high emitting solid fuels, petrol diesel and kerosene are mid-emitting liquid fuels and liquefied petroleum gas (LPG) and CNG are low-emitting gaseous fuels. Transport sector consumes half of the petroleum products in the world, and same is true for India. Petroleum product consumption by motorized vehicles has nearly doubled in India in the last decade.

Fuel and lubricating oil quality have also contributed significantly to transport air pollution in India. Indian gasoline have a high volatility, and the vast majority of gasoline vehicles are carbureted, not fuel-injected (Table 1.1, 1.2). These facts coupled with India's high ambient temperatures increases the potential for evaporating emissions rich in reactive hydrocarbons with the potential to generate ground-level ozone.

Table 1.1: Quality of gasoline in India

	1993	1997	2002	Proposed
Lead mg/L, max	560	150	13	5
Sulfur % by mass, max	0.2	0.15 in unleaded petrol	0.1; 0.02 in notified areas	0.015
Benzene % by volume, max	-	-	1 in notified areas; 3 (metro's); 5(rest of the country)	1
Olefin % by vol, max	-	-	-	21
Aromatics % by vol, max	-	-	-	42
O2 content, % by mass, max	-	-	-	2.7

Source: Badami (2005)

Table 1.2: Quality of diesel in India

	1995	1999	2002	Proposed
Sulfur, total, % by mass, max	1	0.25	0.05 (in metros)	0.035

Source: Badami (2005)

Traffic congestion is yet another problem leading to high vehicular emissions. In Delhi, for example, the average speed for public transport vehicles ranged from 12 to 20 km/hr in the 1990's (RITES, ORGO, 1994). Besides causing loss of time and productivity, traffic congestion increases fuel consumption and carbon monoxide and hydrocarbon emissions per vehicle-km by 200% or more (Faiz et al., 1992). Several studies have shown that maintenance is a significant factor in vehicular emissions.

Epidemiological studies of outdoor air pollution in Western countries, in general, have not considered indoor sources and total exposures. Residents of slum households, who tend to have more health problems due to poverty, might also experience higher outdoor exposure because they live in road-side slums. In such cases, the effect of poverty on health can be confused with the effect of vehicular air pollution. Exposure to indoor air pollution or other factors associated with poverty may also increase the susceptibility of the poor to outdoor air pollution.

Urban aerosols contain not only combustion products. Elevated PM_{10} concentration in Delhi during summer can be attributed to windblown dust excursions from Rajasthan desert areas. These situations are dominated by fugitive dusts mostly associated with coarse particles (i.e. 2.5 µm to less than 10µm particles). Similar situation has been reported in Sun Joakin Valley in the USA (Tinkerton et al., 2000).

1.2.1 Particulate pollutants: the major toxic component of urban air

Particulate matter (PM) is a complex mixture of suspended solid and liquid particle in semi equilibrium with surrounding gases (Brook et al., 2003). The particle constituents vary greatly in size, composition, concentration, depending on origin and age. PM may be classified as primary (particles emitted directly by emission sources) and secondary (particles formed through the atmospheric reaction of gases). The size distributions of airborne particles are important for health impact. The particles larger than 10 µm in diameter are deposited almost exclusively in the nose and throat whereas those smaller than 1 µm

reach the lower regions of the lung. The intermediate size range gets deposited between these two extremes of the respiratory tract. Outdoor (ambient) PM size ranges from approximately 0.001-100 µm in aerodynamic diameter. PM is considered as the single best indicator of potential harm. There are three main size categories for PM measured in urban air:

(a) PM_{10}

They consist of PM with a diameter upto 10 µm. However, for toxicity studies, the most important particles are those having a diameter of less than 10 µm (PM_{10}) because they are respirable whereas the larger particles are not. PM_{10} deposit relatively quickly with a lifetime of less than 2 days, and exposure may lead to adverse responses in the lungs triggering an array of cardio-pulmonary problems (Brunekreef and Forsberg, 2005; Harrabi et al., 2006). PM_{10} has also been associated with emergency hospital admission for asthma, bronchitis, and pneumonia in older people (Ye et al., 2001). For every 10-µg/m^3 increase of $PM_{10,}$ mortality from all causes increases by 0.51% and from cardiopulmonary diseases by 0.68% (Samet et al., 2000). Moreover, the rise in daily mortality from increased concentrations of PM_{10} persists for several days (Zeka et al., 2005).

(b) Accumulation mode or fine particles ($PM_{2.5}$)

They consist of PM with a diameter upto 2.5 µm. Airborne particles smaller than 2.5 µm ($PM_{2.5}$) are usually called fine particles. These particles may penetrate deep inside the airways and are more strongly linked to adverse health effects (USEPA, 1996). Fine particles are composed mainly of carbonaceous materials (organic and elemental), inorganic compounds (sulfate, nitrate, and ammonium), and trace metal compounds (iron, aluminium, nickel, copper, zinc, and lead). There are potentially thousands of different compounds existing on fine particles that may exert harmful biological effects. On any day or location, the PM mass concentration may be similar, yet the composition may vary greatly enough to differentially impact human health (Brook et al., 2003). The relationship between PM_{10} or $PM_{2.5}$ exposure and acute health effects is linear at concentrations below 100 µg/m^3 (Schwela, 2000). A modest rise in PM_{10} or $PM_{2.5}$ level has been shown to be associated with small changes in cardiac function (Mar et al., 2005). Exposure to the fine particles induces oxidative stress (Furuyama, 2006).

(c) Nuclei mode or ultra fine particles (UFP)

The particles in this category are smaller than 0.1 µm. They are also known as ultrafine particle (UFP). UFP are present in great number in polluted urban air (Jaques and Kim, 2000). They have a carbonaceous core with attached inorganic and organic materials that can cause adverse health effects (Oberdorster, 2000). The UFPs have less mass than course particle fraction but they are much greater in number and have a relatively large surface area to mass ratio, making them potential carriers of harmful gaseous compounds. Very tiny particles (UFP) escape alveolar macrophage surveillance, which is very efficient for larger particles (Hahn et al., 1977). Exposure to high doses of UFP can cause severe pulmonary inflammation and hemorrhage, high degree of alveolar and interstitial edema, disruption of epithelial and endothelial cell layers and even death (Oberdorster et al., 1992; Peters et al., 1997, Oberdorster, 2000). Biologic effects of ultrafine particles occur even at modest exposure, such as that occurring in traffic-related air pollution. UFPs cause health effects like cardiovascular problems, pulmonary disease, and development of cancer (Vinzents et al., 2005).

Fate of the particles

Following inhalation, the size of the particles determines where they are likely to deposit in the respiratory tract. Particles larger than 10 micrometer are mainly deposited in the nose and throat and are less likely to affect the health beyond the point of deposition. $PM_{2.5}$ and UFPs are able to penetrate into the

airways all the way to the terminal alveoli. Smaller particles are present in larger numbers and have more total surface area and bioavailability, eliciting greater biological effect.

1.2.2 Other pollutants

(a) Sulfur dioxide (SO_2)

Sulfur dioxide (SO_2) is emitted in direct proportion to the amount of sulfur in fuel. Coal burning is a major source of SO_2 in air. It is an acidic gas, which combines with water vapor in the atmosphere to produce acid rain. SO_2 in ambient air can also affect human health (Routledge et al., 2006), particularly in those suffering from asthma and chronic lung diseases and exacerbates respiratory symptoms and impaired breathing in sensitive individuals (Lipfert, 1994). It can also attach to particle surfaces and may form acidic coatings. It is considered more harmful when particulate and other pollution concentrations are high.

(b) Oxides of nitrogen (NOx)

Nitrogen oxides are formed during combustion processes at high temperatures from the oxidation of nitrogen in air. The major types of oxides of nitrogen are nitric oxide (NO) and nitrogen dioxide (NO_2). They are collectively known as NOx. The main source of NO is road traffic, which accounts for 49% of total NO emissions in Europe and 32% in the USA. It is emitted from both petrol- and diesel engine motor vehicles. Almost all NO_x is emitted as NO, which is rapidly oxidized to more toxic NO_2.

NO_x is a precursor of ozone formed in the troposphere. Oxides of nitrogen are immunotoxic and increase the susceptibility to respiratory tract infection such as influenza. Continued or frequent exposures to high concentrations of NOx in breathing air may cause irritation of the lungs and consequent acute respiratory illness (Hasselblad et al., 1992). In addition, NO_x is a potent and selective vasodilator in pulmonary arterial hypertension (Perez-Penate et al., 2005).

(c) Carbon monoxide (CO)

Carbon monoxide (CO) is a toxic gas emitted into the atmosphere as a result of combustion processes. CO is also formed by the oxidation of hydrocarbons and other organic compounds. CO is produced almost entirely (90%) from road traffic in European cities. It remains in the atmosphere for approximately one month before being oxidized to CO_2. The largest contributors of CO are petrol-fuelled vehicles. CO binds strongly to hemoglobin in red blood corpuscles resulting in the production of carboxyhemoglobin (COHb). This impairs the transport of oxygen within the blood and can result in adverse effect on tissues with high oxygen needs such as the cardiovascular and nervous systems. High concentration (>1000 ppm) for prolonged hours (>8 hr) can give rise to hypoxia. A recent study has shown that chronic exposures to CO may cause adverse birth outcomes such as reduced birth weight and intrauterine growth retardation (Salam et al., 2005).

(d) Polycyclic aromatic hydrocarbons (PAHs)

About 200 different kinds of hydrocarbons are emitted from combustion of petrol and diesel. Of these, the polycyclic aromatic hydrocarbons (PAHs) are of particular interest due to their carcinogenic potential. PAHs are usually adsorbed on the particulate pollutants. They enter the body through inhalation of these respirable particles. These compounds are semi-volatile in nature. Several PAHs like benzo(a)pyrene [B(a)P] are highly carcinogenic (Hrudkova et al., 2004). Incidence of lung cancer has been reported in persons directly exposed to B(a)P from automobile exhausts and biomass (wood, dung, agricultural wastes) fuel burning during household cooking (Cohen and Nikula, 1999).

(e) Volatile organic compounds (VOCs)

VOCs consist of various classes of carbon-containing chemicals that are gases at room temperature. They are released into the environment from petrol and diesel, especially the former, by evaporation or as combustion products. Some VOCs (e.g. benzene) are human carcinogens while others are either respiratory tract irritants or neurotoxic (e.g. toluene, xylene).

Benzene, a VOC, is a minor constituent of petrol. It is produced from combustion and evaporation of both petrol and diesel, especially the former. Combustion of petrol is the largest source (70% of total emissions) of benzene in air. Airborne benzene is primarily absorbed through the respiratory tract and then transported by blood to critical target organs. Therefore, it is possible that cumulative exposure to benzene could lead to systemic changes. True to this apprehension, benzene has been found very harmful for human health for its hematotoxic, neurotoxic, leukemogenic and carcinogenic effects (Wallace, 1984, 1989; Midzenski et al., 1992, Farris et al., 1993). Because of this, a sustained worldwide effort is on to reduce benzene exposure as far as possible.

1.2.3 Air toxics of biological origin

Biological agents present in polluted air may cause several diseases. The biological contaminants include bacteria, moulds, mildew, viruses, animal dander and cat saliva, house dust, mites, cockroaches, and pollen. There are many sources of these pollutants. Pollens originate from plants; people and animals transmit viruses; bacteria are carried by man, animal, soil, and plant debris; and household pets are sources of saliva and animal dander. The protein in urine from rats and mice is a potent allergen. When it dries, it can become airborne.

(a) Bacteria

Along with particulate pollution, numerous airborne bacteria enter the body during respiration. Several of these are pathogenic to humans. For example, *Bordetella pertusis* causes whooping cough, *Corynaebacterium diphtheriae* causes diphtheria, *Mycobacterium tuberculosis* causes tuberculosis and *Mycobacterium pneumoniae* cases bacterial pneumonia. Globally, pneumonia causes two million deaths of children (20% of all child deaths) every year and 70% of them occur in Africa and South-east Asia. The main causative organisms have identified as *Streptococcus pneumoniae.* It has been shown in animal studies that long-term exposures to diesel exhausts increase the risk of pulmonary tuberculosis (Hiramatsu et al., 2005). Tobacco smoke is a proven risk factor for bacterial infection. Smoking is associated with a significant increase in the relative risk of pneumonia (*S. pneumoniae*) and tuberculosis (Trosini-Desert et al., 2004).

(b) Virus

Like bacteria, viral infections have been linked to air pollution. *Mumps* virus (mumps), *Myxovirus influenza* (influenza), *Poliovirus* (poliomyelitis), *Rhinovirus* causing common cold, *Rubella* virus (German measles), *Rubella* (Measles), *Varicella* virus (Chicken pox) and *Variola pox* virus causing small pox, *Haemophilus influenzae*, Respiratory syncytial virus (RSV), influenza, parainfluenza and adenoviruses are some of the viruses which spread through polluted air. Measles infection increases pneumonia morbidity and mortality (Singh, 2005). Air pollution is known to enhance Human papilloma virus (HPV)-mediated cancer of the uterine cervix in women who are chronically exposed to biomass smoke (Velema et al., 2002).

(c) Fungus

Airborne fungi like *Aspergillus fumigatus* cause aspergillosis and *Blastomyces dermitidis* cause blastomycosis. Greater presence of fungi in indoor environment due to poorer house condition, older

house age, relative lack of sun exposure and having no insulation enhances the risk of respiratory illness including oral toxicosis and airway allergy (Howden-Chapman et al., 2005). The problems may turn serious requiring hospitalization (Khalili et al., 2005).

(d) Pollen and other allergens

Airborne pollen and other allergens are major causative agents of bronchial hypersensitivity and asthma. Asthma exacerbation is the most common cause of hospital admission in children. Airborne bacteria and virus infections in allergic asthmatics further increase the risk of hospitalization (Murray et al., 2005). Pollen exposure is usually associated with respiratory tract allergy and eosinophil accumulation in the nasopharynx and the airways (Onbasi et al., 2005).

1.3 AIR QUALITY STANDARDS

National Standard

National Ambient Air Quality Standards (NAAQS) of India has recommended that the annual average concentration of respirable suspended particulate matter (RSPM) with an aerodynamic diameter of less than 10 micrometer (PM_{10}) in ambient air of residential areas should be within 60µg/m^3, while the 24-hr average should be within 120 µg/m^3 (Table 1.3).

Table 1.3: National Ambient Air Quality Standards (NAAQS) of India

Pollutant	Time weighted average	Concentration in ambient air (µg/ m^3)			
		Sensitive areas	Industrial areas	Residential, rural and other areas	Methods of measurement
Sulfur dioxide	Annual 24 hours	15 30	80 120	60 80	* Improved West and Gaeke method * Ultraviolet fluorescence
Nitrogen dioxide	Annual 24 hours	15 30	80 120	60 80	* Jacob and hochheiser modified (Na-Arsenite) method * Gas-phase chemiluminescence
Suspended particulate matter (SPM)	Annual 24 hours	70 100	360 500	140 200	High-volume sampling (average flow rate not less than 1.1m^3 /min)
PM_{10}	Annual 24 hours	50 75	120 150	60 100	Respirable particulate matter sampler
Lead	Annual	0.50	1.0	0.75	Atomic absorption spectrometry after sampling using EPM 2000 or an equivalent filter paper
Carbon monoxide	24 hours 8 hours 1 hour	0.75 (mg / m^3) 1.0 (mg /m^3) 2.0 (mg /m^3)	1.5 (mg /m^3) 5.0 (mg/m^3) 10.0 mg/m^3)	1.0 (mg / m^3) 2.0 (mg/m^3) 4.0 (mg/m^3)	Non-dispersive infrared spectroscopy
Ammonia	Annual 24 hours	400.00 100.00			---

Source: National Ambient Air Quality Monitoring Series: NAAMQS/9/1996 – 97, Central Pollution Control Board, Government of India

Compared with Indian standard, the air quality guidelines advocated by the World Health Organization (WHO, Table 1.4) and the United States Environmental Protection Agency (US EPA) are stricter. According to US EPA, the 24-hour average PM_{10} concentration should be within 150 µg/ m^3 and should not be exceeded more than once per year, and the annual average concentration of PM_{10} should not exceed 50 µg/ m^3 over the course of a calendar year. WHO guideline states that annual and 24-hr average PM_{10} should be within 20 µg/ m^3 and 50 µg/ m^3 respectively.

Table 1.4: WHO Air Quality Guideline (AQG) values

Pollutant	Averaging time	AQG value
Particulate matter		
$PM_{2.5}$	1 year 24 hour (99th percentile)	10 µg/m^3 25 µg/m^3
PM_{10}	1 year 24 hour (99th percentile)	20 µg/m^3 50 µg/m^3
Ozone, O_3	8 hour, daily maximum	100 µg/m^3
Nitrogen dioxide, NO_2	1 year 1 hour	40 µg/m^3 200 µg/m^3
Sulfur dioxide, SO_2	24 hour 10 minute	20 µg/m^3 500 µg/m^3

Source: WHO air quality guidelines global update 2005. Report on a working meeting, Bonn, Germany, World health organization, 2005.

1.4 DELHI, THE CAPITAL OF INDIA

1.4.1 General

Delhi is the capital city of India. The name *Delhi* also refers to the National Capital Territory of Delhi (NCT), which is a special union territory jointly administered by the Central and local government. New Delhi, an urban area within the metropolis of Delhi, is the seat of the Government of India. The name Delhi has its origin either from the Persian word *Dahleez* (threshold, or frontier) or from the name of a Mauryan king, Raja Dhillu. Delhi was once Indraprastha, the capital city built around 5000 BC by the Pandavas of Mahabharata. Archaeological evidence suggests that Indraprastha once stood where the Old Fort is today. The earliest architectural relics of Delhi date back to the Mauryan Period (300 BC) and an inscription of the Mauryan emperor Ashoka (273-236 BC) was discovered near Srinivaspuri in 1966.

The famous Iron pillar near the Qutub Minar was commissioned by the emperor Kumara Gupta I of the Gupta dynasty (320-540) and transplanted to Delhi during the 10th century. Present-day Delhi contains the remnants of seven successive ancient cities including: Qila Rai Pithora built by Prithvi Raj Chauhan in Lal-Kot: Siri Fort built by Alauddin Khilji in 1303; Tughluqabad built by Giyasuddin Tughluq (1321-25); Jahanpanah, built by Muhamad bin Tughluq (1325-51); Kotla Firoz Shah by Firoz Shah Tughluq (1351-88); Purana Qila, built by Sher Shah Suri and Dinpanah built by Humayun both near Indraprastha in 1538-45; and Shahjahanabad incluing Lal qila and Chandni Chowk built by Moghal Emperor Shah Jahan from 1638-1649.

(a) Geography and meteorology

Delhi is situated between the Himalayas and Aravali range in the heart of the Indian sub-continent, surrounded on three sides by the state Haryana and to the east, across the river Yamuna by the state Uttar Pradesh. Its location is between geocoordinates of 76^0 50′ 24″ to 77^0 20′ 37″ E longitude and 28^0 24′ 17″ to 28^0 53′ 00″ N latitude. The major part of the territory lies on the western side of the river Yamuna. Its greatest length is around 33 miles and the greatest width is 30 miles. Delhi's altitude

ranges from 213 to 305 meters above the sea level. Delhi has a semi-arid climate with high variation between summer and winter temperatures. Extreme temperature variations are characteristic of Delhi. Summers are long, from early March till October, with the rainy season in between. Hottest months are April to June when the temperature often rises to 36^0C to 47^0C. Winter starts in November and peaks in January. Temperature falls to 7.9^0C or even lower during December-January. The average annual rainfall is approximately 670 mm (27 inches), most of which falls during the monsoons in July and August. Traditionally, the monsoons are supposed to touch Delhi by June 29 every year. Prevailing wind direction is in W-NW-N sector and E direction with 35% calm conditions. The predominant wind direction in winter season is from W-NW-N sector with 45% calm conditions (NEERI, 2003).

(b) Area

The national Capital Region of Delhi (NCT Delhi) has an area of 1483 sq.km. The city is divided into nine districts. Each district has three subdivisions. Below is the list of the districts and subdivisions of Delhi (Table 1.5, Fig. 1.2):

Table 1.5: Districts of Delhi

Districts	Subdivisions
1.Central Delhi	Darya Ganj, Pahar Ganj, Karol Bagh
2. North Delhi	Sadar Bazar, Kotwali, Sabzi Mandi
3. South Delhi	Kalkaji, Defence Colony, Hauz Khas
4. East Delhi	Gandhi Nagar, Preet Vihar, Vivek Vihar, Vasundhara Enclave
5. North East Delhi	Seelampur, Shahdara,Seema Puri
6. South West Delhi	Vasant Vihar, Najafgarh,Delhi Cantonment
7. New Delhi	Connaught Place, Parliament Street, Chanakya Puri
8. North West Delhi	Saraswati Vihar, Narela, Model Town
9. West Delhi	Patel Nagar, Rajouri Garden, Punjabi Bagh

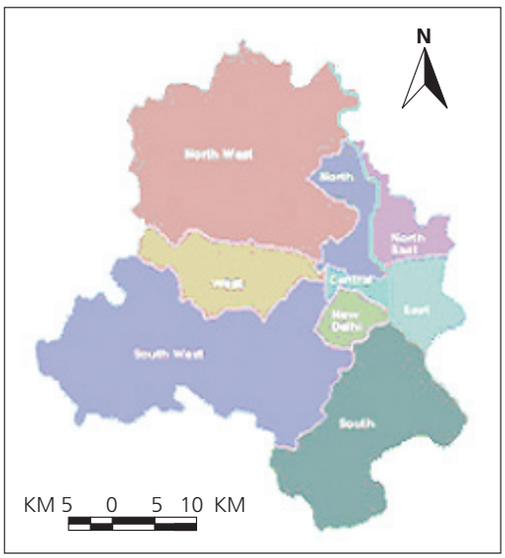


Figure 1.2: District map of Delhi

(c) Population, gender, literacy

Delhi is one of the fastest-growing metropolis in Asia. Delhi's population was 4.05 lakhs (0.4 million) at the beginning of 20th century. According to 2001 census, Delhi's population was 1,37,82,976 (approximately 13.8 million), making it the third largest

city in India after Mumbai and Kolkata. District-wise distribution of population reveals that most thickly populated region is North-Western Delhi. It has 2.85 million inhabitants representing 20.66% of Delhi's total population. Incidentally it is the largest district in Delhi with a total area of 444 sq.km, i.e. 29.66% of Delhi's total area. The next populated regions are South (16.38%), West (15.38%), North-East (12.8%), South-West (12.69%), East (10.51%) and North (5.66%). Central district having only 25 sq.km area had 4.67% of total population, while New Delhi represents only 1.25% of Delhi's total population (Fig. 1.3). According to 2001 Census, the sex ratio (number of females per 1000 males) of Delhi was 821. It was highest in North-East (851) and lowest in South-West district (783). Overall literacy rate in Delhi at the time of 2001 Census was 81.82%, the 5th highest position in the country. Highest literacy rate in Delhi was recorded in East district (85.1%), while North-East district had the lowest literacy rate (77.85%). Literacy rate among males was highest in South-West district (89.93%), and Central district had the lowest literacy (82.5%). In case of females, East district had the highest literacy (79.38%), and North-East district had the lowest female literacy (69.97%).

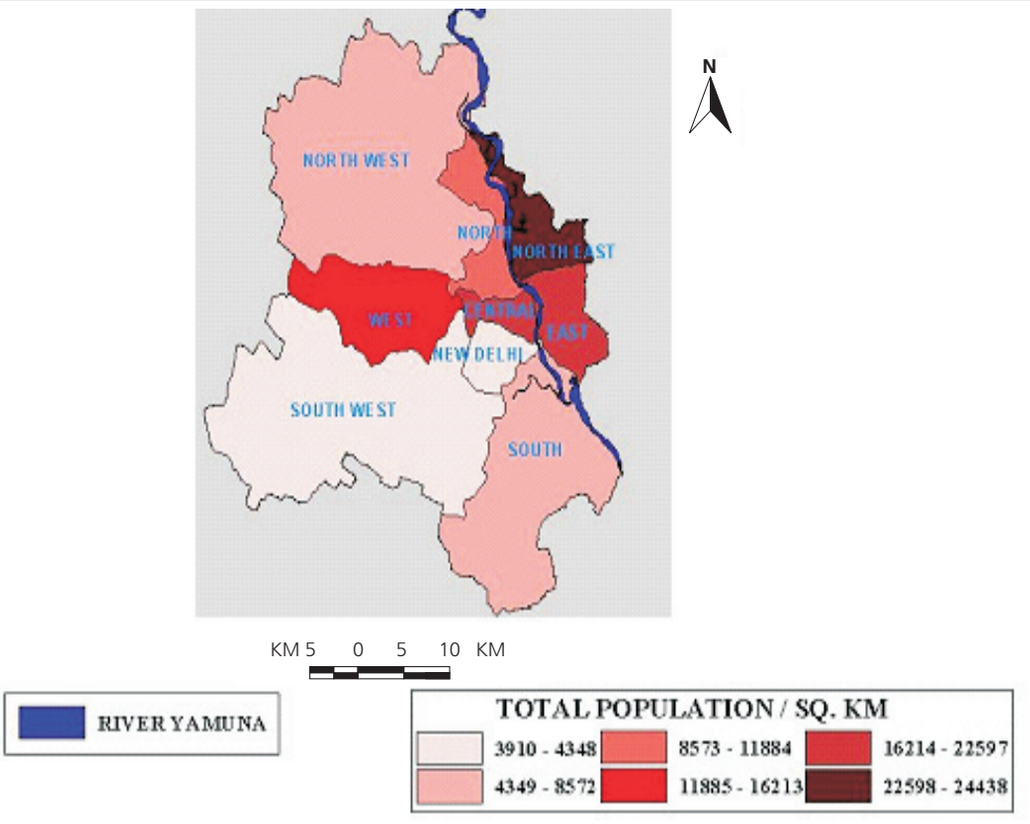


Figure 1.3: Population distribution per square kilometer in Delhi

(d) Administration

The sixty-ninth amendment to the Constitution of India in 1991 granted Delhi the status of a special union territory and officially changed its name to the National Capital Territory of Delhi (NCT). Delhi has its own Legislative Assembly, Lieutenant Governor, Council of Ministers and Chief Minister. However, Delhi is jointly administered by the Union Government of India and the Territorial Government of Delhi. New Delhi, an urban area in Delhi, is the seat of both the State Government of Delhi and the Government of India. Each district is headed by a Deputy Commissioner and has three subdivisions. Each subdivision is headed by the Subdivision Magistrate.

(e) Economy

Delhi's gross domestic product for 2004 was estimated at US $26 billion. Historically, Delhi has been the economic capital of northern India. In recent years, Delhi's service sector has expanded exponentially. Key service industries include information technology (IT), telecommunications, banking, media and life sciences. Delhi and its suburbs account for over 30% of India's IT and IT-enabled services (ITeS) exports - the second largest in the country after Bangalore's 35% share. Delhi's manufacturing industry has also grown considerably as many consumer goods industries have established manufacturing units and headquarters in and around Delhi.

Delhi is one of India's most affluent urban centers and is at the heart of India's largest consumer belt. As an indicator, Delhi has more cars on its roads than India's other metros Kolkata, Chennai and Mumbai combined. Delhi is one of India's largest markets because per capita income is much higher than in other Indian cities. Gurgaon and Noida, two of Delhi's largest satellite cities, have attracted more than $5 billion worth investments in the past three years. The city's booming economy is also the main reason behind so many people migrated to Delhi in recent years in search of better living conditions and employment.

1.4.2 Sources of air pollution in Delhi

Vehicular emissions, industrial emissions, household activities and soil resuspension are the major source of air pollution in Delhi (Balachandran et al., 2000).

(a) Vehicular pollution: motor vehicles in Delhi

As in case of many other Asian cities, motor vehicles are responsible for a substantial part of Delhi's air pollution. Delhi alone with only a little over 1% of India's population account for about 8% of the national motor vehicles (Badami, 2005). The motor vehicle fleet presently stands at 4.2 million, of these 2.7 million are two wheelers (Badami, 2005). Currently vehicular pollution contributes to 72% of the total air pollution load in Delhi (Goyal et al., 2006), which was only 23% in 1970-71 (Table 1.6). It has been estimated that vehicular source is responsible for generating more than 3000 metric tons of pollutants per day (MT /day) in Delhi.

Table 1.6: Relative contribution to air pollution in Delhi

Source	Year			
	1970 - 71	1980 - 81	1990 - 91	2000 – 2001
Vehicle	23%	42%	63%	72%
Industry	56%	40%	29%	20%
Domestic	21%	18%	8%	8%

Source: PHD Chamber of Commerce and Industry Report, November 1991

Since the time of economic liberalization policy introduced by the Government of India, more and more households have graduated to higher income categories. Coupled with changing attitudes towards taking loans, people no longer found it difficult to own their personal car. Sales of passenger cars jumped more than three times from 209,203 units in 1994 to 638,815 units in 2000 - all in a matter of seven years. The National Capital Region of Delhi has the highest number of vehicles in the country - more than Mumbai, Calcutta and Chennai put together.

There has been a steady increase in the number of vehicles in Delhi and their contribution to city's air pollution in the last three decades (Figure 1.4). Till 1990, the rise in the number of motor vehicles in Delhi was proportional with the population growth. Thereafter, the growth of vehicles has been spectacular, surpassing population increase by several folds (Figure 1.5). The population of the city has increased from 3.53 million in 1970 to 13.80 million in 2001, registering a rise of 3.9-fold over a period of 30 years. On the other hand, the number of registered motor vehicles in the city in 2001 was 34.2 lakh against 2 lakh during 1970-71. The growth in the vehicular population of the city during this period, however, has been 17-fold that far surpassed population growth. In 1975, the number of vehicles in Delhi and Mumbai was almost the same. Today Delhi has 3 times more vehicles than Mumbai, although Mumbai has 4 million more inhabitants than Delhi. In 1995 Delhi had 27 lakh vehicles compared with 7.2 lakh in Mumbai, 5.6 lakh in Kolkata and 8.1 lakh in Chennai. In essence, within a span of just 30 years (1971-2001) the city has 3.9-times more residents and 17-times more vehicles. It has been projected that the gap between the growth in human and vehicular population in Delhi will further widen in future.

Figure 1.4: Growth in the number of vehicles (in lakhs) and the relative contribution (%) of road traffic to Delhi's air pollution in the past 30 years (1971-2001)

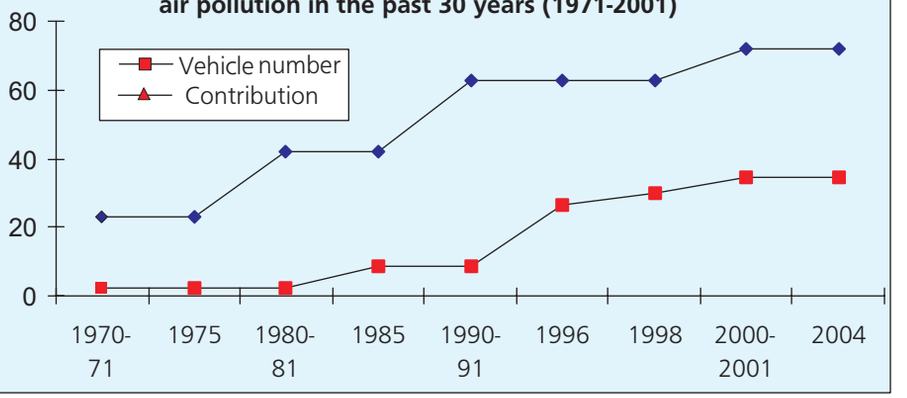
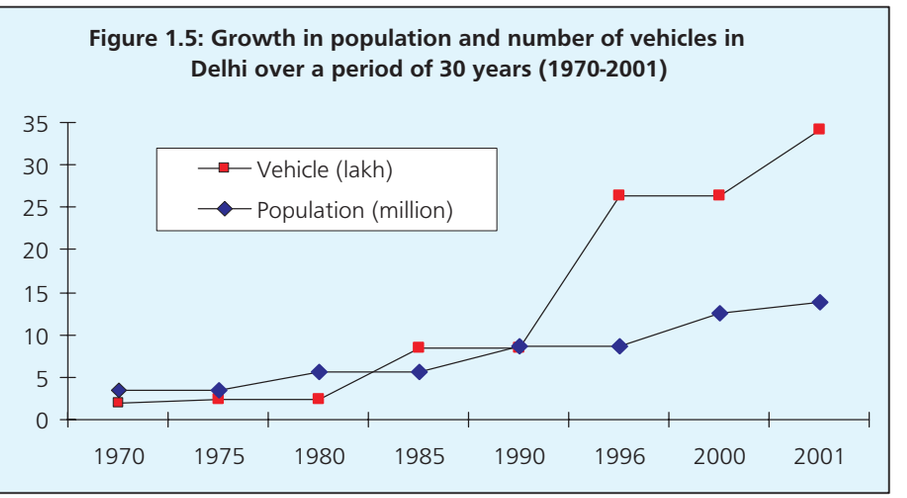


Figure 1.5: Growth in population and number of vehicles in Delhi over a period of 30 years (1970-2001)



(i) Road transportation in Delhi

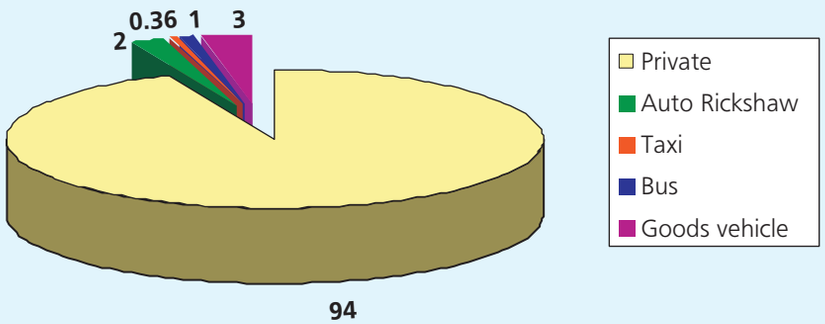
Delhi's road transport includes private vehicles such as 2-wheelers, cars, Jeeps etc.; public transport vehicles, such as bus, taxi, and autorickshaws; and goods transport vehicles such as trucks and tempos.

Public vehicles

Bus

Delhi's buses constitute only 1.2% of the total number of vehicles, but they cater to 60% of the total traffic load. Although personal vehicles such as cars and two wheelers represent nearly 94% of the total number of vehicles of the city (Figure 1.6, 1.7), they cater to only 30% of the travel demand (Dept. of Transport, Govt. of Delhi). Delhi Transport Corporation (DTC) operates the world's largest fleet of compressed natural gas (CNG)-fueled buses. Besides, there are a large number of private-owned CNG-fueled buses plying in Delhi. Delhi's buses pollute much less than diesel-fueled buses of most other cities in India.

Figure 1.6: Percentage share of vehicles plying in Delhi



Taxi and Auto rickshaw

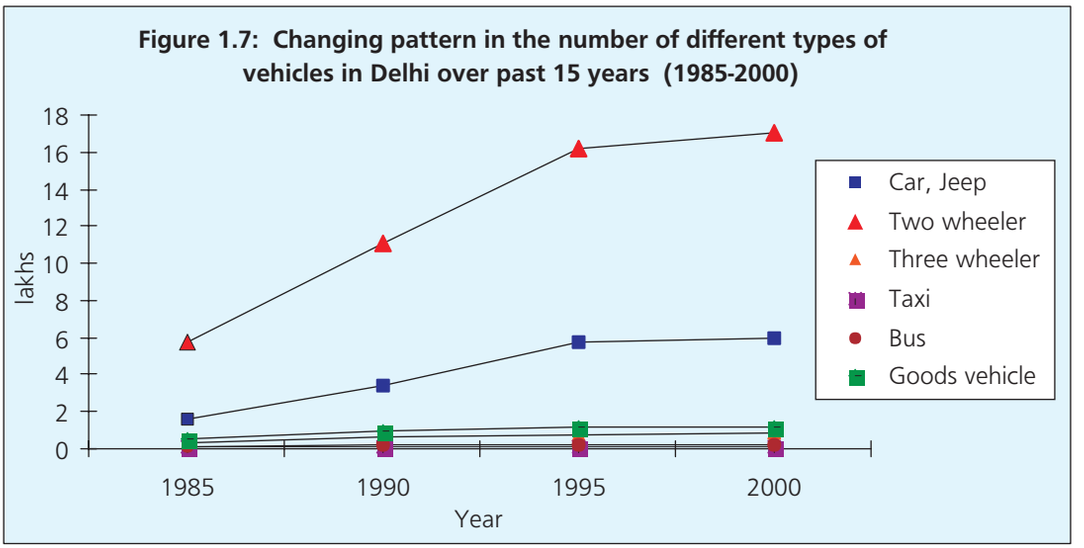
Compared to Mumbai and Kolkata, taxis are fewer in Delhi as people rely more on their own cars. However, Delhi's auto rickshaws provide a very important contribution in mass transport. Because they are CNG-powered, emissions are much less compared to pre-CNG era.

Goods transport vehicles

Lorries and trucks plying on Delhi roads including those coming with goods from adjoining states constitute 3% of city's vehicular population. They are a significant source of air pollution, because the vehicles are run by diesel-powered engines.

Private vehicles

Private vehicles, such as cars, scooters and motor bikes, constitute 94% of Delhi's vehicular population. The numbers of 2-wheelers and cars have increased exponentially in recent times in Delhi, although the number of public transport vehicles has increased only marginally (Figure 1.7). About two-third of the total vehicular population of Delhi is two wheelers. The total number of two wheelers in Delhi in 1993 was 14 lakh, which was 1.5 times more than the combined number of two wheelers in three other metros of India – Mumbai 2.4 lakh, Kolkata 2.2 lakh and Chennai 4.6 lakh.

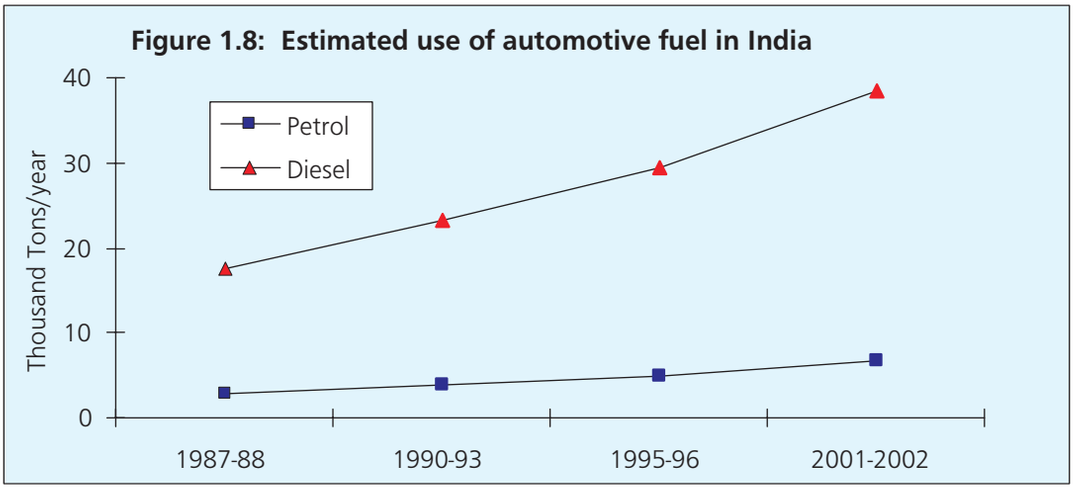


Metro Railways

A metro (mass rapid-transit system) built and managed by the Delhi Metro Rail Corporation is also operating. Three lines are currently operating on elevated as well as underground track totaling a length of 56 km. Line 1 runs east-west for 22 km on an elevated track between Rithala and Shahdara. Line 2 runs north-south for 11km in an underground tunnel between Vishwa Vidyalaya and the Central Secretariat. Line 3, opened on December 31, 2005, runs 22.8 km long on elevated tracks and an underground tunnel, between Indraprastha, Barakhamba Road and Dwarka.

(ii) Automotive fuel

Diesel and petrol (gasoline) were the principal automotive fuels till the introduction of compressed natural gas (CNG) for public transport vehicles in 2001. During 2001-02, the annual consumption of diesel in India was 38,500 tons against 6,640 tons of petrol (Figure 1.8). There has been a steady increase in vehicular pollution and automotive fuel consumption, especially diesel, in Delhi in the last three decades (Table 1.6, Figure 1.8). Diesel is considered more polluting fuel than petrol, because it emits more particulates, especially of fine and ultrafine categories, than petrol during combustion. Petrol, on the other hand, releases more volatile organic compounds, particularly benzene, during combustion and also due to evaporation.



Introduction of CNG

CNG vehicle program was launched in Delhi in 2001. In July 2001, Delhi had 34.2 lakh vehicles out of which CNG was used in 2450 buses, 1178 minibuses, 27,363 three wheelers and 1993 taxis (CPCB). During 2000-2001, over 1.5 million vehicles run on natural gas worldwide. Altogether, 14 countries had 10,000 or more natural gas-fueled vehicles (IANGV, 2001)

Advantages of CNG

Compared with diesel and petrol, CNG is a cleaner fuel as it emits substantially lower amount of particulates carbon monoxide and oxides of nitrogen (Table 1.7). Moreover, these vehicles have low vibrations and fewer odors than diesel engines.

Table 1.7: Emission comparison of diesel and CNG-powered buses

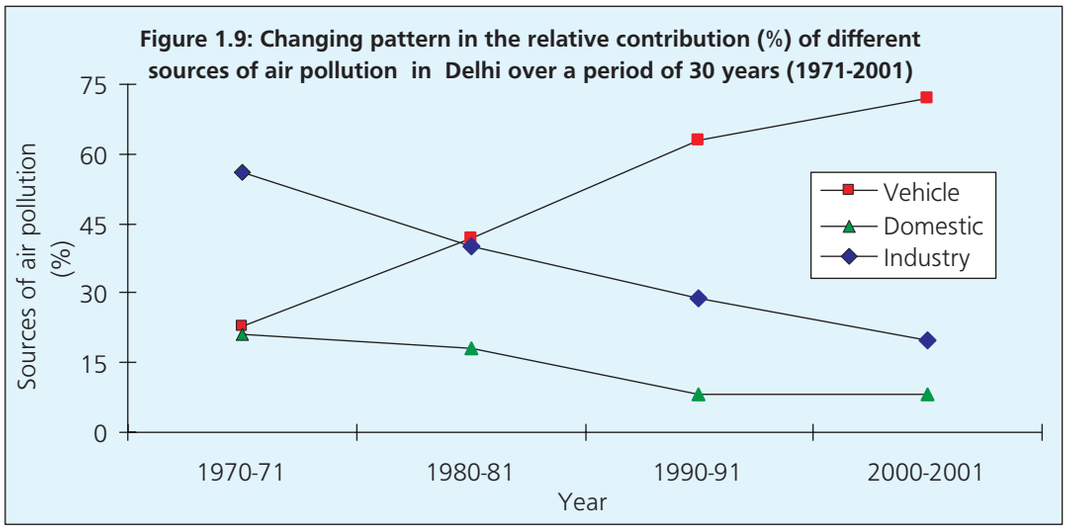
	Diesel	CNG	% reduction
CO	2.4 g/km	0.4 g/km	83
NOx	21 g/km	8.9 g/km	58
PM	380 mg/km	12 mg/km	97

Source: Frailey et al., 2000

(b) Industrial source of air pollution in Delhi

The share of industries as source of air pollution is rapidly declining in Delhi for the past thirty years (Table 1.6). For example, during 1970-71, industrial pollution was the biggest contributor (56%) to city's air pollution load. In contrast, only 20% of Delhi's air pollution is now generated by the industries (Figure 1.9), mainly the three coal-based thermal power plants at Indraprastha, Badarpur and Rajghat.

Figure 1.9: Changing pattern in the relative contribution (%) of different sources of air pollution in Delhi over a period of 30 years (1971-2001)



(c) Domestic source

Like the industries, the contribution of household sector to city's air pollution is steadily declining. Now a days only 8% of Delhi's air pollution is contributed by household sources, compared with 21% in 1970-71 and 18% in 1980-81. Pollution from household sources is mainly due to the use of coal, kerosene and unprocessed solid biomass like firewood, cow dung and agricultural refuse like hay, husk, dried leaves etc.

1.5 ECONOMIC ASPECT OF AIR POLLUTION IN INDIA

Improvement of air quality is associated with reduction in the number of premature deaths, episodes of acute illness such as asthma attacks and the number of chronic respiratory illness cases. The economists evaluate the value of avoiding an illness episode as:

1. the value of work time lost due to the illness by the patient or the caregiver, or both
2. the medical cost of treatment
3. the amount paid to avoid the pain and suffering associated with the illness, and
4. the value of leisure time lost due to the illness by the patient or caregiver.

1.6 HEALTH EFFECTS OF AIR POLLUTION: AN UPDATE

Harmful effects of air pollution on human health are recognized for centuries. It has been estimated that globally 8,000 people die every day from diseases related to air pollution exposure. Each year 60,000 deaths in the United States and 500,000 deaths in China occur due to air pollution. Several epidemiological studies have established a direct relationship between the pollutants and health hazards ranging from morbidity (illness) to mortality (death from illness).

(a) Excess mortality

The London fog incident in 1952 conclusively established an association between air pollution and increased mortality (Logan, 1952). Since then, several epidemiological studies in the USA and Europe have established a clear relationship between air pollution exposure and excess mortality (Samet et al., 1981; Dockery et al., 1982; Wichmann et al., 1989; Archer, 1990; Ostro et al., 1991; Ponka, 1991; Pope et al., 1992; Bobak and Leon., 1992; Schwartz, 1994; Lipfert, 1994; Thurston, 1996; Dockery, 1999). Air pollution is associated with increased risk of acute respiratory infections, the principal cause of infant and child mortality in the developing countries (Bendahmane, 1997). Each 10µg/m^3 increase in annual average $PM_{2.5}$ level may lead to 4%, 6% and 8% rise in the risk of all-cause, cardio-pulmonary, and lung cancer mortally respectively (Pope et al., 2002). An increase in PM_{10} by 10 µg/m^3 has been reported to cause 0.76% excess deaths from cardiovascular causes and 0.58% excess mortality from respiratory diseases (Analitis et al., 2006).

(b) Particulates and health impact

Mortality and morbidity associated with air pollution are primarily due to the toxic effects of particulates (Schwartz, 1991, 1992, 1993; Morgan et al., 1998; Hong et al., 1999; Peters et al., 2000; Arena et al., 2006). Associations have also been reported with gaseous air pollutants viz. ozone (Anderson et al., 1996), nitrogen dioxide (Anderson et al., 1996), sulfur dioxide (Gouvea and Fletcher, 2000) and carbon monoxide (Gouvea and Fletcher, 2000). Compared with particulates, however, the relationship between gaseous pollutants and mortality is less consistent.

(c) Direct relationship between death from heart and lung diseases and air pollution level

Dockery and his co-workers (1993) showed an association between mortality rates and PM_{10} levels not only from lung cancer but also from cardio-pulmonary diseases. They estimated 3.4% excess deaths from respiratory diseases and 1.4% from cardiovascular diseases for every 10 µg/m^3 increase of PM_{10}.

The overall increase in mortality was calculated as 1% for every 10 µg/m^3 rise in PM_{10} (Dockery et al., 1999; Viegi et al., 1999, 2000). Samet et al., (2000) has reviewed the subject and concluded that for every 10 µg/m^3 rise of PM_{10}, there was an increase in mortality from all causes by 0.51% and from cardiopulmonary diseases by 0.68%. This finding is similar to that of Katsouyanni et al., (1997) who had suggested 0.4% increase in mortality with 10 µg/m^3 rise in PM_{10} level. Further, Levy et al., (2000) showed a 0.7% increase in mortality per 10 µg/m^3 increase in PM_{10} concentrations. In the same year Schwartz (2000), in a study in 10 cities in the USA, reported a 0.67% increase in mortality associated with every 10 µg/m^3 increase in PM_{10} concentration.

(d) Most deaths are from heart diseases

The association between air pollution exposure and adverse health outcome has been summarized by Dockery (2001). He noted that although the relative risk for effects of particles are greater for respiratory than for cardiovascular deaths, the actual numbers of death are greater for cardiovascular than for respiratory causes.

(e) Increased morbidity

Besides mortality, air pollution could initiate and/or aggravate several diseases. Excess morbidity is often reflected in absenteeism from school and work, restricted activity spent at home, more attendance to outpatient medical services; emergency visits to clinics and hospitalization (Shy et al., 1978). Air pollution-related pulmonary diseases for which hospital admissions are usually required are acute bronchitis, pneumonia, emphysema, bronchiectasis, chronic airway obstruction and attacks of asthma. Besides lung diseases, air pollution is significantly associated with cardiac and vascular problems (von Klot, 2005; Maheswaran et al., 2005; Mills, 2005).

1.6.1 Air pollution and respiratory system

Primary target of the pollutants: the lung and the airways

Since airborne pollutant generally enters the body through inhalation, lung and the airways are the primary target organs. The airways of the lung are represented by the trachea (windpipe), and beyond it are bronchi (with cartilage cover) and bronchioles (without cartilage). The bronchioles lead to air spaces called alveoli which have an average diameter of 200 micrometer each (Figure 1.10). A recent study has demonstrated that there are approximately 480 million alveoli in both lobes of an adult human lung, and men have more alveoli and larger lung volume than women (Ochs et al., 2003). The mean size of a single alveolus is $4.2x10^6$ µm^3. Alveoli make up approximately 64% of the lung space (Ochs et al., 2004). Human lungs have a total surface area of 1,400 m^2, and everyday we inhale approximately 15 m^3 of air (i.e.15,000 litres). The weight of this inahled air is greater than the food we consume and the water we drink in a day. The lung volume and breathing frequencies of healthy adults at rest are 400-500 ml and 15-17 breaths per minute respectively (Tobin et al., 1983). Recent study has documented that a constant number of respiratory units is maintained from childhood to adulthood while both the smallest bronchioles and alveoli expand in size to produce the increased lung volume with increased age and height (Zeman and Bennett, 2006).

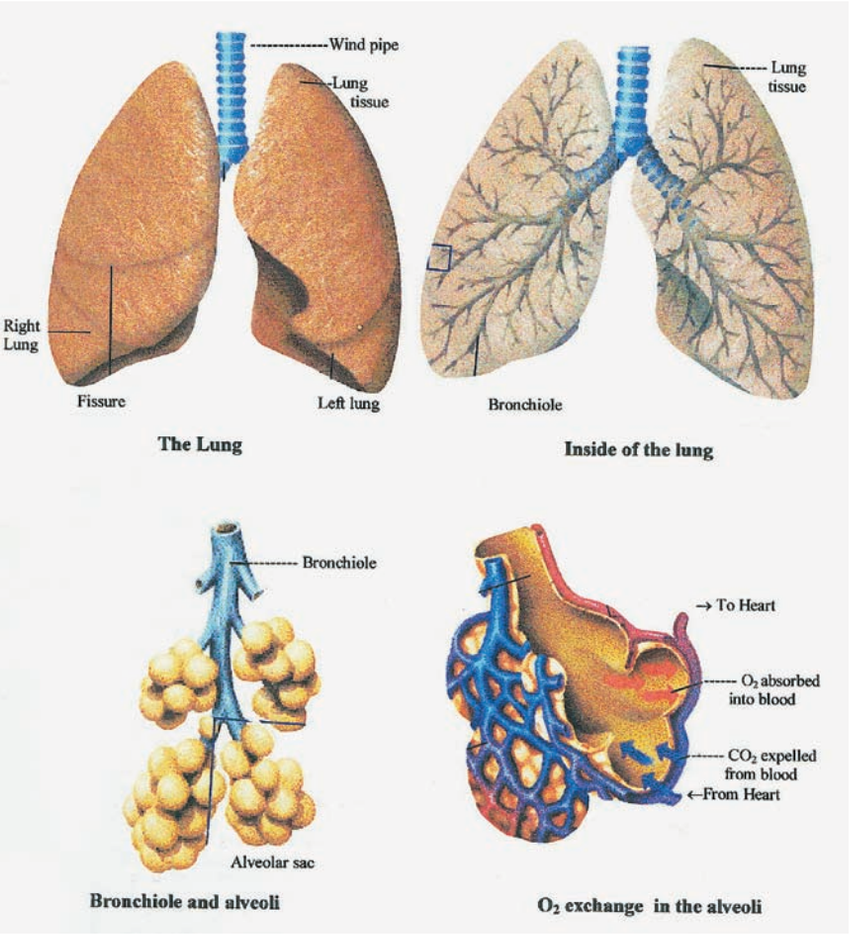


Figure 1.10: Anatomy of the lungs

Respiratory illness following air pollution exposure

Way back in 1981, Samet and co-workers reported statistically significant increase in respiratory emergency room visits in Ohio, USA in association with elevated levels of particulate pollution and SO_2. In 1983, Lutz reported strong positive correlation between weekly particulate pollution levels and the percentage of patients with respiratory illness. Thurston and co-workers (1994) observed similar associations in Toronto and in New York. In 1989, a series of studies in Utah Valley, USA showed that particulate pollution was associated with a wide range of respiratory disorders and hospitalization (Pope, 1989, 1991), lung function decrement and respiratory symptoms (Pope and Dockery, 1992; Pope et al., 1991) and school absenteeism for children (Ransom and Pope, 1992). Dockery et al., (1989); Ostro (1990), Thurston et al., (1994), Abbey et al., (1995) and others have also shown association between air pollution and respiratory morbidity. Particulate air pollution was reported to be associated with emergency hospital visits for asthma in Seattle, USA (Schwartz et al., 1993), and emergency visits for chronic obstructive pulmonary disease (COPD) in Barcelona, Spain (Sunyer et al., 1993) and in Brazil (Martins et al., 2002).

Schwartz (1999) reported that daily variations of PM_{10} and CO were linked to daily hospital admissions among the elderly (more than 65 years of age). A study conducted amongst school children of China

showed positive associations between morbidity prevalence and outdoor levels of particulate matter of all size fractions, but the association was strong for PM_{10} and $PM_{2.5}$ (Zhang et al., 2002). The results also present some evidence that ambient levels of NOx and SO_2 are positively associated with respiratory symptoms in children, but the evidence for these two gaseous pollutants was weak than that for PM_{10} and $PM_{2.5}$ (Zhang et al., 2002). Burnett et al., 2001) showed an association between ozone and hospitalization for acute respiratory diseases in children less than 2 years of age.

(a) Air pollution and rise in the prevalence of respiratory symptoms

Symptoms are a form of signals that act as an indicator of any underlying illness or disease. Epidemiological studies on respiratory and mental health are generally based on collection of data on the prevalence of respiratory and neurobehavioral symptoms to get an estimate of the disease (Bobak and Leon, 1992; Abbey et al., 1995; Dockery et al., 1996).

Type of symptoms

Respiratory symptoms are usually classified into two broad groups: a) Upper respiratory symptoms (URS) which include runny and stuffy nose, sinusitis, sore throat, wet cough, dry cough, cold head, fever, burning or red eyes, and b) Lower respiratory symptoms (LRS) which include wheezing, phlegm, shortness of breath, chest discomfort or pain. Most of the respiratory diseases underlying these symptoms are caused by bacterial, fungal or viral infections, or structural or functional damage to the respiratory system. Very often the symptoms of a multifactor respiratory disease like asthma or chronic obstructive pulmonary disease (COPD) are aggravated following exposure to air pollutant.

Allergic rhinitis

It is an inflammation of the membrane lining the nose and throat due to allergic reaction. The presence of rhinitis is indicated by itchy sensation in the nose, frequent sneezing, watery eyes, and running nose. The symptoms are commonly found following exposure to high level of particulate air pollution (Wongsurakiat et al., 1999) and benzene (Zuskin et al., 1997). Diesel exhaust particle have been identified as the major contributing factor to allergy (Sydbom et al., 2001; Riedl et al., 2005).

Sore throat

If the function of muco-ciliary escalator is impaired by high pollution exposure, the mucous is not cleared from the airways. The retained mucous traps inhaled pathogens resulting in damage to the airways, thickening and scarring of epithelium leading to fibrosis. This is associated by sore throat, an indicator of pharyngitis and tonsillitis, and respiratory or right heart failure.

Chronic cough, bronchitis, sinusitis

Cough is an important symptom frequently reported following exposures to air pollution. It is a reflex response to irritation from mucous or any foreign particle in the upper respiratory tract. When cough is accompanied by production of sputum it is termed productive or wet cough, whereas cough without sputum is known as dry cough. Cough may be caused by inflammation of the upper airways as a result of viral infections like *common cold* and *influenza*. Severe cough may indicate damage to the lungs caused by inflammation associated with *pneumonia* and chronic obstructive pulmonary disease

(COPD). COPD is often indicated by morning cough producing sputum, frequent chest infection, specially during winter producing yellow or green sputum, wheezing particularly after cough, and shortness of breath even after mild exertion. COPD represents two separate lung conditions, *chronic bronchitis* and *emphysema*. Inflamed, congested and narrowed airway is associated with chronic bronchitis causing obstruction to airflow. In emphysema the alveoli are damaged or fused making them less efficient for transfer of oxygen. In acute bronchitis, cough is irritating, persistent and produces clear sputum. The associated symptoms are chest pain or tightness of the chest and wheezing. Morning cough and sputum production is associated with emphysema. High prevalence of chronic cough, phlegm and sinusitis has been reported among garage workers and taxi drivers exposed to vehicular exhaust in United Arab Emirates (Bener et al., 1998). Similarly, high prevalence of cough, sinusitis, bronchitis and asthma has been found in association with traffic-related air pollution in Kolkata (Basu et al., 2001).

Chest tightness, dyspnea

These symptoms along with rhinitis and nose and throat irritation along with lung function abnormality have been observed in workers from a shoe manufacturing plant in Croatia where benzene level was higher than allowable limit (Zuskin et al., 1997). High prevalence of cough with phlegm and rhinitis were recorded among traffic policemen in Bangkok (Wongsurakiat et al., 1999). Similarly high vehicle traffic resulted in asthma, cough and wheeze in children who were additionally exposed to environmental tobacco smoke in Germany (Nicolai et al., 2003).

Bronchial asthma

It results from intermittent narrowing of the airways and consequent shortness of breath. Its early symptoms are wheezing, tightening of chest, shortness of breath, and difficulty in exhaling and dry persistent cough. Although asthma is genetically controlled, exposure to air pollution exacerbate asthma attacks (Cakmak et al., 2004). A strong association between severe asthma symptoms and breath concentration of benzene has been demonstrated in children (Delefino et al., 2002). Asthma and bronchoconstriction have been linked to cumulative exposures to exhaust from diesel-fuelled engines (Riedl et al., 2005) and occupational exposures to VOCs (Cakmak et al., 2004).

(b) Increased risk of lung function impairment

Breathing and gas exchange is achieved by the lungs, chest wall, diaphragm, central nervous system and the pulmonary circulation acting in concert. Pollutants from vehicular exhaust could reach deep inside the lungs and result in the destruction of the alveoli or the proximal airways resulting in the malfunctioning of any of the components thereby hampering the balance. The measurement of mechanics of breathing is collectively known as Lung Function. An instrument called spirometer measures it. A spirometer is integrated with a microprocessor that measures flow rates (Mead et al., 1979).

Types of functional lung impairment

The two major patterns of abnormal lung function are restrictive and obstructive pattern. a) In restrictive lung function, there is a decrease in lung volume (FVC, Forced vital capacity). The subject inhales reduced volume of air due to reduction in total lung capacity. Restrictive lung may be caused by infection and inflammation such as in case of pneumonia or tuberculosis. These infections often leave scar on the lung tissue leading to the loss of elasticity of the lung. Obesity and neuromuscular problems also could lead

to restrictive lung. b) Obstructive lung function is indicated by a decrease in the FEV_1 (Forced expiratory volume in one second)/FVC ratio. Decline in FEV_1/FVC usually results from obstruction in large airways while fall in $FEF_{25-75\%}$ (Forced expiratory flow between 25-75% of FVC, mid expiratory force) signifies small airways obstruction (Dassen et al., 1986; Vedal et al., 1987). Obstructive lung function is common in diseases like asthma and COPD (Figure 1.11).

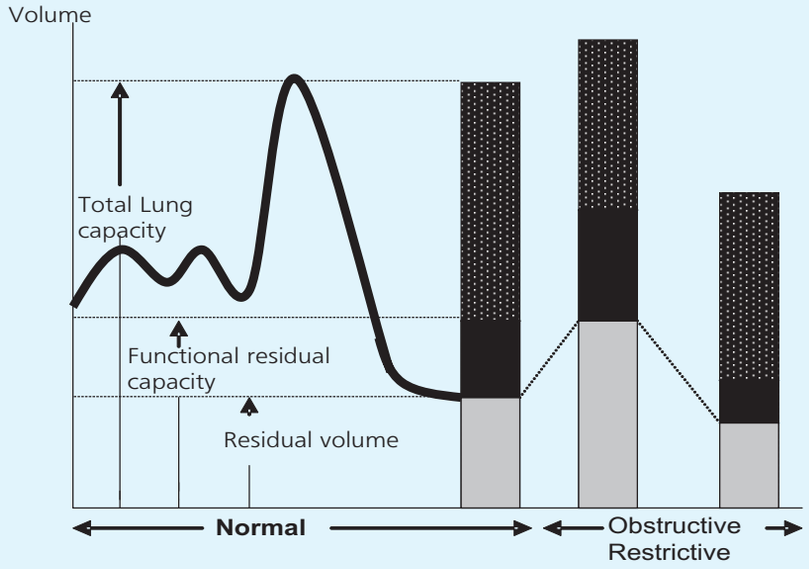


Figure 1 .11: Normal lung volumes and changes that occur in Obstructive and restrictive types of lung function impairments (Adapted from Davidson, 1995)

Reduction in lung function following air pollution exposure

Pulmonary function can be affected by several factors including genetic predisposition, weather, season, time of the day, the basic respiratory health of the subject, smoking status, allergens and air pollution (Schoenberg et al., 1978). Lung function is a very important indicator of the adverse effects of air pollution on the lungs since impaired lung function has been identified as an independent risk factor for morbidity and mortality from heart diseases (Lipfert, 1988).

In the 1980s, a large number of studies have shown a decline in forced vital capacity and forced expiratory volume with increasing concentration of air pollution (Johnson et al., 1982; Lebowitz et al., 1985; Asero et al., 2005). Burnekreef (1991) found a strong association between lung function decrement and particle concentration. Decline in FEV_1 has been reported in school children following a particulate and SO_2 pollution episode in January 1985 in Netherlands (Dassen et al., 1986; Hoek and Brunekreef, 1994). Diesel exhaust exposure resulted in airway obstruction in railroad workers (Kilburn, 2000). Studies by Frye et al., (2003) have related decrement in lung function following urban air pollution episode. A study in Delhi has shown the adverse effect of air pollution on spirometric lung function, which was aggravated by smoking habit. The study also reported greater airway obstruction in bidi smokers (Chhabra et al., 2001). Decreased lung function in response to air pollution has also been reported by investigators from Germany (Ibald-Mulli et al., 2002) and Iran (Golshan et al., 2002).

Workers from a shoe manufacturing plant in Croatia revealed several respiratory symptoms along with lung function abnormality where benzene level was found higher than permissible limit (Zuskin et al., 1997). Another study reported inverse relationship between peak expiratory flow rate and ambient VOC including benzene in children (Delefino et al., 2002). The additive effect of cigarette smoking and air pollution on reduction of FEV_1 was shown in a survey of 7,685 individuals in Canada (Gan et al., 2005)

(c) Cellular lung response to air pollution: Changes in pulmonary cytology

In the course of development of diseases in the lung the cells of the airways usually undergo a number of changes that are accurately reflected in the sputum. Thus, sputum cytology is immensely helpful to study the initiation and progression of the underlying diseases in the airways and inner lung (Lahiri et al., 2000). Sputum contains mucus, inhaled substances including various microorganisms, epithelial cells, alveolar macrophages and immune cells like neutrophil, eosinophil and lymphocytes. Microscopic examination of these exfoliated cells and immunocytes provides useful information regarding the pathophysiological changes in the lung tissues.

(i) Cell types in a normal lung

More than 40 cell types have been identified in the normal lung and airway. The major cell types are: basal cell, Intermediate cell, ciliated and non-ciliated columnar epithelial cells, goblet cell, Type I and Type II pneumocytes and alveolar macrophages.

Pneumocytes

A rich capillary network surrounded by endothelial cells cover the alveolar space. The alveolar space is lined by a basement membrane on which lie the epithelial cells (pneumocytes).

Type I pneumocytes

These cells cover about 95% of alveolar surface and because of their extensive surface area they are thought to be at high risk of chemical and particulate attack despite being covered by a protective epithelial lining fluid and pulmonary surfactant. The cells permit efficient gaseous exchange by the red blood cells present in the alveolar capillary and contain enzymes, which are important in mediating toxicant biotransformation (Dinsdale, 1998).

Type II pneumocytes

They cover only 3-5% area of alveolar surface and are located at the corners of the alveoli. These are secretory cells and synthesize pulmonary surfactant and also the components of the underlying basement membrane (Crouch et al., 1987). Type II cells also contain a spectrum of P-450 isoenzymes (Miller et al., 1986) and a high concentration of reduced glutathione (Horton et al., 1987). All these are important for the breakdown and biotransformation of inhaled or circulating chemicals.

Surfactant

Pulmonary surfactant or surface-active substance is a lipoprotein mixture rich in phospholipids. It has a 'detergent' property of lowering surface tension in the fluid layer that line the alveoli once air enters the lungs. Its ability to form a monomolecular layer at the interface between air and the alveolar lining fluid

allows some air to be retained within the alveolus at all times. Surfactant is produced continually from Type-II epithelial cells because it has a half-life of only 14-24 hours. Deficiency of surfactant is associated with respiratory distress syndrome.

Interstitium

It represents the space between the alveoli. It is composed of fibroblasts and connective tissue components such as proteoglycans, collagen and elastin, which are responsible for maintaining the skeletal backbone of the innermost region of the lung.

(ii) Cells normally found in sputum

Squamous epithelial cells

Squamous epithelial cells originate from normal lining of the mouth or pharynx. A mixture of intermediate and squamous epithelial cells is often found in sputum. The intermediate cells are characterized by round to oval nucleus and cyanophilic cytoplasm. Superficial cells have pyknotic nuclei and orangeophilic cytoplasm. Enucleated squamous and nucleated parabasal cells are occasionally present.

Ciliated columnar cells

These cells are shed singly, or in loose clusters or sheets from the lining of the respiratory tract, mainly as a result of traumatic exfoliation or inflammation. Following extensive damage to the respiratory epithelium these cells may be found in large numbers in sputum.

Goblet cells

Goblet cells are non-cilated epithelial cells. Together with the submucosal glands, they produce and secrete high molecular weight mucus glycoproteins, called mucins. Mucin helps entrapment of inhaled pollutants including particles and microorganisms, and their disposal by mucociliary clearance. These cells also produce lipids and small glycoproteins. Mucins are tightly packed in the intracellular granules of the goblet cells. Goblet cells discharge mucus in response to wide spectrum of stimuli- irritant gases, inflammatory mediators, reactive oxygen species, nerve activation, and changes in the biophysical environment (Rogers, 1994). Mucins are discharged rapidly (within milliseconds) and vast quantities of mucus are released.

The ability of goblet cells to give rise to ciliated cells, to rapidly produce vast quantities of mucus in response to acute airway insult, and to change in number according to variations in chronic insult indicates that these cells are vitally important front-line defenders of the airways (Rogers, 1994).

Alveolar macrophage (AM), the first line of cellular defense in lung

Pulmonary macrophages include i) alveolar macrophages (AMs), ii) airway macrophages and iii) interstitial macrophages. These cells are present in sputum in varying numbers and their presence indicates the adequacy of sputum sample. AMs are the dominant phagocytic cells, which act as first line of cellular defense in the lungs. Their mobility, secretory behavior and bactericidal properties are essential for the maintenance of clean and sterile alveoli. Macrophages by their immense secretory potential may cause tissue injury while performing their defensive role.

Heterogeneity

Macrophages have an average diameter of 10-45 µm (mean diameter 20µm). Cline in 1975 grouped it into three types: Type A – with a mean diameter of 20-25 µ, accounting for 94-98% of mononuclear cells, Type B – with a mean diameter of 30 µ constitutes 5% and Type C – with a mean diameter of 40 µ accounting for less than 10% of the mononuclear cells. The nucleus is oval or irregular and usually eccentric in position with nucleus: cytoplasmic ratio 1:3. Cytoplasm shows numerous granules and ingested particles.

Number and origin of AM

In adult human lung there are approximately 480 million alveoli and each alveolus is defended by about 73 macrophages. Therefore, it has been estimated that human lung contains 35.0 billion macrophages for its defense against inhaled pollutants.

Macrophages originate from peripheral blood monocytes. Monocytes like other cells present in the blood originate from the bone marrow. The precursor stem cells in the bone marrow give rise to monoblasts that differentiate into promonocytes and then to monocytes. The monocytes circulate in blood for 36-104 hours and then leave the blood vessels, migrate through the endothelial barrier to interstitial space and then across the epithelium to the tissues where maturation and differentiation occur in response to environmental stimuli and they are called macrophage or histiocytes. The transformation is regulated by hematopoietic growth factors like colony stimulating factor (CSF) and interleukins (ILs) [Frampton, 2001; Zareba et al., 2001; Goldberg et al., 2001; Dockery, 2001; Schwartz, 2001; Donaldson et al., 2001; von Klot et al., 2005, Ballester et al., 2006].

The AM pool in lung is maintained by division of pre-existing interstitial macrophages or by influx of monocytes from blood to the lung (Lewis, 1995). Following exposure to inhaled pollutants, AM number in lung is increased either by cell division, or recruitment of monocytes from circulation or by enhancing the life span of existing cells by suppressing apoptosis (Foster, 1999). They have a normal life span ranging from months to years and are destroyed in the lymphatics.

Function of AM

AMs represent the first line of cellular defense in the lung. They play a key role in particle clearance from the inner airways by phagocytosis, generation of oxygen radicals, and endocytosis of insoluble particles and dust (Becker, 1995). They possess potent antimicrobial activities via local release of degradative enzymes and reactive oxygen metabolites. AM is perhaps the most secretory cell in the body. They actively participate in inflammation, wound healing and tissue repair through their vast array of secretory products (Laskin and Pendino, 1995). They release inflammatory cytokines like tumor necrosis factor-alpha (TNF-□), interleukin-1 (IL-1), chemotactic factors like leukotrine B-4 (LTB_4), platelet-activating factor (PAF) and chemotactic cytokine such as interleukin-8 (IL-8) that strengthens the pulmonary defense (Strieter et al., 1990). Macrophages also act as effector cells for killing of tumor cells in the lung (Shellito and Kaltreider, 1985), and known to regulate cellular and humoral immunity by processing and presenting antigen, interacting with helper/inducer T-lymphocytes (Thepen et al., 1994; Lewis, 1995). They also perform various metabolic and homeostatic functions including lipid and iron metabolism (Madden et al., 1991).

Enzymatic marker of AM

The enzymatic marker is non-specific esterase (NSE) found in cytoplasmic granules of the cell. The enzyme comprises of three carboxylic ester hydrolases, namely carboxylesterase, arylesterase and acetylesterase. These three cytoplasmic enzymes are widely distributed in many tissues. The application of NSE method is helpful in defining cells of monocytic origin and thus detects histiocytes and macrophages. The type of NSE detected in AM is likely to be arylesterase as it acts on aromatic esters like □-naphthyl acetate.

Inflammatory cells

Neutrophils, eosinophils and lymphocytes are present in varying proportion depending upon the inflammatory or allergic reaction in the lung. The magnitude of inflammation can be assessed from the extent of rise in the number of these cells particularly the neutrophils.

(iii) Change in airway cells following air pollution exposure

Both chronic and acute exposures to air pollution have been shown to directly affect the structural integrity of the respiratory system. Continued chemical exposure can cause necrosis and subsequent sloughing off of ciliated epithelial cells. Microscopic examination of these exfoliated cells in spontaneously expectorated sputum provides important information regarding the pathophysiological changes in the lung tissue and development of lung disease including malignancy (Roby et al., 1990). Saccomanno and his co-workers (1970) demonstrated cytological changes in the respiratory epithelium following air pollution exposure. Air pollution exposure was associated with presence of abnormal columnar epithelial cells and squamous metaplasia in sputum of young adults and children (Plamenac et al., 1973, 1978). Exposure to diesel exhaust showed a significant increase in neutrophils, mast cells, CD4+ and CD8+ T-lymphocytes, along with upregulation of endothelial adhesion molecule, ICAM-1 and VCAM-1 in airway lavage (Salvi et al., 1999; Takizawa et al., 2000). Changes in airway epithelial cells were reported in sputum samples of smokers (Kulawik et al., 2003). Higher prevalence of sputum eosinophilia and neutrophilia along with marked rise in the prevalence of respiratory symptoms have been reported both in adults and children of Kolkata, and the changes have been attributed to the city's high air pollution level (Lahiri et al., 2000a, 2000b). Controlled exposure of healthy human volunteers to diesel exhaust particles (DEP) for 1 hour produced marked cellular inflammatory response in the airways involving neutrophils, mast cells and lymphocytes (Salvi et al., 1999). DEP exposure was accompanied by increased expression of endothelial adhesion molecules and their complementary ligands on inflammatory cells. It has been demonstrated that DEP elicits remarkable increase in neutrophils in the proximal airways, while the lymphocyte and histamine responses were found in the distal airways (Salvi et al., 1999).

AM response to air pollution exposure

A pioneering study by Mylius and Gullvag (1986) demonstrated several fold increase in the number of AM in sputum of persons exposed to industrial air pollution. The AM count was found to rise with increasing level of particulate pollution in the workplace. They advocated the number of AM as a reflection of the reaction of lung to air pollution. Following this study sputum AM has been used as a biomarker of pollution exposure and effect as it involves non-invasive procedure, the cells can be analyzed through relatively simple microscopic techniques, the cytological alterations in sputum accurately reflect the changes within the lung and the whole procedure is cost–effective. Hence

the procedure seems well suited for large population based studies especially in the developing world. A comparative study by the current researchers among the residents of highly polluted city of Kolkata and the inhabitants of Sunderban islands in Bay of Bengal where air pollution level was significantly lower revealed several-fold rise in AM number in sputum of urban subjects (Lahiri et al., 2000a). Similar changes were observed in children exposed to high level of urban air pollution of Kolkata (Lahiri et al., 2000b). In another study, traffic- related air pollution was shown to be associated with 6.5- fold rise in AM number in traffic policemen and street hawkers of Kolkata compared with relatively less exposed office workers (Basu et al., 2001). Thus, AM number in sputum appears to be a sensitive biomarker of cumulative exposure to air pollution. The number of alveolar macrophages with phagocytosed PM_{10} has been correlated with atherosclerotic lesions in PM_{10} exposed rabbits (Suwa et al., 2002).

Functional impairment of AM following ultrafine particle exposures

Ingestion of ultrafine particles by the AM may lead to impairment of phagocytic activity of these cells, particularly after infection that induce an increased production of Interferon gamma (Lundborg et al., 2001). Cytoskeletons of the macrophages are important for migration, phagocytosis of foreign materials and intra cellular transport and digestion. Ultrafine particles in urban dust and diesel exhaust have been shown to cause cytoskeletal toxicity leading to impaired function of the macrophages, compromising lung defects (Moller et al., 2002).

Siderophages in sputum: indicator of pulmonary hemorrhage

AM process hemoglobin from phagocytosed erythrocytes to form degradation products like iron (Perez-Arellano et al., 1992). These iron-containing macrophages are known as siderophages. Their presence in sputum in high numbers is indicative of either past intrathoracic bleeding or extravasations of red blood cells into the alveoli due to a sluggish blood flow (Grubb, 1994). Therefore, the presence of siderophages is of considerable value in detecting covert pulmonary hemorrhage. An earlier study by the present research team has demonstrated the presence of large number of siderophages in sputum of subjects chronically exposed to Kolkata' air pollution (Roy et al., 2001). It was concluded that abundance of siderophages in sputum and associated microscopic hemorrhage in the inner airways is an indicator of adverse lung reaction to air pollution (Roy et al., 2001). Hemosiderin-laden AM were observed in sputa of traffic policemen in Italy (Giovagnoli, 1999) and automobile service station workers and hawkers in Kolkata (Roy et al., 2001); they were also found in steel workers in cases of pulmonary hemorrhage (Corhay, 1992). Becroft and co-workers (1997) reported abundance of intra-alveolar siderophages as a cause of sudden death in infants. Animal studies have shown accumulation of iron in the rat lung following exposure to DEP. Deposition of iron causes oxidative stress, inflammation, and neutrophilic lung injury (Ghio et al., 2000).

Elastase: a tissue-degrading enzyme

Elastin is a fibrous protein that is widely distributed in the elastic tissues of lung. Elastase, a proteolytic enzyme found in the lysosomes of neutrophils and AM is capable of destroying elastin, collagen and fibronectin. Elastase is also involved in wound healing and disposal of damaged cells and debris. Uptake of non-degradable irritant materials from the atmosphere has been shown to enhance elastase production and secretion by the AM (Werb and Gordon, 1975). Release of excess elastase from neutrophils and

activated AM promotes the development of emphysema as it leads to degradation of alveolar wall (Laskin and Pendido, 1995). Neutrophil and macrophage elastase plays a major role in emphysema in smokers (Churg et al., 2002; Shapiro et al., 2003; Valenca et al., 2004).

1.6.2 Systemic effects of air pollution

(a) Cardiovascular changes

(i) Air pollution, increased plasma viscosity, hypertension

There are reports that indicate air pollution may affect blood pressure. Indeed, high blood pressure (hypertension) is common among persons cumulatively exposed to high level of air pollution (Pope et al., 1999a). Hypertension is defined as systolic blood pressure at least 140 mm Hg, diastolic blood pressure at least 90 mm Hg, or both (Chalmers et al., 1999). Elevated plasma viscosity, increased heart rate (>80 beats/min), reduced heart rate variability, and increased risk of arterial hypertension have been reported in association with chronic air pollution exposure (Pope et al., 1999; Gold et al., 2000; Ibald-Mulli et al., 2001; Brook et al., 2003). Increase in heart rate in response to air pollution has been shown to be most marked in individuals who have high blood viscosity. Hypertension is one of the predictors of cardiovascular mortality with relative risk of up to 2.0 or more (Blazer et al., 2001).

Air pollution exposure and cardiovascular diseases (CVD) are intimately related, and it is a growing concern worldwide (Zareba et al., 2001; Dockery, 2001; Goldberg et al., 2001; Donaldson et al., 2001). CVD associated with air pollution are angina, cardiac insufficiency, hypertension and myocardial infarction (MI) i.e. heart attack. Studies conducted from the late nineties have consistently shown that PM_{10} is associated with overall hospital admissions for CVD (Schwartz, 1999; Burnett et al., 1999; Moolgavkar, 2000; Linn et al., 2000). Air pollution has been associated with sudden death in patients with stable angina (Benchimol et al., 2000) and myocardial infarction (Lind et al., 2001, Peters et al., 2001, 2004). In combined analyses across six eastern US cities, each 10 $\mu g/m^3$ increase in $PM_{2.5}$ was found to be associated with 2.1% increase in total mortality from ischaemic heart diseases (Schwartz et al., 1996). Among air pollution-related deaths in the US in 1997, only 8.5% were from respiratory diseases (COPD, pneumonia, influenza etc.) while cardiovascular deaths (heart, cerebrovascular and arterial diseases) accounted for 39.5% of all deaths (Greenle et al., 2000). Thus, CVD, rather than respiratory ailments, are the most important cause of death from air pollution exposure. A study in Delhi with high level of air pollution has reported higher risk of CVD and deaths at a younger age (Cropper et al., 1997).

(ii) Underlying mechanism of cardiovascular effects of air pollution

The mechanism by which air pollution affects the cardiovascular system is largely unknown. Seaton and co-workers (1995) first proposed that pollutant exposure induces a transient increase in blood coagulability, and this was subsequently corroborated by the study of Schwartz (2002) who demonstrated an association between PM_{10} and all three markers of cardiovascular risk: higher level of fibrinogen in blood plasma, greater number of platelets in circulation, and elevated WBC count. Several other workers have linked air pollution, especially the PM_{10} level, with markers of adverse cardiovascular events like increase in peripheral white cell counts (Salvi et al., 1999), elevation of

interleukin-6 level (Ernst and Resch, 1993; Ridker et al., 2000), upregulation of C-reactive protein (CRP; Seaton et al., 1999; Peters et al., 2001), rise in plasma viscosity due to increase in fibrinogen level (Ghio et al., 2000; Peters et al., 2001; Schwartz, 2001), and alteration in cardiovascular autonomic control (Ibald-Mulli et al., 2001). Raised fibrinogen and neutrophil levels is common in patients with coronary heart disease (Danesh et al., 1998). CRP, an index of inflammation, is an acute phase protein produced in the liver in response to injury and infection. A rise in CRP level increases the risk of coronary artery disease (Ridker and Haughie., 1998; Mendall et al., 1996). A survey in England has shown 1.5-fold increase in the incidence of coronary artery disease for each doubling of CRP level (Mendall et al., 1996).

Inhaled particles elicit pulmonary inflammation, penetrate the blood stream, interact with platelets and trigger systemic increase in coagulability and procoagulant alteration in blood rheology that can promote the genesis of atherosclerosis leading to cardiac arrhythmias, arterial dysfunction, and acute MI (Seaton et al., 1995, Ruckerl et al., 2006). The important role of ultrafine particles (UFP), especially those containing transition metals, in mediating cardiovascular changes associated with urban air pollution has been supported by the work of Donaldson and co-workers (2001). They have shown that oxidative stress generated by UFP and subsequent oxidation of low-density lipoprotein (LDL) cholesterol destabilizes atheromatous plaque, which leads to an ischemic event. Alternatively, particles may induce activation of nuclear factor kappa beta (NF-kB) transcription factor facilitating generation and release of proinflammatory cytokines that stimulates the liver to generate more coagulation factors giving rise to an ischemic event (Donaldson et al., 2001). Besides PM_{10}, exposure to SO_2 was found to be significantly associated with rise in WBC, NO_2 with raised platelet count and fibrinogen, while ozone had no effect on any of the outcomes (Schwartz, 2002).

The proposed mechanism of air pollution- related cardiovascular events (Frampton, 2001; Donaldson et al., 2001) is as follows.

- Air pollution exposure causes deposition of particulates in airways
- The particulates mediate generation of reactive oxygen species (ROS) by airway macrophages
- ROS inflicts injury to airway and alveolar epithelial cells
- Nuclear factor kappa beta (NFkB) is activated and proinflammatory cytokines are elaborated causing upregulation of adhesion molecules in vascular endothelium and circulating leucocytes
- Immigration of neutrophil, eosinophils, lymphocytes and monocytes from blood to airways follows
- Production of C-reactive protein (CRP), and fibrinogen in liver is increased, eliciting activation of platelets and coagulation cascade and oxidation of low-density lipoprotein (LDL) cholesterol
- Generation of foam cells, plaque formation in arteries, atherosclerosis
- Rupture of plaques leading to angina and myocardial infarction

(iii) Susceptible groups

People at highest risk tend to be those with pre-existing cardiovascular disease and elderly. In addition, persons with diabetes are also more susceptible to CVD mediated by airborne pollutants (Singh et al., 2000; Zanobetti and Schwartz, 2001).

(b) Hematotoxicity of air pollution

Air pollution exposure is associated with a multitude of hematological alterations.

(i) Neutrophilia

PM_{10} exposure in laboratory animals increases circulating neutrophils (Polymorphonuclear cells, PMN), band cells, and an increase in the size of bone marrow mitotic pool of PMNs (Suwa et al., 2002). Repeated exposures to PM_{10} in rabbits stimulates the bone marrow to increase the production of PMN in the marrow and accelerates the release the more mature PMN into the circulation, the magnitude of this changes was related to the amount of particles phagocytosed by AM (Mukae et al., 2001). Exposure to DEP for only 1 hour produced a marked increase in the neutrophils in the peripheral blood. Concomitantly a 4-fold increase in their numbers in the bronchial epithelium and a 3- fold increase in the submucosa was recorded (Salvi et al., 1999). This suggests that DEP is a potent stimulus for release of neutrophils from the bone marrow and the transit from blood to the airway tissues. Acute exposure to ambient particles accelerates the transit of PMN from marrow to the circulation, whereas chronic exposure expands the size of the bone marrow pool of PMN (van Eden and Hogg, 2002). In vivo and in vitro studies have demonstrated that alveolar macrophages produce the mediators implicated in the bone marrow response to PM_{10} exposures. The transit of neutrophils and other inflammatory cells from the blood into tissue occurs in a highly regulated manner involving sequential upregulation of several adhesion molecules on the endothelial cells and their respective ligands in the leucocytes, along with release of chemoattractant from the epithelial and inflammatory cells. Following acute exposure to DEP significant decrease in endothelial ICAM-1 and VCAM-1 expression was observed (Salvi et al., 1999). A combination of several pollutants present in diesel exhaust may be more potent in producing a biological response than each pollutant in isolation. Moreover, chronic low dose exposure to air pollution with intermittent acute high-dose exposures may elicit a response that is different from acute response seen in several experimental and epidemiological studies.

(ii) Altered platelet count

Exposure to DEP has been associated with markedly elevated platelet number in peripheral blood, i.e. thrombocytosis (Salvi et al., 1999). Thrombocytosis has been described as an acute phase reaction and has been linked to a range of inflammatory lung disorders (Sutor, 1995). Peters and colleagues (2000) have demonstrated elevated platelet count and increased plasma viscosity during rise in particulate pollution in ambient air. After rupture of an arterial sclerotic plaque in a coronary artery, platelets play a crucial role in the subsequent thrombus formation, leading to MI. Thrombocytosis, especially in elderly people compromised with cardiovascular function, may increase their risk of developing strokes and coronary vessel thrombosis, thereby increasing cardiovascular mortality and morbidity (Peters et al., 2000). Road repair workers exposed to asphalt fumes have higher platelet volume but lower platelet count (Chase et al., 1994). An increased mean platelet volume is an indicator of larger and more reactive platelets, and persons with these changes are at higher risk of myocardial infarction (Endler et al., 2002).

(iii) Hematotoxicity of benzene

Volatile organic compounds (benzene, toluene and xylene) are haematotoxic, and exposures to these pollutants are associated with higher prevalence of hematological abnormalities like alterations in WBC,

RBC and platelet count in children (Lee et al., 2002). Rapidly proliferating stem cells in the bone marrow are most vulnerable to benzene toxicity (Marcus, 1990). Benzene cause bone marrow suppression, decreased erythrocyte, hemoglobin and hematocrit levels leading to anemia, suppression of WBC counts (leukopenia), and reduction in platelet number (thrombocytopenia). Suppression of all three elements (RBC, WBC and platelets) is called pancytopenia. Benzene exposure often leads to pancytopenia. Pancytopenia accompanied by necrosis of the bone marrow, is diagnostic of aplastic anemia caused by benzene. Bone marrow dysplasia including dyserythropoiesis, eosinophilic dysplasia and abnormal cytoplasmic granulation of neutrophilic precursors have been reported following occupational benzene exposure (Aksoy, 1991).

Other hematological changes observed in humans exposed chronically to benzene include decreased leukocyte osmotic resistance, decreased phagocytic function of neutrophils, reduced glycogen content and decreased activity of peroxidase of neutrophils, and increased delta aminolevulinic acid activity in erythrocytes (Aksoy, 1991). A report by Farris et al., (1996) showed significant reduction in the number of reticulocyte in blood, B-lymphocytes in the marrow and spleen and increased frequency of micronucleated reticulocyte in bone marrow following benzene exposure. Workers occupationally exposed to high level of benzene had higher incidence of anemia (Pyszel et al., 2005) and they had disturbances in the heme metabolism in lymphocytes and zinc level in plasma (Muzyka et al., 2002). Severe bone marrow dysplasia is frequently accompanied by clonal T cell expansion and alterations in T lymphocyte subsets which suggest that autoimmune-mediated bone marrow injury is an early or predisposing event in the pathogenesis of benzene-induced persistent hematopoietic disease (Irons et al., 2005).

(c) Immunotoxicity of air pollution

(i) Cells of the immune system

Lymphocyte

Lymphocytes are the key cells of the immune system responsible for acquired immunity and immunologic attributes of diversity, specificity, memory and self/non-self recognition. They are usually small, round or partially ovoid, slightly motile and non-phagocytic and non-granular leukocytes (except for a group of natural killer cells). There are approximately 10^{10} lymphocytes in the human body. The size ranges from 6-15 µm. Lymphocytes can be broadly divided into T- (thymus-dependent), B- (Bursa of Fabricius in birds and bone marrow-dependent in mammals), and NK (Natural Killer) cells. These cells have specific surface marker glycoproteins (Cluster Determinants or CDs) by which they can be identified. The T-cells comprise of T-helper cell (CD4+) and T-cytotoxic cell (CD8+).

Table 1.8: Lymphocyte subtypes

CD marker	Name	Characteristic
CD4+	T-helper 1 (Th-1)	Production of interferon
CD4+	T-helper 2 (Th-2)	Production of Interleukin
CD8+	T-cytotoxic (Tc)	Cytotoxicity, production of perforin
CD19+/ CD20+	B-cell	Generation of immunoglobulin
CD16+ CD56+	Natural killer (NK) cell	Antibody-independent killing of virus and tumor cells

CD4+ cells help in recognizing the antigen presented by antigen presenting cell (APC) like macrophages. It releases cytokines to activate the B-lymphocytes (CD19+ and CD20+ cells) to produce a clone of B-cells, mature to plasma cell and produce the specific type of antibody against the antigen. CD8+ T- cell mediate cellular cytotoxicity crucial for host defense against viral agents. NK cells (CD16+CD56+) are involved in natural killing of tumor cells and viruses (Table 1.8). A general increase in the number of lymphocytes is known as lymphocytosis whereas a decrease is lymphocytopenia.

It has been shown that measurement of CD4+T lymphocytes provide a useful biological marker of past exposure to aromatic amines (Araki et al., 1993). Benzene, styrene and PAHs activate peripheral lymphocyte and caused changes in T-cells in workers exposed to aromatic hydrocarbons (Biro et al., 2002). The human immunodeficiency virus (HIV) destroys T cells (specifically, CD4+ lymphocytes). Without this key defense, the body is susceptible to opportunistic diseases. An increase in lymphocytes is usually a sign of a viral infection. In some rare cases, leukemias are found through an abnormally raised lymphocyte count in an otherwise normal person.

Monocyte / macrophage

A monocyte is the largest of all leukocytes measuring 12 to 20 µm in diameter. On a Wright's stained peripheral blood smear they show a bluish-gray cytoplasm with a large cytoplasm-to-nucleus ratio. The nucleus is ovoid or kidney shaped either centrally or eccentrically located. During hematopoiesis in the bone marrow, the granulocyte-monocyte progenitor cells differentiate into promonocytes, enter the blood stream where they further differentiate into mature monocytes. Monocytes circulate in the blood stream for about 8 hours during which they enlarge migrate into tissues and differentiate into specific tissue macrophages at different anatomical locations. They serve different functions in different tissues and are named according to their tissue location: alveolar macrophages in the lung, histiocytes in connective tissue, Kupffer cells in the liver, mesangial cells in the kidney, microglial cells in the brain and osteoclasts in bone. Monocytes are responsible for phagocytosis (ingestion) of foreign substances in the body. Monocytes can perform phagocytosis using intermediary (opsonising) proteins such as antibodies or complement that coat the pathogen, as well as by binding to the microbe directly via pathogen-recognition receptors. Monocytes are also capable of killing infected host cells via antibody, termed antibody-mediated cellular cytotoxicity. Vacuolization may be present in a cell that has recently phagocytosed foreign matter. Monocytes, which migrate from the blood stream to other tissues, are called macrophages. Macrophages are responsible for protecting tissues from foreign substances but are also the predominant cells involved in atherosclerosis.

Polymorphonuclear neutrophil (PMN)

Polymorphonuclear neutrophils (PMN) are granulocytes with a diameter of 12-15 µm in peripheral blood smears. PMNs in turn account for 55-65% of all leukocytes in humans. PMN are active phagocytes, capable of only one phagocytic event, expending all of their glucose reserves in an extremely vigorous respiratory burst. The respiratory burst involves the activation of an NADPH oxidase enzyme, which produces large quantities of superoxide, a reactive oxygen species. Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages.

Low PMN counts are termed neutropenia. This can be congenital or due to acquired factors. It can also be a side effect of medication, including chemotherapy. Functional disorders are often hereditary. They are disorders of phagocytosis or deficiencies in the respiratory burst as in chronic granulomatous disease, a rare immune deficiency. In alpha 1-antitrypsin deficiency, the important neutrophil enzyme elastase is not adequately inhibited by alpha 1-antitrypsin, leading to excessive tissue damage in the presence of inflammation - most prominently pulmonary emphysema. Cytokines produced in the lung due to deposition of ambient particles also appear in the circulation and act on the bone marrow. PMN recently released from the bone marrow following the action of the cytokines are preferentially sequestered in pulmonary capillaries, are less chemotactic, contain more damaging granular enzymes, leading to damage of the alveolar tissue (van Eden and Hogg, 2002).

Eosinophil

Eosinophils are granulocytes responsible for combating allergen exposure and parasitic infestations in the body. Eosinophils make up about 2-4% of the all white blood cells, and are about 10-12 µm in size. The nucleus is bilobed with a narrow connection in between. The cytoplasm contains coarse granules, rounded in shape, staining reddish thereby indicating an acidophilic nature of the granules. These granules contain histamine and proteins such as eosinophil peroxidase, RNase, DNases, lipase, plasminogen, and major basic protein that are toxic to both parasites and the host's tissue.Eosinophils play a role in fighting viral infections, which is evident from the abundance of RNAses they contain within their granules. Eosinophils also play a role in the allergic response, and in fibrin removal in inflammation. Eosinophils are considered the main effector cells in asthma pathogenesis. An increase in eosinophils, i.e. the presence of more than 500 eosinophils/µl of blood is called an eosinophilia, and is typically seen in people with a parasitic infection of the intestines, a collagen vascular disease (such as rheumatoid arthritis), malignant diseases such as Hodgkin's Disease, extensive skin diseases (such as exfoliative dermatitis), Addison's Disease and with the use of certain drugs such as penicillin. Eosinopenia is a decrease in eosinophil number, which occurs characteristically when glucocorticoids are administered.

Basophil/mast cell

Basophils are the least common of the granulocytes, representing about 1% of circulating leucocyte. They contain large cytoplasmic rounded granules, which stain deep blue or violet indicating its basophilic nature. The granules obscure the nucleus under the microscope. However, when unstained, the nucleus is visible and it usually has 2 lobes. A cell in tissues, the mast cell, has many similar characteristics. For example, both cell types store histamine, a chemical that is secreted by the cells when stimulated in certain ways (histamine causes some of the symptoms of an allergic reaction). Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed.

Basophils tend to appear in specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. While the exact purpose of basophils has never been proven, they appear often in tissues where parasites are found. They can be found in unusually high numbers at sites of exoparasite infection, e.g., ticks. They also appear in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE antibody very tightly. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins.

When activated, basophils secrete histamine, several proteoglycans, lipid mediators like leukotrienes, and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system. There are other substances that can activate basophils to secrete which suggests that these cells have other roles in inflammation.

(ii) Immune response to air pollution

Small number of T-lymphocyte resides in the bronchial tissue of normal subjects: CD4+ cells predominate in the submucosa and CD8+ cells in the epithelial (Salvi et al., 1999). Following DEP exposure among human volunteers, T-lymphocytes, mostly CD4+ cells, infiltrate the submucosa and bronchial epithelium (Salvi et al., 1999). The number of B-lymphocytes in the bronchial tissue did not change, but their numbers increased in the bronchoalveolar lavage fluid with a corresponding decrease in the blood, suggesting trafficking of circulating B-cells to the bronchial lumen following DEP exposure (Salvi et al., 1999).

In recent decades, increased prevalence of allergic conditions has been observed in developed countries. Although lifestyle, exposure to infection, and diet are important confounders, a strong link between industrialization and allergy has been established (Fernvik et al., 2002). The underlying mechanisms of hypersensitivity involved pollutant-mediated stimulation of interleukin-5 production, immunoglobulin-E synthesis, eosinophil recruitment and bronchial hyperactivity (Fernvick et al., 2002).

Chronic exposure to vehicular pollution is associated with airway inflammation, and diesel exhaust particles play an important role in this response (Salvi et al., 1999). The inflammatory response by DEP was not due to NO_2, as NO_2 alone did not show any cellular inflammatory response in the airways (Blomberg et al., 1996). Instead, the particulate component of diesel exhaust is responsible for the action (Salvi et al., 1999). *In vitro* studies have shown that UFP elicit a greater inflammatory response in the alveolar space when compared with larger-sized particles, probably due to an interaction between a larger surface area of small particles within AM and interstitial cells (Albelda, 1994). PM_{10} collected by environmental sampling generate hydroxyl radicals in aqueous solution by an iron-dependent process (Gilmour, 1996). These free radicals cause activation of redox-sensitive transcription factors such as NF kB and AP-1 (Meyer et al., 1994) NFkB is important in transcription of many cytokines and chemokine genes, including inducible nitric oxide synthase (iNOS), cyclooxigenase-2 (COX2), and vascular and adhesion molecules (Lee et al., 1994). Oxidative stress induced by these free radicals increases the permeability of epithelial cells that would further facilitate the transfer of particles into the Interstitium (Lee et al., 1995). The proximity of the interstitial inflammatory cells to the endothelium and the blood spaces means that signals such as cytokines could be released into the blood causing systemic effects.

(d) Reproductive toxicity

Particulate matter can significantly increase the adverse reproductive outcomes in both males and females. Studies show relatively low level of air pollution (higher than 40 µg PM_{10}/m^3) result in intrauterine growth retardation (IUGR) in the first gestational month in females and YY8 disomy in the sperms (Sram et al., 1999). Exposures to ambient air pollutants have also been associated with adverse birth outcomes.

Investigation for the effects of air pollutants on birth weight mediated by reduced fetal growth among term infants who were born in California showed O_3 exposure during the second and third trimesters and CO exposure during the first trimester were associated with reduced birth weight and an increase of IUGR (Salam et al., 2005). Air pollutants viz. benzene exerts toxic effects on mammalian fetuses (Dobrzanska-Tatarczuch and Starek, 1991; Brown-Woodman et al., 1994). Disruption of embryonic development following exposure of pregnant women to aromatic hydrocarbons is well recognized. Benzene exposure induces a decrease in mean gestational age (Wang et al., 2000). Exposures to low level of benzene in work places interrupt the function of hypothalamic-pituitary-ovarian axis and affect normal levels of follicle stimulating hormone (FSH), pregnandiol-3-glucuronide (PgD) and estrone conjugate (EIC) with shortened luteal phase in female workers (Chen et al., 2001). Benzene has been detected above the maximum allowable concentration in semen of workers exposed to organic solvents, and the change has been attributed to abnormal pregnancy outcome among wives of the benzene-exposed workers (Xiao et al., 2001). Similarly, benzene exposure from the rubber industry resulted in alteration in viscocity, liquefaction capacity, sperm count, sperm motility and proportion of sperm with normal morphology in the workers (De Celis et al., 2000). Moreover, benzene induces numerical aberration in the chromosomes of sperm cells (Zhao et al., 2004). Diesel exhaust particles contain substances with estrogenic, antiestrogenic and antiandrogenic activities. An exposure to low level of diesel exhaust (0.1 mg DEP/m^3) have been shown to reduces the expression of several genes which play a key role in gonadal development and also a reduction in estrogen receptor mRNA expression (Takeda et al., 2004)

(e) Neurotoxicity

Besides physical health, air pollution exposure may lead to impairment of mental health, because toxic effects of particulate matters on central and peripheral nervous system has been reported. Difficulties with recall, response, concentration, and sleep disorders suggest central nervous system impairment due to vehicular emission (Kilburn, 2000). Benzene produced discrete changes in norepinephrine (NE) and dopamine (DA) turnover in certain areas of the hypothalamus (Andersson et al., 1983). Tyrosine hydroxylase is the key enzyme for biosynthesis of catecholamine and the hypothalamus is one of the major association centers in the central nervous system. Inhalation of benzene in high doses (>500 ppm) affects the functions of all these centers at an inhalation (Anderson et al., 1983). DEP selectively damages dopamine neurons through the phagocytic activation of microglial NADPH oxidase and consequent oxidative insult (Block et al., 2004). Several environmental toxicants promote or interfere with neurotransmitter function (Korpela et al., 1986; Sun et al., 1992) and evoke neurodevelopmental abnormalities by disrupting the timing or intensity of neurotrophic actions. This developmental neurotoxicity extends to late phases of brain maturation including adolescence (Slotkin, 2004). A recent study from this laboratory has demonstrated short-term memory loss in mice exposed to benzene (Banik and Lahiri, 2005).

(f) Change in antioxidant status

Oxidative stress refers to the injury caused to cells resulting from increased formation of reactive oxygen species (ROS) and/or decreased antioxidant reserve. Reactive oxygen metabolites (O_2^-, $OH^\cdot$, H_2O_2) are products of aerobic metabolism and are continuously produced *in vivo* which, if not neutralized with the endogenous antioxidants, may endanger the cellular integrity. These free radicals may cause

oxidative DNA damage, lipid peroxidation and enzymatic oxidation leading to cellular damage. Air pollution containing a variety of toxic substances on their surface induce production of ROS by AM and neutrophils and induce an inflammatory reaction.

Living organisms have a well-orchestrated machinery of antioxidant defense mechanism for their survival against oxidative stress. Superoxide dismutase (SOD) is a member of the family of metallo-enzymes and is the most important antioxidant present in the body to act against superoxide (O_2^-) radicals. It accelerates the dismutation of superoxide anion into hydrogen peroxide (H_2O_2) and molecular oxygen.

$$2\ O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Other antioxidants enzymes are catalase and glutathione peroxidase. In addition there are several antioxidants present in food such as vitamin E, C and A, flavonoids.

Several studies have shown PM induces cytotoxic, oxidative stress, and inflammatory responses in human epithelial lung cells. PM-exposed cells showed concentration- and time-dependent changes in lipid peroxidation, superoxide dismutase activity, 8-hydroxy-2'-deoxyguanosine formation, and poly(ADP-ribosylation, tumor necrosis factor-alpha secretion, inducible nitric oxide synthase activity, and nitric oxide release. These findings suggested that oxidative stress and inflammatory responses precede cytotoxicity in PM-exposed L132 cells. $PM_{2.5}$ has been shown to significantly increase lipid peroxidation levels and decrease SOD, catalase and glutathione peroxidase activities in laboratory animals (Liu and Meng, 2005). These results led to a conclusion that airborne $PM_{2.5}$ was a systemic toxic agent, both to respiratory and cardiovascular systems. An exposure to DEP causes oxidative stress (Furuyama et al., 2006). A study conducted among petrol pump workers reported change in the antioxidant status (Georgieva et al., 2002). A fall in SOD level in liver has been documented following benzene poisoning (Pan et al., 2003). In a recent study, Holovska (2005) has shown decrease in glutathione peroxidase (GSHPx) and glutathione-S-transferase (GST) activities, but not in SOD and thiobarbituric acid reactive substances (TBARS), in the liver of rats after benzene exposure via inhalation.

(g) Genotoxic effects of air pollution

Besides affecting the respiratory system, exposure to vehicular emission may cause genetic changes as long-term adverse health effect. Urban atmospheres contain complex mixtures of air pollutants including mutagenic and carcinogenic substances such as benzene, diesel soot, heavy metals and PAHs (Klumpp et al., 2006). Different chemical agents or their metabolites may cause DNA strand breaks, impairment of DNA repair system, dysregulation of cell cycle and induction of programmed cell death i.e. apoptosis (Eastman and Barry, 1992; Tovalin et al., 2006). DNA strand break usually occurs when reactive oxygen species interact with DNA (Moller and Wallin, 1998). Diesel exhaust particles, urban particulate cause DNA damage (Don Porto et al., 2001).

Chromosomal changes

Chromosomal aberration and sister chromatid exchange have been reported following in vitro exposure of benzene metabolites to bovine lymphocytes (Sivikova, 2005). In human the loss or gain of a whole

chromosome (aneuploidy) is common in the development of leukemia and other cancers. Chromosome 5 and 7 are highly sensitive to loss (monosomy) following hydroquinone and benzenetriol exposure in vitro whereas chromosomes 8 and 21 are highly sensitive to gain (trisomy) (Smith et al., 2000; Zhang et al., 2005).

(i) Micronucleus formation and other nuclear anomalies

Micronucleus (MN) formation is the accepted laboratory practice to detect chromosomal breaks in a cell. MN is defined as microscopically visible, round or oval chromatin masses in the cytoplasm next to the nucleus (Schmid, 1975). MN consists of a part of the chromosome or chromatid, or a whole chromosome that has not been incorporated in the spindle apparatus due to aberrant mitosis (Schmid, 1975). Assessment of the number of MN is widely used to identify the genotoxic damages and its formation is considered a simple biomarker of mutagenic effect of environmental pollutants (Stich et al., 1982; Belien et al., 1995). Since the air pollutants enter the body via nasal-oropharyngeal route, the epithelial cells of the buccal mucosa are in constant touch with the foreign particles. Therefore, genotoxic effects of air pollutants can best be evaluated by MN test in buccal epithelial cells.

Criteria for identifying micronucleus

MN are round or oval in shape, consist of nuclear material that is fully separated from the parent nucleus and covering a total area <1/5th of the parent nucleus (Tolbert et al., (1991). Both structural and numerical chromosomal aberrations have been observed fairly consistent in the lymphocytes and bone marrow cells of the individuals exposed to benzene at workplace. It is now generally accepted that benzene is a human clastogen (IARC, 1987). Increases in the number of both unstable and stable chromosomal aberrations were observed in men, even 2 years after cessation of workplace exposure (Tough and Court Brown, 1965). Somatic mutations as an endpoint of benzene-induced genotoxic effects in heavily exposed workers was studied recently by Rothman et al., 1995). They used the glycophorin A (GPA) mutation assay. The results suggested that benzene induces gene-duplicating mutations, presumably through recombination mechanisms, but not gene-inactivating mutations due to point mutations or deletions.

Tolbert and his associates (1991) found nuclear anomalies in buccal epithelial cells of snuff users. In another study they proposed that the nuclear anomalies were as common as micronucleus (Tolbert et al., 1992). The induction of karyorrhexis and 'nuclear anomalies' in colonic crypt cells has been correlated positively with the induction of colonic tumors by chemical treatment (Duncan et al., 1985). Formation of nuclear anomalies like pyknotic and karyorrhectic nuclei in intestinal epithelial cells has been reported in rats intraperitoneally injected with benzidine (Percy et al., 1989) and aromatic hydrocarbons (Blakey et al., 1985). Further, Subrahmanyam (1991) and his colleagues showed that benzene exposure in human and animals result in structural and numerical chromosomal aberration in lymphocytes and bone marrow cells. Analyses of micronucleus (MN) frequencies in peripheral lymphocytes by use of the cytokinesis-block technique among 49 traffic police revealed that MN frequency was significantly higher among the traffic police and frequency was found to increase with age, but no influence was observed for gender or smoking (Maffei, 2005).

(ii) DNA damage, assay technique

Induction of DNA damage is an important initial event in the pathway of carcinogenesis. A considerable battery of assays exists for the detection of different genotoxic effects of compounds in experimental systems. Among these, single cell gel electrophoresis (SCGE) or Comet assay is technically simple, relatively fast, cheap, and sensitive method for detection of DNA strands breaks. The assay can be undertaken in virtually all mammalian cell types without requirement for cell culture. Detection of programmed cell death by apoptosis is also done by this method, as degradation of nuclear DNA by endonuclease enzymes is the hallmark of apoptosis.

In this method the cells are embedded in agarose and lysed, generating nucleus-like structures in the gel (referred to as nucleoids). Following alkaline electrophoresis, the DNA strands migrate toward the anode, and the extent of migration depends on the number of strand breaks. The migration is visualized and scored in a fluorescence microscope after staining. Although not all types of genotoxic exposures should be expected to result in DNA damage in mononuclear blood cells, the Comet assay seems to be a valuable tool for detection of genotoxic exposure in humans. The Comet assay has been successfully applied for detection of DNA damage in mammalian cells elicited by environmental exposures, including diet, exercise, hypoxia, and sunlight (Moller, 2005). Five of the metabolites of benzene *viz.* muconic acid, hydroquinone, catechol, p-benzoquinone and benzenetriol caused DNA damage *in vitro* in lymphocyte Comet assay (Anderson et al., 1995; Gaskell et al., 2005). Increase in DNA damage by cumulative benzene exposure has been confirmed *in vivo* in laboratory animals (Plappert et al., 1994; Tuo et al., 1996) and in human subjects employed in printing press (Joo et al., 2004, Sul et al., 2005). DNA strands break in liver cells has been recorded in benzene-exposed mice (Plappert et al., 1994; Vestergaard et al., 2002).

(h) Air pollution and cancer incidence

An association between air pollution exposure and cancer has been reported in laboratory animals and in human subjects by epidemiologic studies. Air pollution exposure has been linked to carcinoma of the lung (Kuper et al., 2002; Vineis et al., 2005; Chen, 2005), larynx (Zheng et al., 2001, Kuper et al., 2002), nasopharynx (Kuper et al., 2003; Sasco et al., 2004), esophagus (Kuper et al., 2002; Guo et al., 2004), oral cavity (Kuper et al., 2002), urinary bladder (Mastrangelo, 1996, Guo et al., 2004), uterine cervix (Sasco et al., 2004), breast (Johnson, 2005; Grant and Garland 2005; Bonner 2005), and leukemia and lymphoma (Guo et al., 2004; Kasim et al., 2005; Fritschi et al., 2005). Diesel exhaust exposure has been linked to increased incidence of lung cancer (Garshick et al., 2002; Garshick et al., 2004; Laden et al., 2004).

Carcinogens in air

There are a large number of carcinogens present in automobile exhausts, industrial and household emissions. For example, cigarette smoke contains nearly 4,200 chemicals and 44 of these chemicals are carcinogenic. The most important environmental carcinogens are benzene and benzo(a)pyrene. Benzene is a Class 1 carcinogen (confirmed human carcinogen) while benzo(a) pyrene and diesel exhaust particles belong to Class 2A (probably carcinogenic to humans) human carcinogens according to International Agency for Research on Cancer (IARC, 1982). The association between chronic benzene exposure and development of human leukemia has been established by epidemiological and case studies (IARC 1982),

most of which have dealt with industrial exposures. Of the two major classes of leukemia (myeloid and lymphoid), the most consistent evidence for causal relationship in humans has been found between benzene exposure and myeloid leukemia (Goldstein, 1988).

Carcinogenicity of benzene results from its cellular metabolism (Whysner, 2004). It is particularly carcinogenic to the hematopoietic system. Chronic exposure to benzene results in progressive decline of hematopoietic function inducing leukemia, aplastic anemia and myelodysplastic syndromes (Kang, 2005). Damage to macromolecules resulting from benzene metabolites and misrepair of DNA lesions may lead to changes in hematopoietic stem cells (HSCs) that give rise to leukemic clones (Faiola et al., 2004). Cumulative benzene exposures are strongly associated with acute myeloid leukemia (Descatha et al., 2005) and to a lesser extent with acute and chronic lymphocytic leukemia (Glass et al., 2003). It affects the bone marrow through the action of its highly reactive metabolites, especially p-benzoquinone (Xie et al., 2005).

Wong (1987) reported a significant dose-response relationship between cumulative exposure to benzene and mortality from leukemia and all lymphopoietic cancers combined. In retrospective cohort study from China encompassing 28,460 workers exposed to benzene in 233 factories, 30 cases of leukemia (23 acute, 7 chronic) were found, as compared to four cases in the reference cohort of 28,257 workers in machine production, textile and cloth factories (Yin et al., 1987). A statistically significant excess risk for aplastic anemia and leukemia has been reported in studies conducted among shoe factory workers in Italy who were occupationally exposed to high level of benzene (Paci et al., 1989; Seniori et al., 2003). Similarly, benzene exposure may cause acute non-lymphocytic leukemia, aplastic anemia and myelodysplastic syndrome (Yin et al., 1996; Hayes et al., 2000; Steffen et al., 2004; Crosignani et al., 2004; Bernardini et al., 2005). In contrast, a study of 454 petroleum refinery workers in the USA employed between 1952 and 1978 showed no excess deaths from leukemia. However, the median exposure to benzene was relatively low 0.45 mg/m^3 (0.14 ppm) (Tsai et al., 1983). Besides leukemia, benzene exposure is believed to be responsible for cancers of the brain, lung, paranasal cavity and esophagus (Forni et al., 1996; Beach and Burstyn, 2006). A study reported a high risk of traffic police developing cancer due to contact with traffic benzene vapor during daily work (Wiwanitkit et al., 2005).

1.6.3 Additive and synergistic effects of airborne pollutants

Following inhalation, air pollutants act on the target tissues in unison rather than individually. The pollutants may also react with each other and some of the compounds generated in the process may be more toxic than the primary pollutants (Hazucha, 1999).

The additive or cumulative response to a mixture is the sum of the effects induced by the individual components of the mixture. Conceptually, the additive effect occurs only when the action of each pollutant is independent. When a pollutant does not elicit a response when acting alone but increases the effect of another co-occurring pollutant, the effect is called potentiation. Synergism refers to any combination of action in which the result is more than which would be attained if the actions were entirely independent of each other. In other words, in a synergistic process the whole is greater than the sum of its parts. As for example smoking and exposure to vehicular emission or air pollution result

in a greatly increased probability of lung cancer compared to the risk of either smoking or asbestos exposure alone.

Human exposure to complex mixtures of air pollutants is a challenge to the toxicologists and epidemiologists because of the enormous range of variations and confounding factors making exposure assessment, study design and data interpretation difficult. Therefore, it is debatable whether the observed changes in human subjects could be attributed to benzene alone. To explore these points parallel experiments need to be conducted in experimental animals under controlled laboratory conditions where the animals are exposed to measured doses of benzene in drinking water and also inhalation. Comparing the health response following controlled benzene exposure to those obtained from vehicular emission exposed population, can give an insight into the possible health effects of benzene from vehicular emission.

1.6.4 Health effects of air pollution: modifying factors

Age and gender

The effect of age and gender on response to particulate air pollution exposure is significant. Young people (mean age 24 year) exposed to 1 hour to 300 μg/m^3 PM_{10} from disel engine showed rise in platelet and neutrophil number in peripheral blood (Salvi et al., 1999). $PM_{2.5}$ exposure in young girls (aged 24 year) resulted in incresased RBC and hemoglobin levels while no change was observed in men of similar age (Soresen et al., 2002). In contrast, decrease in platelet, RBC, and fibrinogen was reported in air pollution exposed aged (>60 years) individuals (Seaton et al., 1999). Personal exposure to fine particles ($PM_{2.5}$) in an ambient and/or indoor air can lead to changes in blood (Sorensen et al., 2003). However, except for elevated fibrinogen level no change was observed in hemoglobin, RBC, WBC, platelets in pollution exposed human volunteers (Ghio et al., 2000). In general, RBC and hemoglobin are higher in men while platelets, fibrinogen and oxidised protein in plasma are higher in women (Sorensen et al., 2003). It has been reported that for each 10 microgram per meter cube rise in $PM_{2.5}$ level RBC is elevated by 2.3% and hemoglobin by 2.6% in women but no such change was observed in men (Sorensen et al., 2003).

The average lung size is 23% smaller in women than men. The functional residual capacity of lung varies between 3.0-3.9 litres with a range of 2.9-5.9 litres in men and 2.1-3.9 litres in women (Jaques and Kim, 2000). The total lung deposition of particulate matter especially ultrafine particles is greater in women (Jaques and Kim, 2000). Lung function gradually detoriates with age even if we donot expose our lungs to tobacco smoke or other environmental pollutants that may provoke lung inflammation. Evidence of decline in lung function in healthy individuals becomes apparent in the fourth decade of life, thereafter the decline gradually progresses. Some individuals are much more susceptible to declining lung function as a function of age than others. Certain types of inflammatory disorders and bacterial lung infections tend to occur more frequently in older people (>60) and they have more CD4, neutrophils, IL-6 and IL8 in lungs than young people (Meyer, 1996).

1.6.5 Mechanism of air pollution-related health injury

Several recent studies suggest that general mechanistic pathways of air pollution-related diseases of the lungs and heart probably include pulmonary and systemic oxidative stress and inflammation, enhanced

initiation and promotion of atherosclerosis, and altered cardiac autonomic functions (Pope 2004a, Brook et al., 2004, Tao, 2003). The consensus opinion is that the coarse and fine particles (PM_{10} and $PM_{2.5}$ respectively) are the primary mediators of toxicity in the lungs and the airways, while fine and ultrafine particles (aerodynamic diameter less than 0.1µm) generally mediate the toxicity on the heart and blood vessels (Pope 2004a, Brook et al., 2004). It was also observed that exposures to fine particles from outdoor sources of combustion and from tobacco smoke might invoke similar pathophysiological processes (Pope 2004a, Brook et al., 2004). Indeed, airway inflammation, an important factor in mediating air pollution effects on lung, is a common finding among smokers as well as in persons who have lived for long in polluted environments (Gauderman et al., 2004).

The most accepted mechanism of adverse health effect by particulate pollution is by oxidative stress. This is mediated partly by particle induced inflammation in the lungs causing macrophages to release reactive oxygen species, and partly by transitional metals such as iron, cobalt, nickel and manganese adsorbed on the particle surface which are capable of generating ROS through Fenton reaction (Donaldson et al., 2001; Li et al., 1997). Long-term exposure to particles increases the risk of developing atheroscelortic plaques through increased oxidation of low density lipoproteins. The involvement of oxidative stress is evidenced by the report that progressive atherosclerosis in heavy smokers by supplementing with combination of antioxidant vitamins C and D (Salonen et al., 2000). Other possible mechanisms of cardiovascular effects are increased blood viscosity due to lung inflammation (Peters et al., 1997) or direct effect of inhaled particles on platelets and red blood cells (Salvi et al., 1997; Seaton et al., 1999).

1.7 OBJECTIVE OF THE STUDY

Respirable particulates present in high concentrations in urban air are recognized as hematotoxic, neurotoxic, and carcinogenic. Several million Indians residing in the cities are exposed to these pollutants both occupationally and environmentally.

With this background, the present study was undertaken in Delhi with following objectives:

- To prepare a database on air pollution related respiratory symptoms among the residents of Delhi
- To assess the degree of lung function impairment in persons chronically exposed to city's air
- To explore the underlying mechanism of air pollution related pulmonary dysfunction at the cellular and subcellular level

CHAPTER-2.0

MEASUREMENT OF AMBIENT AIR QUALITY OF DELHI

2.1 AMBIENT AIR QUALITY MONITORING IN DELHI

Data on the concentration of ambient air pollutants with respect to RSPM (respirable particulate matter with an aerodynamic diameter of less than 10 µm, i.e. PM_{10}), carcinogenic organic compounds like polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs), oxides of nitrogen (NOx), sulfur dioxide (SO_2), suspended particulate matter (SPM) and ozone in study areas during and preceding months of this study were obtained from different air quality monitoring stations operated under National Air Quality Monitoring Programme (NAMP). Central Pollution Control Board and National Environmental Engineering Research Institute (NEERI) operate these stations under NAMP at the following locations as mentioned in Table 2.1.

Table 2.1: Air Quality Monitoring Stations in Delhi

	Name	Area	Operated by
1	Ashok Vihar	North	CPCB
2	ITO	Central	CPCB
3	Nizamuddin	South-east	CPCB
4	Shahadara	North-east	CPCB
5	Janak Puri	West	CPCB
6	Shahzada Bagh	North	CPCB
7	Siri Fort	South	CPCB
8	Sarojini Nagar	South	NEERI
9	Town Hall Library	North	NEERI
10	Mayapuri Industrial Area	West	NEERI

(a) Air quality measurements in control areas

Air quality data of rural areas of West Bengal were obtained from monitoring stations of the West Bengal State Pollution Control Board. Additionally, real-time particulate pollutant concentration in air by portable, battery-operated laser photometer (DustTrak™ Aerosol monitor, model 8520, TSI Inc., MN, USA) were measured, particularly for those areas where monitoring stations were absent. The instrument contains 10-mm nylon Dor-Oliver cyclone, operates at a flow rate of 1.7 liter/min and measures particle load in the concentration range of 1µg-100 µg/m^3. PM was measured with the aerodynamic diameter of less than 10 µm (PM_{10}), less than 2.5µm ($PM_{2.5}$) and les than 1.0 µm (PM_1). The monitor was calibrated to the standard ISO 12103-1 A1 test dust. The monitor was placed in open space at least 30 feet away from the roads, 3 feet above the ground level on a wooden stool. The monitoring was carried out 8 hours/day (07:30 –15:30 hours) for three consecutive days in a week and 3 alternate weeks in a season (summer, monsoon, and winter seasons). Monitor could not be used for longer periods due to the limitation of battery power.

(b) Efficiency of real-time aerosol monitor and reliability of data

Real-time Dust Trak monitors of TSI, USA was experimentally used in California, USA in late 90's simultaneously with traditional monitors using filter-based gravimetric method. Researchers of Harvard School of Public Health have reported satisfactory performance (Kim et al., 2004). They measured $PM_{2.5}$ simultaneously by DustTrak direct-reading aerosol monitor Model 8520 (which was used in this study) and filter-based gravimetric method. Spearman correlation proved the two methods as highly correlated. A subsequent study in Canada by Zhu et al., (2005) confirmed the reliability of data generated by real-

time monitor. They monitored concentrations of fine particles in diesel exhausts with Dust Trak real-time monitors, and recorded the measured data every 5 seconds. Test variation of real-time monitoring in different test days was found similar to that measured by filter-based traditional gravimetric method, whereas the repeatability of the monitored data within the same day was better than that of gravimetric method (Zhu et al., 2005).

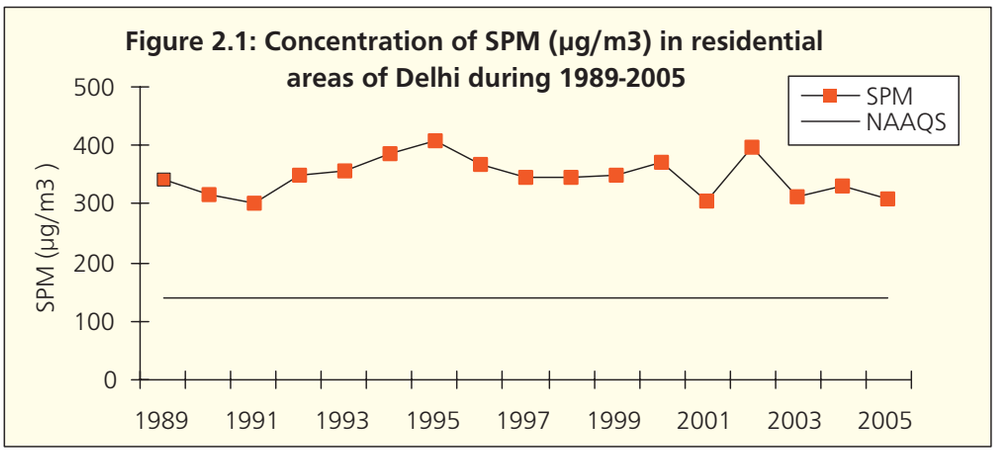
(c) Statistical analysis

The results are expressed as mean ± Standard Deviation (SD). The data were statistically analyzed by student 't' test and $p<0.05$ was considered significant.

2.2 RESULTS

(a) Suspended Particulate Matter (SPM)

The data shows that the concentration of SPM in Delhi's air had varied between 300 and 409 µg/m^3 from 1989 to 2005. All through these years the SPM level exceeded National Ambient Air Quality Standards (NAAQS) in residential areas as depicted Figure 2.1 and 2.2. SPM levels at various monitoringstations in Delhi is given in Table 2.2.



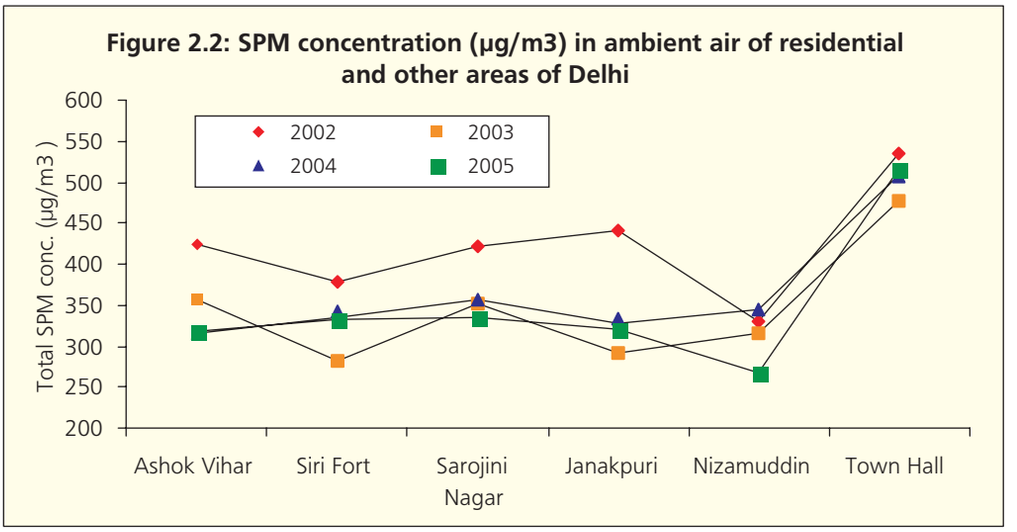


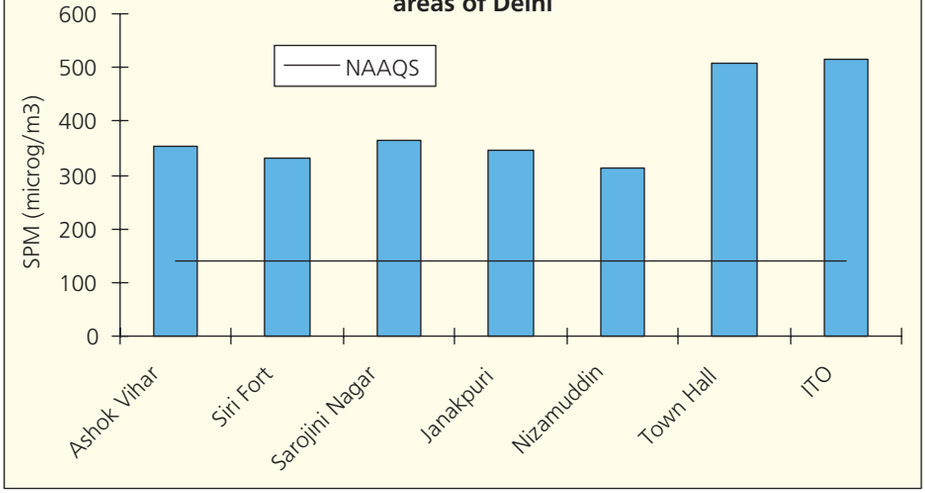
Table 2.2: Suspended Particulate Matter concentrations (in µg/m³) in Delhi's air during 2002-2005

Area	2002	2003	2004	2005	4-yr (Mean ± SD)
Residential areas					
Ashok Vihar	425	356	315	318	354 ± 51
Siri Fort	378	281	334	333	332 ± 40
Sarojini Nagar	421	352	356	335	366 ± 38
Janakpuri	442	291	328	320	345 ± 66
Nizamuddin	329	315	345	268	314 ± 33
Town Hall	534	478	508	516	509 ± 23
Mean ± SD	422 ± 69	346 ± 72	364 ± 72	348 ± 86	370 ± 35
Industrial area					
Mayapuri	NA	425	484	523	477 ± 49
Shahzada Bagh	468	354	338	308	367 ± 70
Shahdara	415	343	357	300	354 ± 47
Mean ± SD	442 ± 37	374 ± 45	393 ± 79	377 ± 127	396 ± 31
Traffic intersection					
ITO	533	509	500	512	514 ± 14
Overall	438 ± 67	370 ± 77	387 ± 78	373 ± 101	392 ± 32

*Source, CPCB, Delhi ; NA, data not available; *p<0.05 compared with residential area*

During 2002-2005, Delhi had an average concentration of 392 ± 32 µg/m³ (mean ± SD) of SPM in ambient air. Average SPM concentration in residential areas was 370 ± 35 µg/m³, which was higher than the National Standard for SPM in residential areas. Among the residential areas monitored during this period, highest SPM level was found in Town Hall (4-year average 509 µg/m³) in northeast Delhi, followed by Sarojini Nagar (366 µg/m³) and Ashok Vihar (354 µg/m³) in northern part of the city. During this period, the average concentration of SPM n residential areas declined from 422 to 348 µg/m³, showing a reduction of 18%. SPM levels (4 year average) at various monitoring statons in Delhi is deopicted in Figure 2.3.

Figure 2.3: SPM levels (4 year average) in different areas of Delhi



In Industrial area, mean SPM concentrations (4 year average) was 396 µg/m^3. However, highest SPM level of the city was found in traffic intersection point at ITO (514 ± 14 µg/m^3, 4-year average).

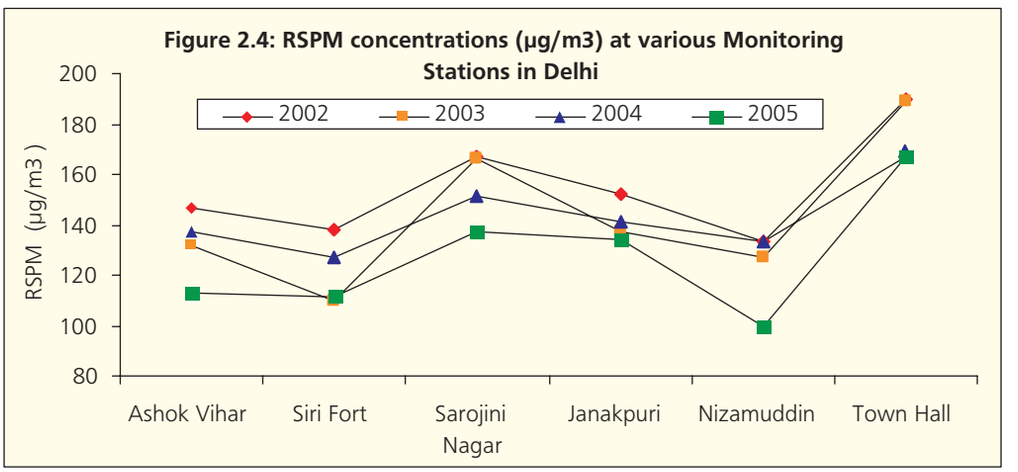
(b) Respirable Suspended Particulate Matter (RSPM/PM_{10})

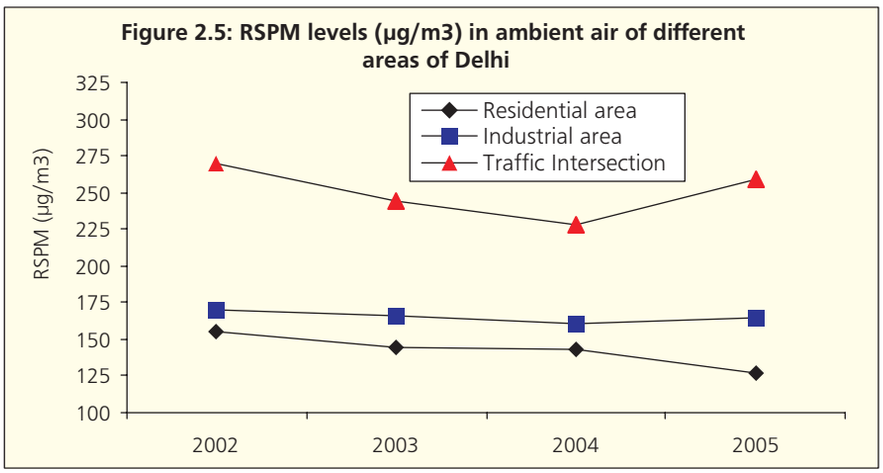
RSPM levels at various monitoring stations are given in Table 2.3. Out of the 10 air quality monitoring stations operative in Delhi, 6 were located in residential areas. The mean annual average respirable suspended particulate matter (RSPM) level during 2002-2005 in these areas of Delhi was 142 µg/m^3, which exceeded NAAQS. RSPM levels at various monitoring stations and areas are depicted in Figure 2.4 and 2.5 respectively. Among the residential areas, highest RSPM concentration was reported at Town Hall (4-year mean 178 µg/m^3), followed by Sarojini Nagar (155 µg/m^3) and Janakpuri (141 µg/m^3).

Table 2.3: Respirable suspended particulate matter (RSPM) concentrations (µg/m^3) in Delhi's air during 2002-2005

Area	2002	2003	2004	2005	4-yr (Mean ± SD)
Residential and other areas					
Ashok Vihar	147	132	137	113	132 ± 14
Siri Fort	138	110	127	111	122 ± 13
Sarojini Nagar	167	166	151	137	155 ± 14
Janakpuri	152	137	141	134	141 ± 8
Nizamuddin	133	127	133	100	123 ± 16
Town Hall	190	189	167	167	178 ± 13
Mean ± SD	155 ± 21	144 ± 29	143 ± 14	127 ± 24	142 ± 11
Industrial area					
Mayapuri	NA	212	213	233	219 ± 12
Shahzada Bagh	186	151	138	130	151 ± 25
Shahdara	153	136	131	131	138 ± 10
Mean ± SD	170 ± 23	166 ± 40	161 ± 45	165 ± 59	165 ± 4
Traffic intersection					
ITO	270	244	228	259	250 ± 18
Overall	171 ± 42	160 ± 42	157 ± 36	152 ± 53	160 ± 8

*Note:- NA- Data not available; *p<0.05 compared with residential area*





There was a declining trend in RSPM level in residential areas in the preceding four years. Compared with 2002 average (155 µg/m^3), the RSPM level has been decreased by 18% in 2005 (127µg/m^3). Industrial areas had a 4-year mean of 165 µg/m^3 of RSPM, which was higher than the NAAQS. At ITO crossing, the 4-year annual average RSPM concentration was 250 µg/m^3, the highest in Delhi. Taking 2000 data as the baseline, a sharp decline in RSPM level was recorded in 2001, the year in which CNG was introduced for public transport vehicles in Delhi. However, in the subsequent years the benefit was diluted perhaps due to the rise in the number of personal vehicles. The concentration was highest in traffic intersection point at ITO, followed by industrial areas and residential areas. RSPM level was lowest in residential areas. PM_{10} distribution in Delhi is depicted in Figure 2.6.

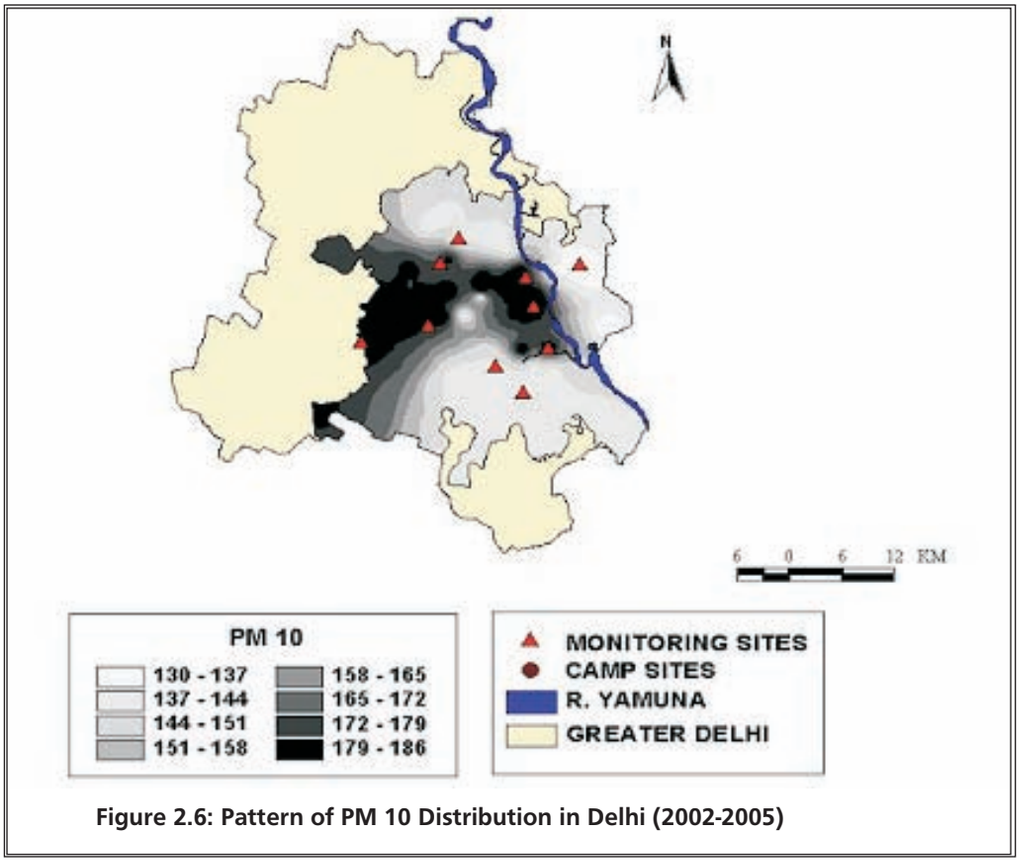


Figure 2.6: Pattern of PM 10 Distribution in Delhi (2002-2005)

RSPM to SPM ratios are given in Table 2.4. RSPM:SPM ratio was appreciably higher at traffic intersection areas (0.49) than that of residential (0.39) and industrial areas (0.39), suggesting that the proportion of respirable particles in SPM was higher at traffic intersection.

Table 2.4: Four-year (2002-05) average SPM and RSPM concentrations at different areas of Delhi

Area	SPM (µg/m³)	RSPM (µg/m³)	RSPM/SPM ratio
Residential and other areas			
Ashok Vihar	354	132	0.37
Siri Fort	332	122	0.37
Sarojini Nagar	366	155	0.42
Janakpuri	345	141	0.41
Nizamuddin	314	123	0.39
Town Hall	509	178	0.35
Industrial area			
Mayapuri Industrial Area	477	219	0.46
Shahzada Bagh	367	151	0.41
Shahdara	354	138	0.39
Traffic intersection			
ITO	514	250	0.49

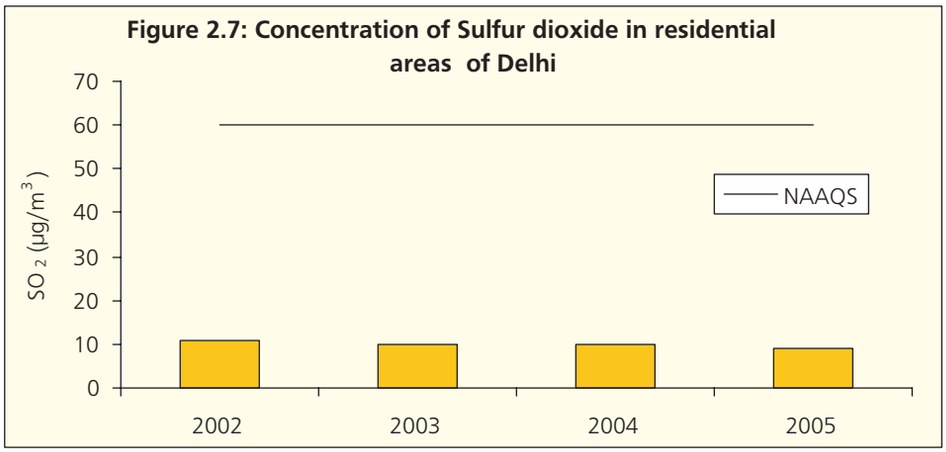
(c) Sulfur dioxide (SO_2)

The concentrations of SO_2 in Delhi's air were within the NAAQS during 2002-2005. The ambient SO_2 levels are given in Table 2.5. The 4-year (2002-05) average concentration of SO_2 in residential areas was 10µg/m³. SO_2 levels at ITO and industrial areas were 9 and 11 µg/m³ respectively. Trend in ambient SO_2 (4 year average) levels in residential areas is depicted in Figure 2.7. SO_2 levels were within the prescribed NAAQS during all the years.

Table 2.5: Concentration of Sulfur dioxide (µg/m³) in ambient air in different areas of Delhi during 2002- 2005

Area	2002	2003	2004	2005	4-yr mean±SD
Residential and other areas					
Ashok Vihar	6	6	10	8	8 ± 2
Siri Fort	12	9	8	9	10 ± 2
Sarojini Nagar	7	7	7	5	7 ± 1
Janakpuri	14	12	10	11	12 ± 2
Nizamuddin	13	12	11	10	12 ± 1
Town Hall	12	12	11	8	11 ± 2
Mean ± SD	11 ± 3	10 ± 3	10 ± 2	9 ± 2	10 ± 1
Industrial area					
Mayapuri	NA	13	12	14	13 ± 1
Shahzada Bagh	10	7	10	8	9 ± 2
Shahdara	17	11	10	9	12 ± 4
Mean±SD	14 ± 5	10 ± 3	11 ± 1	10 ± 3	11 ± 2
Traffic intersection					
ITO	10	9	8	9	9 ± 1
Overall	11 ± 3	10 ± 3	10 ± 2	9 ± 2	10 ± 1

Note:- NA- data not available



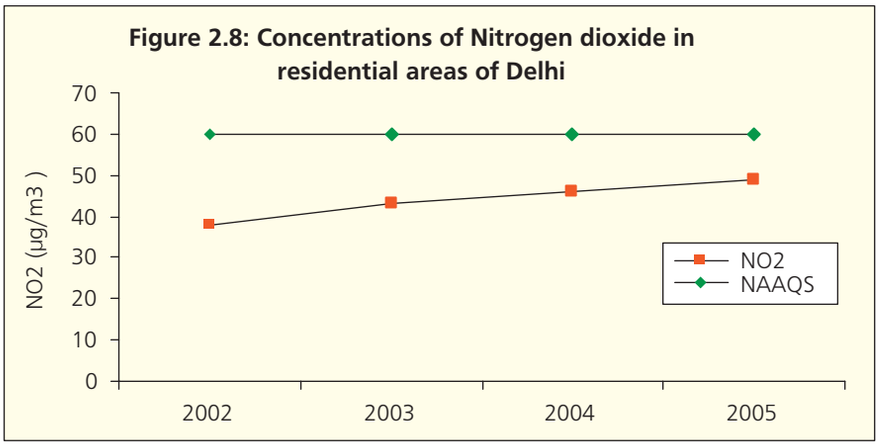
(d) Nitrogen dioxide (NO_2)

The ambient NO_2 levels are given in Table 2.6. NO_2 levels in Delhi's air in residential areas varied between 32 and 59 µg/m^3 during 2002-2005. The 4-year average in residential areas was 44 µg/m^3. NO2 levels at ITO was higher than other locations. NO_2 levels at ITO ranged from 75 µg/m^3 in 2002 and 83 µg/m^3 in 2005. Trend in ambient NO_2 (4 year average) levels inresidential areas is depicted in Figure 2.8.

Table 2.6: Concentrations of nitrogen dioxide (µg/m^3) in ambient air of different areas of Delhi during 2002- 2005

Area	2002	2003	2004	2005	4-yr (Mean±SD)
Residential and other areas					
Ashok Vihar	26	32	39	49	37± 10
Siri Fort	27	32	35	35	32 ± 4
Sarojini Nagar	43	46	53	54	49 ± 5
Janakpuri	40	44	41	48	43 ± 4
Nizamuddin	39	43	45	45	43 ± 3
Town Hall	53	59	60	64	59 ± 5
Mean±SD	38 ± 10	43 ± 10	46 ± 9	49 ± 10	44 ± 5
Industrial area					
Mayapuri	NA	45	56	49	50 ± 6
Shahzada Bagh	34	39	47	46	42 ± 6
Shahdara	36	33	39	36	36 ± 2
Mean±SD	35 ± 1	39 ± 6	47 ± 9	44 ± 7	41 ± 5
Traffic intersection					
ITO	75	94	89	83	85 ± 8
Overall	41 ± 15	47 ± 19	50 ± 16	51 ± 14	47 ± 4

Source, CPCB and NEERI, Delhi; NA, data not available



The annual average concentration of NO_2 in ambient air of Delhi's was within the NAAQS at most of the locations.

(e) Polycyclic aromatic hydrocarbons (PAHs)

(i) Total PAHs

SPM-laden PAH concentrations in air of Delhi in winter (December 2004 and January 2005) are presented in Table 2.7. The mean value of PAHs in residential areas of the city during this period was 23.8ng/ m^3. PAH concentration was highest in ITO traffic intersection area (54.4 ng/m^3), followed by Shahdara (44.4 ng/ m^3) and Nizamuddin (42.6 ng/ m^3). Lowest PAH level (10.8 ng/ m^3) was recorded in Siri Fort in South Delhi. Ashok vihar and Shahzada Bagh both in North Delhi had 18.0 and 17.0 ng / m^3 of PAHs (2-month average) respectively.

Table 2.7: Concentration of SPM-laden total polycyclic aromatic hydrocarbons (ng/m^3) in Delhi's air

Location	December 2004	January 2005	Mean
Residential Areas			
Siri Fort	6.6	14.9	10.8
Ashok Vihar	27.9	8.1	18.0
Nizamuddin	65.2	20.0	42.6
Residential areas, mean	33.2	14.3	23.8
Industrial Areas			
Shahzada Bagh	22.7	11.3	17.0
Shahdara	72.3	16.5	44.4
Industrial areas, mean	47.5	13.9	30.7
Traffic Intersection			
ITO	41.8	66.9	54.4
Overall	39.4	23.0	31.2

(ii) Benzo(a)pyrene (B(a)P)

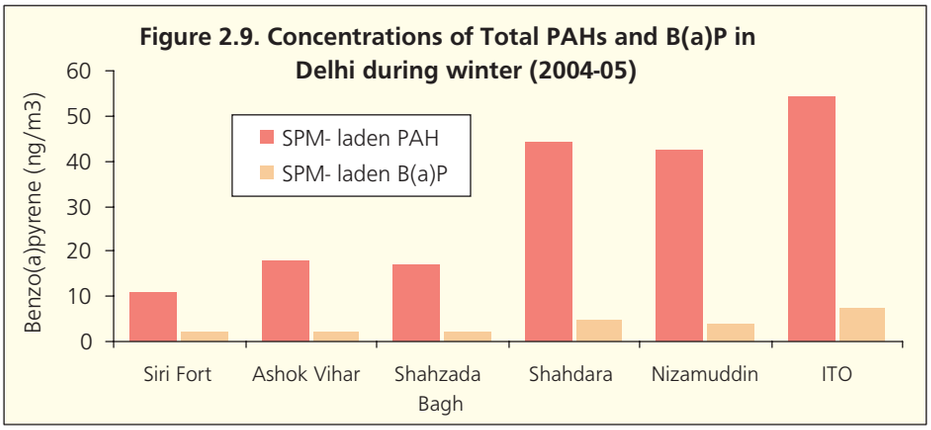
B(a)P is a potential carcinogenic PAH. B(a) P levels in Delhi are presented in Table 2.8. Its concentration was highest in traffic intersection area of ITO (7.3 ng/m^3), and mean value in residential area was 2.77 ng/ m^3 in three residential areas monitored. Among the residential areas, Nizamuddin had highest level

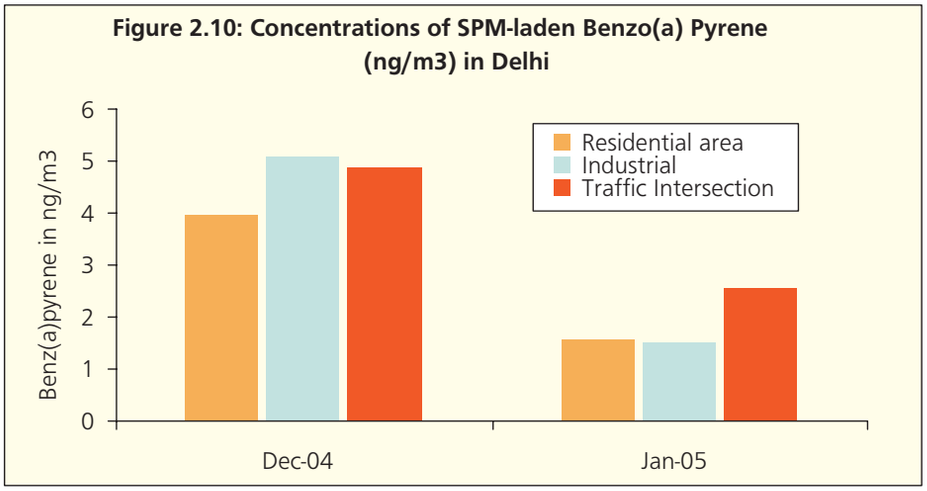
of this carcinogen in its air (3.96 ng/m^3), followed by Siri Fort (2.30 ng/m^3) in southeast. The lowest B(a) P level (1.98 ng/m^3) was found in Shahzada Bagh in north Delhi.

Concentration of Total PAHs and B(a)P is depicted in Figure 2.9. B(a)P levels in residential and industrial areas are depicted in Figure 2.10. Pattern of B(a)P distribution in Delhi is depicted in Figure 2.11.

Table 2.8: Concentrations of SPM-laden Benzo (a) pyrene (ng/m^3) in Delhi's air

Location	December 2004	January 2005	Mean
Residential areas			
Siri Fort	2.74	1.87	2.30
Ashok Vihar	3.34	0.78	2.06
Nizamuddin	5.86	2.07	3.96
Residential areas, mean	3.98	1.57	2.77
Industrial areas			
Shahzada Bagh	2.74	1.22	1.98
Shahdara	7.41	1.80	4.60
Industrial areas, mean	5.08	1.51	3.29
Traffic intersection			
ITO (SPM)	7.04	7.58	7.31
Overall	4.86	2.55	3.70





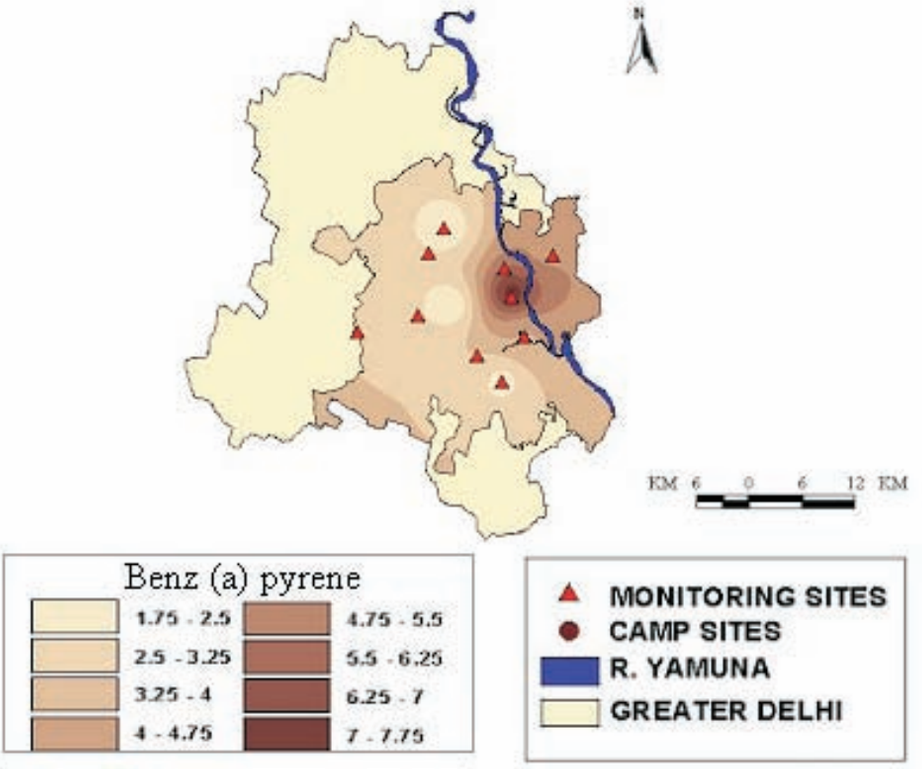


Figure 2.11: Pattern of B(a)P Distribution in Delhi

(f) Volatile organic compounds (VOCs): Benzene and toluene

VOCs contain several organic chemicals like benzene, toluene, xylene (BTX), formaldehyde etc. Out of these, benzene and toluene levels have been measured in some selected areas of Delhi by CPCB. Benzene is a highly toxic compound for human health. Sustained exposure to benzene may cause damage to the bone marrow that may lead to life-threatening diseases like aplastic anemia and leukemia.

(i) Benzene

Benzene (µg/m^3) level in Delhi during 2002-2004 are presented in Table 2.9. Analysis of benzene data for the past four years (2002-05) shows a slow but steady decline in airborne benzene level till 2004 in residential localities and traffic intersection area of ITO. Benzene data of residential areas shows that north Delhi (Ashok Vihar and Moti Nagar) had higher benzene level in air than that of east (East Arjun Nagar) and JNU, south Delhi. JNU area had the lowest benzene level in the city. Benzene levels in residential areas and ITO are presented in Figure 2.12.

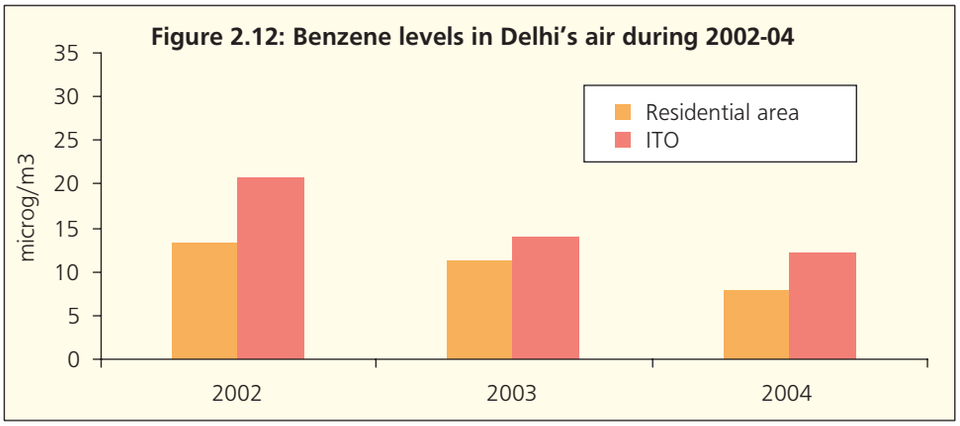
Table 2.9: Benzene (µg/m^3) level in Delhi during 2002-2004

Area	2002	2003	2004
Traffic intersection area			
ITO crossing	20.7	14.0	12.3
Residential and other areas			
Siri Fort	10.9	11.0	8.1
Ashok Vihar	NA	13.0	8.1

Area	2002	2003	2004
Moti Nagar	NA	13.0	9.5
East Arjun Nagar	NA	11.0	7.3
JNU	NA	6.0	4.7
Town Hall	15.8	13.0	8.8
Residential and other area, mean	13.4	11.2	7.8
Overall	15.8	11.6	8.4

Note: NA- Data not available

Figure 2.12: Benzene levels in Delhi's air during 2002-04



Compared with the residential areas, however, benzene concentration was higher in traffic intersection area of ITO. Pattern of Bezene distribution in Delhi is depicted in Figure 2.13.

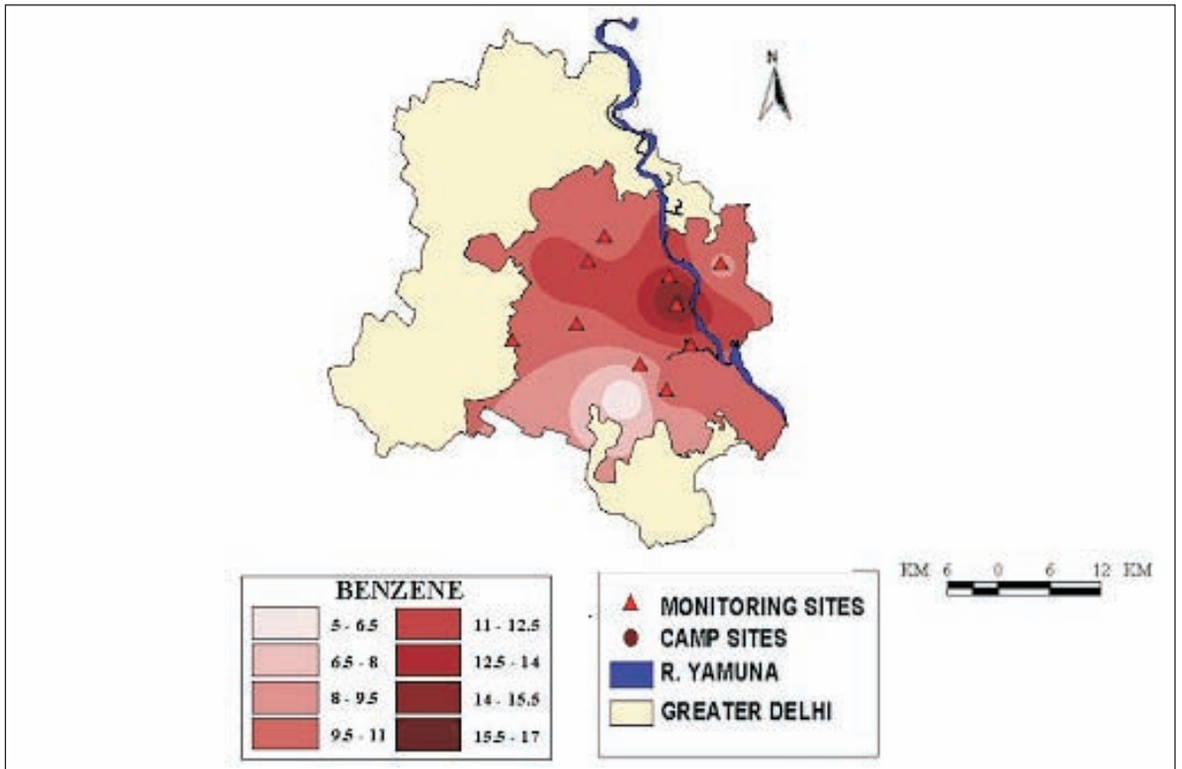


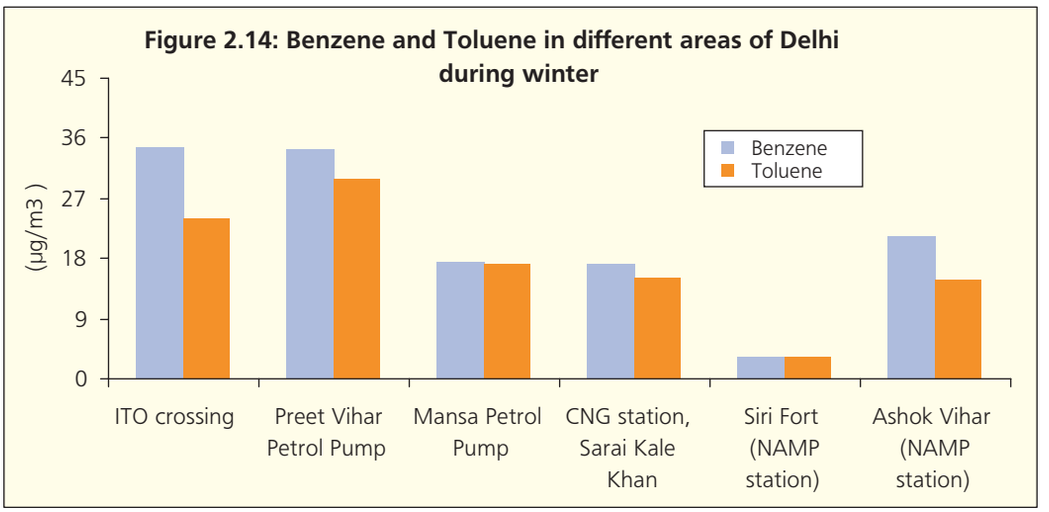
Figure 2.13: Pattern of Benzene Distribution in Delhi

(ii) Toluene

Benzene and Toluene levels in Delhi are presented in Table 2.10. Toluene level was relatively low in residential areas, and higher in traffic intersection area of ITO, and petrol/CNG refueling stations of the city. The petrol pump and CNG refueling stations had benzene: toluene ratio of 1.01-1.15. In contrast, roadside measurements at ITO showed a higher ratio of 1.44. Benzene and Toluene levels in different areas of Delhi are presented in Figure 2.14.

Table 2.10: Volatile organic compound in ambient air in Delhi during winter (Feb 3-10, 2005)

Location	Benzene ($\mu g/m^3$)	Toluene ($\mu g/m^3$)	Benzene: Toluene
Residential areas			
Siri Fort (NAMP station)	3.39	3.13	1.08
Ashok Vihar (NAMP station)	21.44	14.82	1.45
Petrol Pump			
Preet Vihar Petrol Pump	34.38	29.81	1.15
Mansa Petrol Pump	17.40	17.21	1.01
CNG refueling station			
Sarai Kale Khan	17.15	15.15	1.13
Traffic intersection area			
ITO crossing	34.57	24.02	1.44



(g) Meteorological Data

Meteorological data of Delhi such as temperature, relative humidity, wind speed, wind direction and visibility during the study period was obtained from Indian Meteorological Department at Mausam Bhawan, Lodhi Road, New Delhi and the website wunderground.com and the meteorological data are presented in Table 2.11. For the rural areas of West Bengal data supplied by the regional Meteorological office at Alipore, Kolkata was used.

Table 2.11: Meteorological data of Delhi during 2004-2005.

	Summer (Mar-Jun)	Monsoon (Jul-Oct)	Winter (Nov-Feb)
Temperature (^{0}C)			
Minimum	17.5	22.2	9.7
Maximum	42.6	36.5	26.0
Average	31.4	28.2	18.3
Relative humidity (%)			
Minimum	14.2	47.6	29.0
Maximum	72.9	94.8	97.0
Average	38.7	63.6	64.2
Wind speed (km/h)			
Maximum	29.3	31.5	38.3
Average	5.9	4.6	3.4
Wind direction	WNW / W / WSW	WSW /ENE/ ESE	WSW / ENE
Visibility (km)	3.1	3.3	2.1

Source: wunderground.com

(h) Air Quality of Control Areas

The mean (SD) concentrations of SPM and PM_{10} in air of the control areas of West Bengal were 179.8±24.7 and 82.5±14.2 µg/m^3 respectively. Mean $PM_{2.5}$ concentration of these areas was 45.8±5.9 µg/m^3. Concentrations of SO_2 and NO_2 in these areas during the study period were 5.6± 2.2 and 30.3±5.2 µg/m^3 respectively.

Comparison of the air quality of residential areas of Delhi with that of control areas during 2002-2005 revealed significantly higher levels of all the pollutants in residential areas as compared to control areas. Concentrations of SO_2 and NO_2 were within standards in Delhi as well as in control areas, but Delhi had a substantially higher level as compared with control.

2.3 SUMMARY

1. Mean concentration of total suspended particular matter (SPM) in Delhi's air during 2002-2005 was 370 µg/m^3 in residential areas, 396 µg/m^3 in industrial areas, and 514 µg/m^3 in traffic intersection point at ITO.
2. Mean concentrations of the respirable suspended particulate matter (RSPM, particulate matter with less than 10 µm diameter, PM_{10}) during this period were 142, 165, and 250 µg/m^3 in residential, industrial, and traffic intersection point respectively.
3. Mean concentrations of sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) in Delhi's air during 2002-2005 were 10 and 47 µg/m^3 respectively. In the control areas the concentrations of SO_2 and NO_2

were 5.6 and 30.3 µg/m^3 respectively. The levels of these two pollutants were within the Standard in Delhi as well as in control areas.

4. A small decline in the concentrations of SPM and RSPM in ambient air has been recorded in residential areas of Delhi during 2002-05.
5. The average concentration of benzo(a)pyrene in ambient air of Delhi was 3.70 ng/m^3 during December 2004 and January 2005. The concentration was highest at ITO (7.31 ng/m^3).
6. Benzene levels were 7.8 µg/m^3 in residential areas of Delhi in 2004. Highest concentration was found in traffic intersection point at ITO.

CHAPTER-3.0

QUESTIONNAIRE SURVEY FOR PREVALENCE OF RESPIRATORY SYMPTOMS

3.1 INTRODUCTION

Chronic exposures to outdoor and indoor air pollution have been implicated to several respiratory ailments. Diseases of the lungs and the airways are often manifested by one or more symptoms that can be easily recognized. Thus, the presence of a particular symptom or a group of symptoms helps in identifying the presence of an underlying disease in respiratory organs. This has been utilized by several epidemiological studies in which the prevalence of respiratory symptoms has been assessed in order to get an insight into the occurrence of a disease in the airways and the lungs. In this study the prevalence of respiratory symptoms were assessed in urban and rural subjects through questionnaire survey. Questionnaires were used because they are the most commonly used subjective instruments of measurement in respiratory epidemiology. Questionnaires represent a convenient tool for investigating large sample population due to low cost, easy to use by the investigator, and good compliance of the participants (Liard and Neukirch, 2000).

3.2 MATERIALS AND METHODS

(a) Organization of health camps

The study was conducted in differents parts of Delhi and rural West Bengal simultaneously between November 2002 and August 2005. The sample areas were divided into different homogeneous strata based on air pollution level. Then sampling was done from each stratum randomly. Thus stratified random sampling procedure was followed under the general plan of Simple Random Sampling Without Replacement method. Altogether 7,051 people aged between 21 and 67 years participated in this study. To enrol the participants health camps were organized at different localities. For this different organizations such as local panchayat, cultural clubs, housing societies, trade unions, office and institutional authorities, and traders' associations and others were approached beforehand. Researchers discussed with them the objective and plan of work. Almost in all cases, these organizations informed and invited local people to attend a makeshift health camp held usually in a roadside corner, market place, basement of an office or in a club room from early morning till evening. The people's response to this investigation was so overwhelming that in several places in Delhi the camps had to be continued till 10.00 PM in the face of popular demand (Fig. 3.1, 3.2).

Figure 3.1 Health check-up camp in progress at Central Pollution Control Board, East Arjun Nagar, Delhi during the first phase of the work

Figure 3.2 Health check-up camp at offices of CSIR, Pusa Road, New Delhi

(b) Inclusion and exclusion criteria

Citizens of Delhi who were residing in the city for the last ten years or more without interruptions were included. Only apparently healthy individuals were included in this study. Subjects under treatment for tuberculosis, cancer, and serious heart, lung or kidney ailments were excluded. Also, pregnant and lactating women were not included as these conditions might modify some of the measured parameters.

Participants in Delhi

A total number of 6005 adults who were residents of Delhi for the past 10 years or more volunteered to participate in this study. Among the participants, 4467 (74.4%) were men and 1538 (25.6%) were women. The age of the participants was between 21 and 66 years (Figure 3.3).

Figure 3.3 Health check-up camp organized at Shahzada Bagh in west Delhi

Health camps were organized at the following localities of Delhi and the number of subjects indicated in the Table 3.1 volunteered to participate.

Table 3.1: Number of participants in Delhi

Area	Sampling month and year	No. of participants
EAST DELHI		
East Arjun Nagar	11/ 02, 2/03, 4/03, 3/04, 12/04	749
Shahdara	12/04	147
Gandhi Nagar	12/04	169
Vasundhara Enclave	2/03	35
Total, East		**1100**
CENTRAL DELHI		
ITO	7/04, 9/04,3/04	346
Ajmeri Gate	4/03, 9/03, 7/04	351
Nizamuddin	3/04	173
Hailey Road	9/03	121
Old Rajinder Nagar	9/04	164
New Rajinder Nagar	11/02	191
Pusa Road	2/03, 8/05	106
Karol Bagh	7/04, 8/05	332
Hari Nagar	7/04	125
Total, Central		**1909**
NORTH DELHI		
Chandni Chowk	12/04	120
Civil Lines	7/04	67
Darya Ganj	8/05	24
Kamla Nagar	12/04	62
Kalyan Vihar	12/04	15
Ashok Vihar	3/04, 8/05	72
Rohini	12/04	72
Shalimar Bagh	8/05	58
Sangam Park Extension	9/03	34
Shahzada Bagh	7/04, 12/04	269
Total, North		**793**
WEST DELHI		
Paschim Vihar	9/03	70
Janak Puri	3/04, 9/04, 8/05	240
Virendra Nagar	9/04	75
Tilak Nagar	9/04	77
Jaidev Park	9/04	49
Kangan Heri	9/03	81
Inder Puri	9/04	173
Total, West		**765**
SOUTH DELHI		
R.K.Puram	3/04, 8/05	201
Sarojini Nagar	9/03	38

Area	Sampling month and year	No. of participants
Vasant Place	7/04	175
Safdarjung Enclave	7/04, 12/04	129
Lajpat Nagar	3/04, 7/04	160
Green Park	7/04	165
Yusuf Sarai	3/04	113
Tughlakabad Ind. Area	11/02	80
Nehru Place	2/03, 9/03, 3/04, 8/05	207
Lodhi Road	7/04	170
Total, South		**1438**

Control group

As control, 1046 apparently healthy subjects from the rural areas of North and South 24-Parganas, Hooghly, Nadia, West and East Medinipur districts of West Bengal were enrolled (Table 3.2). Within the control group, 775 (74.1%) were men and 271 (25.9%) were women. The age of the participants varied between 21 and 67 years (Fig. 3.4). Health camps were organized in the following control areas:

Table 3.2: List of participants in the control areas

Area	District	Sampling month/ year	No. of participants
Taki	24-Parganas (N)	7/03, 12/04	143
Ganga Sagar Island	24-Parganas (S)	4/04, 11/04	169
Gayeshpur	Nadia	2/03, 5/03	148
Dhaniakhali	Hoogly	5/04, 12/04	116
Sabang	East Medinipur	8/04, 6/05	107
Kharaberia	West Medinipur	2/05, 9/05	165
Panskura	East Medinipur	1/04, 7/05	198
Total			**1046**

Figure 3.4: Collection of sputum samles of rural subjects at Taki, Indo-Bangladesh border, North24 Parganas, West Bengal

(c) Questionnaire survey protocol

Respiratory health of the subjects was assessed through questionnaire survey. For this a modified, structured respiratory questionnaire was developed based on the respiratory questionnaire of British Medical Research Council (BMRC, Cotes, 1987), the American Thoracic Society (ATS) and National Heart and Lung Institute (NHLI) Division of Lung Diseases (DLD) questionnaire (ATS-DLD-78-C; Ferris, 1978), and Compendium of Respiratory Standard Questionnaires (CORSQ) for adults. The questions formulated to elicit information about respiratory symptoms and general health covering the last 12-month period of life of the subject. The 2-page questionnaires were prepared in English, Hindi, (for Delhi) and Bengali (for control group) were administered to the participants. The researchers described each question to the participants clearly so that they can comprehend and answer them properly. For illiterate and poorly educated participants, the researchers filled up the questionnaires on their behalf. The project personnel collected the filled up questionnaires from the participants and scrutinized whether every question has been attended to. Incompletely answered questionnaires were returned to the participants for completion. Thereafter the filled up questionnaires were collected and brought back to the laboratory for entry into computer programs for statistical analysis and interpretation.

Information sought through questionnaire:

- Prevalence of upper respiratory symptoms like sinusitis, rhinitis (runny or stuffy nose), common cold and fever and sore throat in past three months and in the preceding one year
- Prevalence of lower respiratory symptoms like wet or dry cough, wheeze, heaviness in chest or chest pain, breathlessness in the preceding three months and one year
- Prevalence of symptoms related to carbon monoxide exposure like headache, dizziness and eye irritation
- Prevalence of current asthma through history of dyspnea attacks associated with wheezy breathing at any time in the last twelve months (Golshan et al., 2002), and prevalence of medically diagnosed asthma from subjects answer to the questions "Has a doctor ever told you that you had asthma?" And " have you ever been treated by a medical practitioner for asthma?"
- Information about subject's residential proximity to main road, type of cooking fuel use at home, the amount of time spent outside, and overall activity and behavior
- Other variables taken into account were age, sex, smoking habit, height and weight, calculated body mass index in kg/m^2
- Passive smoking was defined by number of smokers at home

Establishment of socio-economic status

Socio-economic status (SES) was ascertained following the procedure of Srivastava (1978) and Tiwari et al., (2005) by scoring 0 to 10 of seven indicators: house, material possession (household gadgets, conveyance etc.), education (score 0 for illiterate, 10 for Ph.D., M.D, M.E. etc.), occupation (0 for no gainful employment, and 10 for Class I or equivalent jobs) monthly income (per capita income of the family Rs.500/- and below got a score of 2, and >15,000 in urban and >10,000 in rural got a core of 10), land/house cost (0 for no land/house, 10 for costing >50 lakh), social participation and understanding. Scores of seven profiles were added and classified into 3 categories of SES-low, medium and high.

(d) Statistical analysis

The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software, and expressed as mean± standard deviation. Generalized estimating equations (GEE) more specifically generalized linear model (GLM) were used to examine the relationship between respiratory symptoms and possible confounders like RSPM levels.

For these model-based methods, data were stratified according to age, sex, region, SES, RSPM levels etc, adjusting for continuous as well as discrete variables. After screening for outliers that might lead to misleading results, bivariate correlation for measuring the association between particulate exposure and outcome (e.g. respiratory symptoms, lung function impairment) was done following Spearman's correlation (rho) test. For categorical variables Chi-square test was done.

When the number of degrees of freedom was close to the total degrees of freedom available, conditional logistic regression model was followed. The step-up conditional logistic regression model was developed using variables that were significantly associated with the outcome variable (for example; presence of a respiratory symptom) in univariate analysis. Moreover, odds ratio and 95% confidence interval as calculated from logistic regression models were used to determine the degree of association between exposure and outcome. $p<0.05$ was considered as significant.

(e) Ethical clearence

The Institutional Ethics Committee of Chittaranjan National Cancer Institute (CNCI), Kolkata approved the study protocol.

3.3 RESULTS

3.3.1 Demographic characteristic

Demographic characteristics of control subjects (n=1046) and the residents of Delhi who took part in this study (n=6005) are compared and presented in Table 3.3. It is evident that both groups were comparable with respect to age, sex, BMI, smoking and food habit. But they differed with respect to tobacco chewing, alcohol use, education, fuel use at home and income.

Table 3.3: Demographic characteristics of the participants

Characteristics	Control (n=1046)	Delhi (n=6005)	p-value
Median age in year (range)	37(19-67)	35 (19-66)	NS
Gender (%): Male	74.1	74.4	NS
Female	25.9	25.6	NS
Median BMI in kg/m^2	20.9	23.4	NS
Tobacco smoking/chewing habit (%)			
Current smoker	25.0	23.6	NS
EX-smoker	0.8	0.7	NS
Never smoker	74.2	75.7	NS
Chewer	6.9	11.3	<0.05
Regular use of alcohol (%)	1.2	4.2	<0.05
Level of education (% individuals)			
Up to 5 years' of schooling	30.5	32.6	NS
10 years' of schooling	32.6	15.6	<0.05
Graduate	27.5	36.2	<0.05
Postgraduate	5.6	4.1	NS
Professional	3.7	11.5	<0.05

Characteristics	Control (n=1046)	Delhi (n=6005)	p-value
Religion (%)			
Hindu	88.6	87.8	NS
Muslim, Sikh, Jain and others	11.4	12.2	NS
Food habit (%)			
Vegetarian	4.7	6.4	NS
Mixed	95.3	93.6	NS
Cooking fuel use at home (%)			NS
LPG	92.8	99.4	<0.05
Kerosene	0.5	0.4	NS
Biomass	6.6	0.2	<0.05
Average family income/month (Rs.)	5200	9450	<0.05

NS, statistically not significant (p>0.05)

(a) Age distribution of the participants

The age of the participants varied between 19 and 66 years in Delhi, and between 19 and 67 years in control group. The median age of the participants was 35 years in Delhi, and 37 years in control group. The difference in median age between control and Delhi' participants was not significant (p>0.05 in Chi-square test).

(b) Sex

There were 4467 men and 1538 women among the participants in Delhi. In the control group 775 were men and 271 were women. The male: female ratio was 2.90 in Delhi and 2.85 in control.

(c) Occupation

The occupation of the participants of Delhi is listed in Table 3.4. It is evident that 31.2% were office employees, 12.1% were traders and businessman, 8.8% were students and unemployed persons, and 8.65 were housewives.

Table 3.4: Occupation-wise distribution of participants in Delhi

Area	Number of participants	% of total
Office employee	1873	31.2
Teacher	294	4.9
Student, unemployed	529	8.8
Housewife	518	8.6
Police personnel	172	2.9
Autorickshaw driver	318	5.3
Taxi, car driver	101	1.7
Roadside hawker	265	4.4
Trader, businessman	726	12.1
Factory worker	48	0.8
Laborer	705	11.7
Retired person	167	2.8
Other	289	4.8
Total	**6005**	**100.0**

In the control group, 25.9% (271/1046) were farmers and agricultural laborers, 19.1% were housewives and maids, 17% were teachers, 4.3% were office employees, 10% were students and unemployed, and remaining 23.6 % were artisans engaged in cottage industries like handloom, pottery etc.

(d) Regional distribution of the participants in Delhi

About 32% of the participants of Delhi were from central part of the city, 18.3% from eastern, 12.7% from western, 13.2% from northern, and 23.9% were from southern Delhi (Table 3.5).

Table 3.5: Area-wise distribution of participants in Delhi

Area	Number of participants	% of total
East	1100	18.3
West	765	12.7
North	793	13.2
South	1438	23.9
Central	1909	31.8
Total	**6005**	**100.0**

(e) Seasonal distribution

The study was conducted in all three seasons i.e. summer, monsoon and winter. Out of the total 6005 participants in Delhi, 30% (1798) were examined in winter, 25.2% (1515) in summer and 44.8% (2692) in monsoon. In control, 32.9%, 20.4%, and 46.7% individuals were examined during winter, summer and monsoon respectively (Table 3.6).

Table 3.6: Distribution (%) of participants according to season of examination

Season	Control (n=1046)	Delhi (n=6005)
Summer (March-June)	20.4	25.2
Monsoon (July-October)	46.7	44.8
Winter (November-February)	32.9	30.0

(f) Socio-economic status (SES) of the participants

SES of control and Delhi groups are presented in Table 3.7. About 44% of the participants of Delhi (2659) and 49% of control group (515) were from low SES. Persons belonging to medium and high SES in Delhi were 29.2% and 26.5% respectively in Delhi, and 32.8% and 18% in control.

Table 3.7: Distribution (%) of participants according to socio-economic status

	Low	Medium	High
Control (n= 1046)	49.2	32.8	18.0
Delhi (n=6005)	44.3	29.2	26.5

(g) Habits

(i) Smoking

Among male participants of Delhi, 31.3% (1398/4467) were current smokers, 0.8% (37) were ex-smokers, and 67.9% (3032) were never-smokers. On the other hand, 1.4% of female participants of

Delhi (22/1538) were current smokers, 0.2% (3) were ex-smokers, and 98.4% (1513) were never-smokers. Overall, 1420 participants (23.6%) were current smokers, 40 (0.7%) were ex-smokers and 4545 (75.7%) were never-smokers.

In control, 33.8% (262/775) males were current smokers, 1% (8) were ex-smokers, and 65.2% were non-smokers. None of the control women was current or ex-smoker. Overall, 262 of1046 control subjects (25.0%) were current smokers, 0.8% were ex-smokers, and remaining 776 (74.2%) were never-smokers. Both in Delhi and control group, smoking of bidi was common in persons belonging to low SES, while individuals belonging to medium and high SES preferred cigarettes. Overall, bidi smoking was more common in control group while cigarette smoking was more common in Delhi.

(ii) Tobacco chewing

In Delhi, 3.1% of females (48/1538) and 14.1% of males (630/4467) were habitual tobacco chewers. Overall, 11.3% of Delhiites who participated in this study were tobacco chewers. In contrast, 8.4% of the males and 6.3% of the females were tobacco chewers in the control group, with an overall prevalence of 6.9%. Thus, tobacco-chewing habit was more common in Delhi ($p<0.05$). People belonging to lower and medium SES were more addicted to smokeless tobacco both in Delhi and control groups. Tobacco-containing pan-masala (guthka), khaini, and zarda were the common tobacco products consumed by the urban subjects, while zarda, gurakhu, guthkha, and snuff use was more common in the villages.

(iii) Drinking of alcohol

About 4.2% participants of Delhi (252/6005) were regular alcohol users against 1.2% of controls (13/1046).

3.3.2 Prevalence of respiratory symptoms, in general

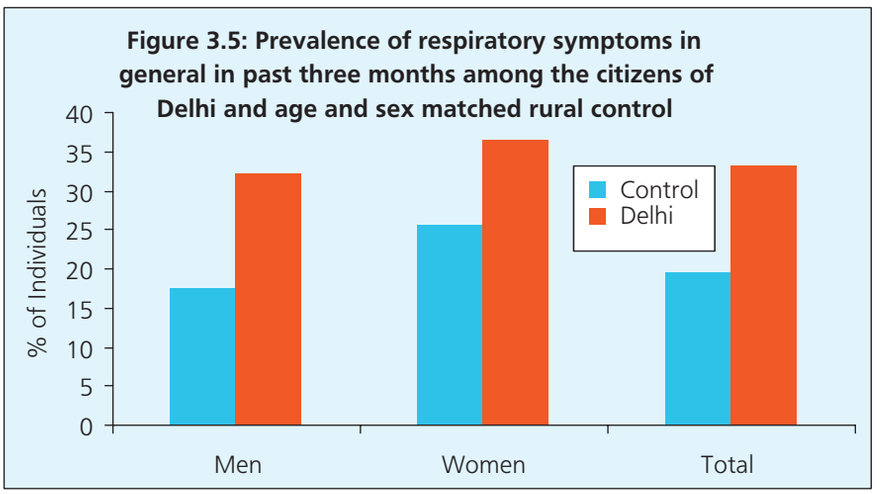
Symptoms more prevalent in Delhi

The prevalence of respiratory symptoms in recent past (preceding 3 months) was recorded through questionnaire survey and personal interview by the researchers. One or more respiratory symptoms were present during this period in 33.2% participants of Delhi and 19.6% of control subjects (Table 3.8, Figure 3.5). Thus, the citizens of Delhi had 1.7- times more prevalence of respiratory symptoms compared with rural controls, and the difference between these two groups with respect to respiratory symptom was highly significant ($p<0.001$), Fig. 3.6.

Table 3.8: Prevalence (%) of respiratory symptoms in general in past three months

	Control (n=1046)	Delhi (n=6005)
Respiratory symptoms, total		
Men	17.5	32.1*
Women	25.5	36.4*
Overall	19.6	33.2*

Many subjects had more than one symptom; $p<0.05$ compared with control in the Chi-square test



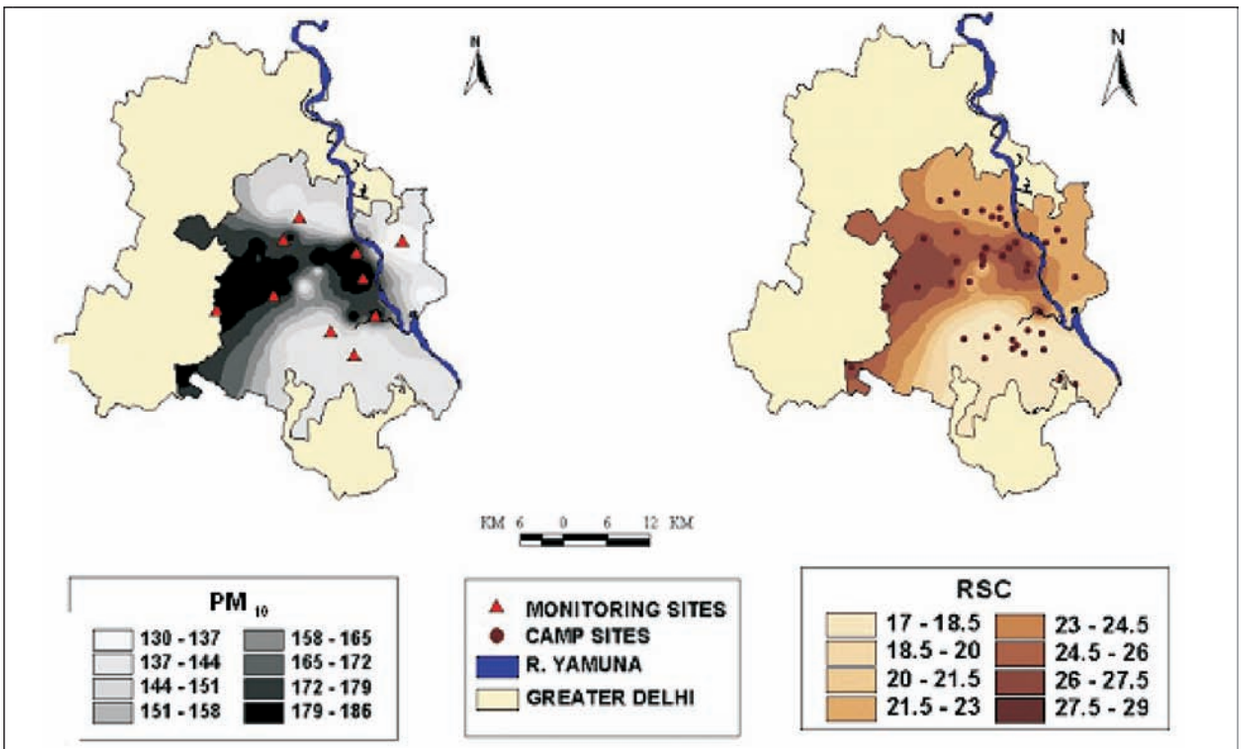


Fig. 3.6: Pattern of MP_{10} distribution and prevalence of respiratory symptom complex among residents of Delhi

Women had more symptoms than men

The prevalence of respiratory symptoms was greater in women than in men. This is true both in rural and urban settings. For example, respiratory symptoms in general were present in 36.4% women of Delhi against 32.1% of city's men. Similarly, 25.5% of women in control group had respiratory symptoms compared with 17.5% of control men (Table 3.8, Figure 3.5). The women: men ratio in symptom prevalence was 1.13 in Delhi and 1.46 in control group. Thus, the gender difference in the prevalence of respiratory symptoms was wider in rural areas. In summary, respiratory symptoms were significantly

more prevalent among the citizens of Delhi compared with the controls as one third of the citizens had respiratory symptoms in the previous three months, and women suffered more than the men in both rural and urban settings.

(a) Prevalence of upper respiratory symptoms (URS)

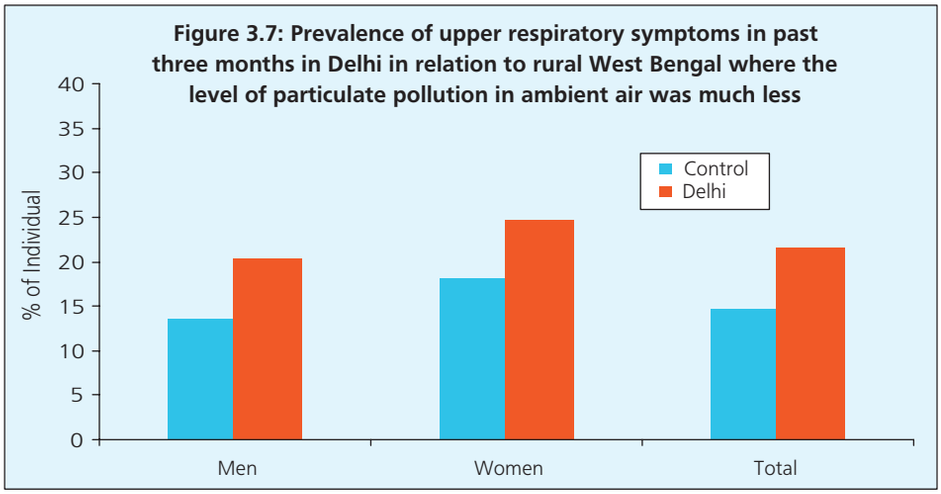
(i) URS more prevalent in Delhi, especially in women

Sinusitis, rhinitis (runny or stuffy nose), sore throat and common cold with fever were the most prevalent upper respiratory symptoms (URS). In general, 21.5% participants from Delhi (1291/6005) had experienced one or more of these URS in the past 3 months. In comparison, 14.7% of control subjects (154/1046) had URS (Table 3.9, Figure 26). The difference in the prevalence of URS between urban and control groups was significant ($p< 0.05$) in Chi-square test. Compared with control, the relative risk (RR) of URS in Delhi was 1.5 with an odds ratio (OR)=1.59, 95% confidence interval (95%CI) 1.32-1.91.

Table 3.9: Prevalence (%) of upper respiratory symptoms (URS) in past three months

Group	Control (n=1046)	Delhi (n=6005)
Men	13.5	20.4*
Women	18.1	24.6*
Overall	14.7	21.5*

**, p<0.05 compared with respective control in Chi-square test*



URS was more prevalent in women than in men. In Delhi, 24.6% women had URS compared with 20.4% of men. Similarly 18.1% women in control group had URS against 13.5% of men, and the gender difference in this regard was significant ($p<0.05$), implying that URS had female predominance (Figure 3.7). Conditional logistic regression analysis revealed that the higher prevalence of URS among Delhi's women compared with their male counterparts was significant (OR=1.28, 95% CI 1.13-1.46).

(ii) Smokers had more URS than non-smokers

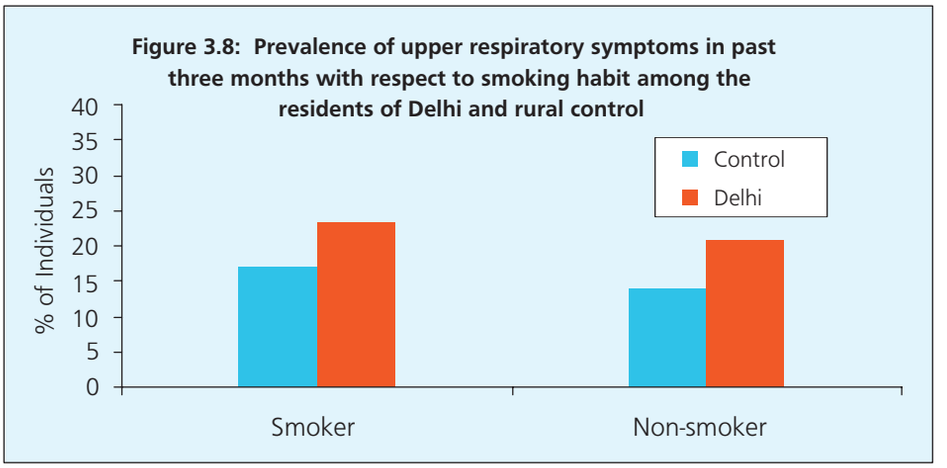
There were 262 and 1420 current smokers in control group and Delhi respectively. URS was more prevalent in current smokers of cigarette and bidi than in never-smokers and past smokers. URS

prevalence was 13.9% in current smokers versus 17.0% in ex- and never-smokers among control subjects. In Delhi, 23.4% current smokers had URS against 20.9% of ex-and never-smokers (Table 3.10, Figure 3.8). Greater prevalence of URS among smokers in Delhi relative to non-smokers was significant in conditional logistic regression (OR=1.34, 95% CI 1.17-1.64).

Table 3.10: Prevalence (%) of URS in past three months with respect to smoking habit

Group	Current smoker	Ex- and never-smoker
Control	17.0	13.9
Delhi	23.4*	20.9*

**, p<0.05 compared with respective controls*



URS was most prevalent among the residents of North Delhi, and least in inhabitants of South Delhi. The regional difference in the prevalence of URS was significant in conditional logistic regression analysis (OR=1.55, 95% CI 1.27-1.89).

Socioeconomic conditions of the people had a significant impact on URS prevalence. The symptoms were most prevalent in subjects belonging to low SES, and least prevalent in high SES (OR= 1.49, 95% CI 1.32-1.87). Similarly, the extent of outdoor exposure was positively associated with symptom prevalence. Individuals with high outdoor exposure had highest prevalence of URS, while URS was least prevalent in people with minimum outdoor activities (OR=1.58, 95%CI 1.31-1.87).

Air pollution, especially particulate pollution, appeared to be the most significant contributing factor to the genesis of URS. A direct positive correlation between the level of RSPM in breathing air and the prevalence of URS among the residents. Considering OR=1 for 50-75 $\mu g/m^3$ of RSPM, Odds Ratio (OR) of 2.69 with 95% CI 1.91-3.79 was observed for RSPM level above 150 $\mu g/m^3$ (Figure 3.9). Thus the risk factors for URS were female gender, smoking habit, low SES, high outdoor exposure and elevated RSPM level in ambient air.

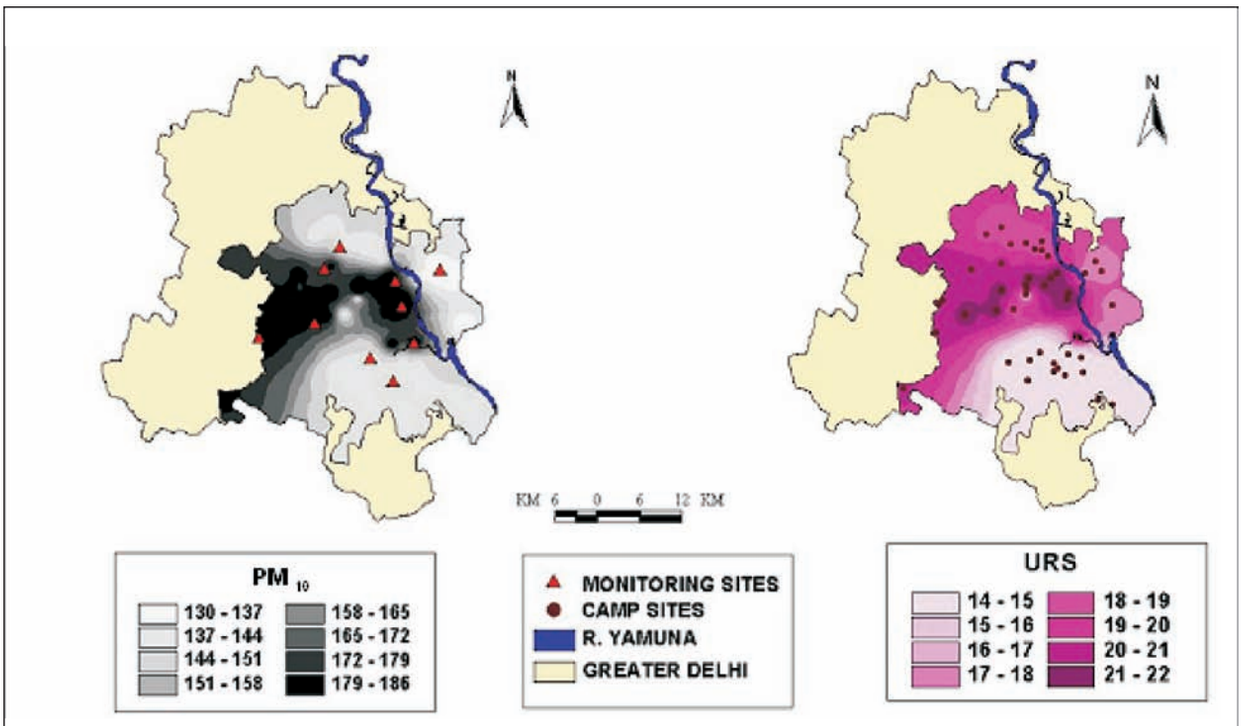


Fig. 3.9: Pattern of MP_{10} distribution and prevalence of upper respiratory symptoms among residents of Delhi

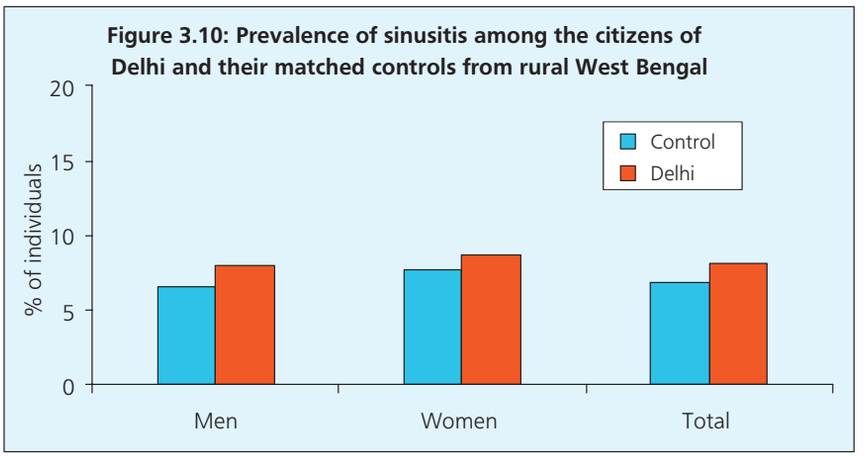
(iii) Prevalence of five individual symptoms under URS

U1. Sinusitis

Sinusitis is inflammation of one or more of the paranasal sinuses. The symptoms are purulent rhinorrhea, anosmia, nasal congestion, facial pain, headache, fever, cough and purulent discharge. Sinusitis can be subdivided into acute (symptoms present for less than 4 weeks), subacute (symptoms for 4-8 wk), chronic (symptoms for 8 wk or longer of varying severity) and recurrent (3 or more episodes of acute sinusitis per year). The diagnosis is based on symptoms, clinical history and physical examination of the subject. Sinusitis was present in 8.2% residents of Delhi compared with 6.9% in control, and the difference was not significant ($p>0.05$: Table 3.11; Figure 3.10). Its prevalence was slightly higher in women than in men both in control and urban groups.

Table 3.11: Prevalence (%) of sinusitis in past three months

Group	Men	Women	Total
Control	6.6	7.7	6.9
Delhi	8.0	8.7	8.2



Sinusitis was most prevalent during monsoon (9.9% and 8.8% in Delhi and control group respectively) and least prevalent during winter both in Delhi and control group (6.7% and 4.4% respectively, Table 3.17). The relative risk of sinusitis among the residents of Delhi was 1.2, OR=1.21 (95%CI 0.93-1.57), suggesting that the rise in the prevalence of sinusitis in urban subjects was not significant when compared with that of rural controls (Table 3.16).

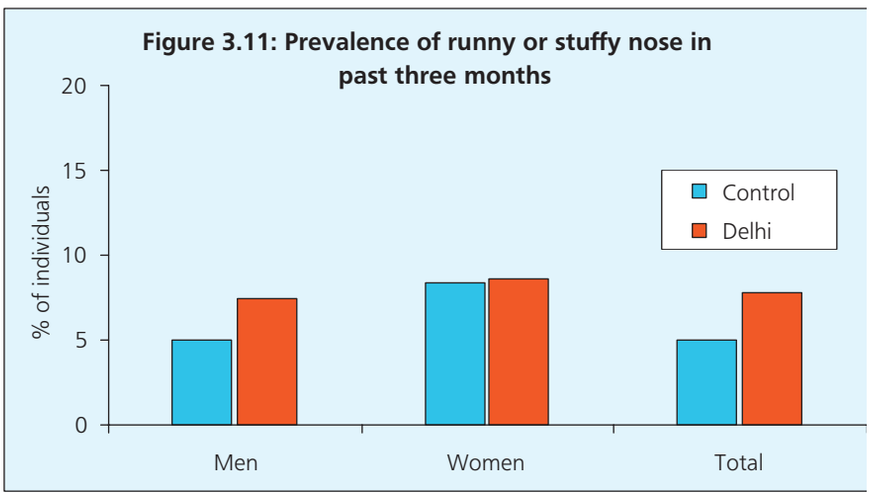
U2. Running or stuffy nose

Running or stuffy nose and sneezing are symptoms associated with rhinitis. In nearly 90% cases, rhinitis is caused by hypersensitivity i.e. allergic reactions to a host of environmental allergens. Frequent presence of the symptom was recorded in 7.8% citizens of Delhi compared with 5.0% in rural control ($p<0.05$, Table 3.12; Figure 3.11). Women suffered more from this problem than men both in Delhi and control, especially in the latter group.

Table 3.12: Prevalence of runny or stuffy nose in past three months

Group	Prevalence (%)		
	Men	Women	Total
Control	5.0	8.4	5.0
Delhi	7.5*	8.6	7.8*

**p<0.05 compared with respective control in Chi-square test*



Running or stuffy nose was more common in winter (9.7%) and monsoon (7.6%) than in summer (5.7%) in Delhi. In controls, highest prevalence was recorded during monsoon (6.1%), followed by summer (4.7%) and winter (3.5%; Table 3.17; Figure 3.15). The rise in the prevalence of running or stuffy nose in Delhi was significant with a RR of 1.6, OR= 1.62 (95% CI 1.19-2.19, Table 3.16).

U3. Sneezing

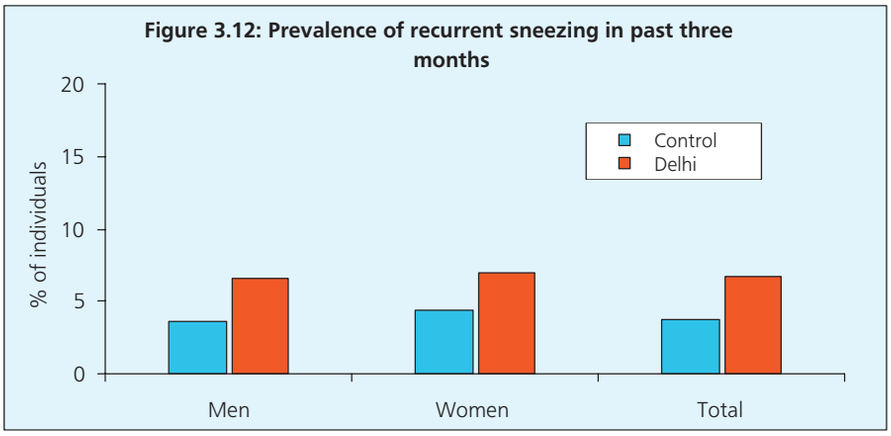
Recurrent sneezing was found in 405 participants of Delhi (6.7%) and 40 in control group (3.8%, Table 3.13). Thus, citizens of Delhi had 1.7-times more prevalence of sneezing than control, and the difference was highly significant ($p<0.01$). Like runny or stuffy nose, sneezing was more prevalent in women than the men both in rural and urban settings. In control group, 4.4% women (12/271) had this symptom compared with 28 (3.6%) men. In Delhi, 108 women (7%) and 297 men (6.6%) had this symptom, but the difference was not significant ($p>0.05$; Figure 3.12).

Prevalence of sneezing was more or less similar in winter and monsoon in Delhi (7.1% and 6.9%) respectively, while a lesser prevalence was recorded during summer months (6.1%). In contrast, sneezing prevalence among control subjects was highest in summer (5.2%) and lowest (3.2%) in winter (Table 3.17, Figure 3.15).

Table 3.13: Prevalence (%) of sneezing

Group	Men	Women	Total
Control	3.6	4.4	3.8
Delhi	6.6*	7.0*	6.7*

**, p<0.05 in Chi-square test compared with respective control group*



The greater prevalence of recurrent sneezing among the residents of Delhi, as compared with that of controls, was significant with a RR of 1.8, OR= 1.77 (95% CI 1.22-2.34, Table 3.16).

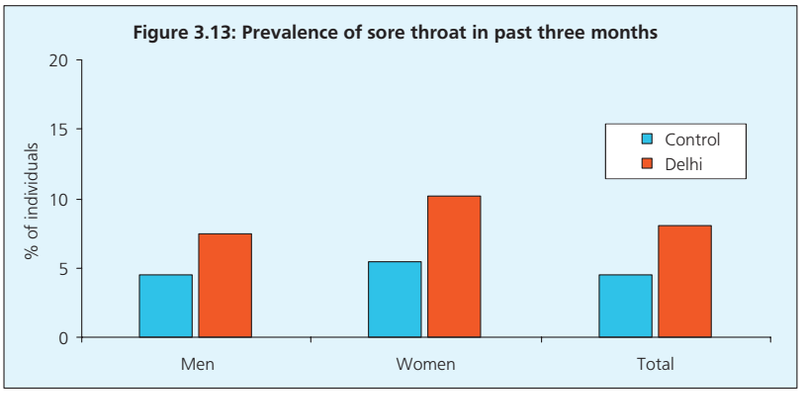
U4. Sore throat

Sore throat was reported by 8.1% of Delhi's residents (487/6005) against 4.5% in controls ($p<0.05$; Table 3.14). The prevalence was slightly higher in women both in Delhi and in control areas (Figure 3.14). The prevalence of sore throat in Delhi was highest during winter (12.4%) when ambient air pollution level was highest. Conversely, lowest prevalence (5%) was recorded during monsoon when the city's air was least polluted. In summer, the prevalence of sore throat was 8.6%. In control group also, highest (6.1%) and lowest (3.3%) and intermediate (5.6%) prevalence levels were recorded during winter, monsoon and summer respectively (Table 3.17, Figure 3.15). The results suggest a close relationship between the prevalence of sore throat and particulate pollution level.

Table 3.14: Prevalence of sore throat among individuals in past three months in Delhi and rural (control) areas of West Bengal

Group	Prevalence (%)		
	Men	Women	Total
Control	4.5	5.5	4.5
Delhi	7.4*	10.2*	8.1*

**p<0.05 compared with respective control in Chi-square test*



The greater prevalence of sore throat among the residents of Delhi was significant with a RR of 1.8, OR=1.88 (95%CI 1.37-2.58, Table 3.16).

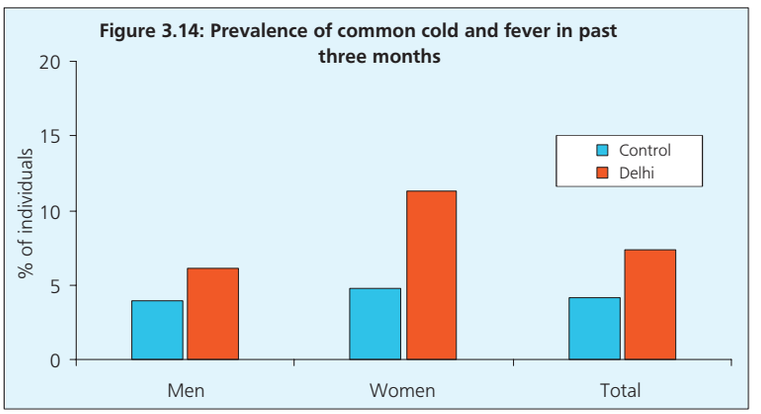
U5. Common cold and fever

Common cold and fever were present in 7.4% citizens of Delhi in past three months. In contrast, 4.1% of control subjects had this symptom during this period (Table 3.15), and the difference in symptom prevalence was significant (p<0.05). The symptom was more prevalent among females than in males in Delhi as well as in control group, particularly the former. In Delhi, 11.3% of the women participants had this symptom against 6.1% of men, and the difference was highly significant (p<0.01; Figure 3.14).

Table 3.15: Prevalence of common cold and fever in past three months

Group	Prevalence (%)		
	Men	Women	Total
Rural	3.9	4.8	4.1
Urban	6.1*	11.3*	7.4*

**p<0.05 compared with rural in Chi-square test*



The prevalence of the symptom was highest during the winter months both in Delhi (10.1%) and control (5.8%), intermediate in summer (7.8% and 4.2%), and lowest during monsoon (5.3% and 2.9% in Delhi and control respectively, Table 3.17, Figure 3.15). Prevalence of common cold and fever was inversely associated with socio-economic conditions.

The greater prevalence of common cold with fever among the residents of Delhi was significant with a RR of 1.8, OR=1.87 (95%CI 1.34-2.61, Table 3.16).

(iv) Summary of the prevalence of URS

Prevalence

Except for sinusitis, prevalence of remaining four symptoms under URS was significantly elevated among the residents of Delhi (Table 3.16). In general, the symptoms were most prevalent during winter when the air pollution level was highest, and lowest in monsoon when the air was least polluted (Figure 3.15). However, sinusitis was most prevalent during monsoon when the level of fungal aeroallergen is relatively high.

Table 3.16: Prevalence (%) of upper respiratory symptoms in past three months

Symptom	Control	Delhi	Relative risk (RR)	OR (95% CI)
Sinusitis	6.9	8.2	1.2	1.21 (0.93-1.57)
Runny or stuffy nose	5.0	7.8	1.6	1.62 (1.19-2.19)*
Sneezing	3.8	6.7	1.8	1.77 (1.22-2.34)*
Sore throat	4.5	8.1	1.8	1.88 (1.37-2.58)*
Common cold & fever	4.1	7.4	1.8	1.87 (1.34-2.61)*
URS, total	14.7	21.5	1.5	1.59 (1.32-1.91)*

*More than one symptom was present in many subjects; *, p<0.05*

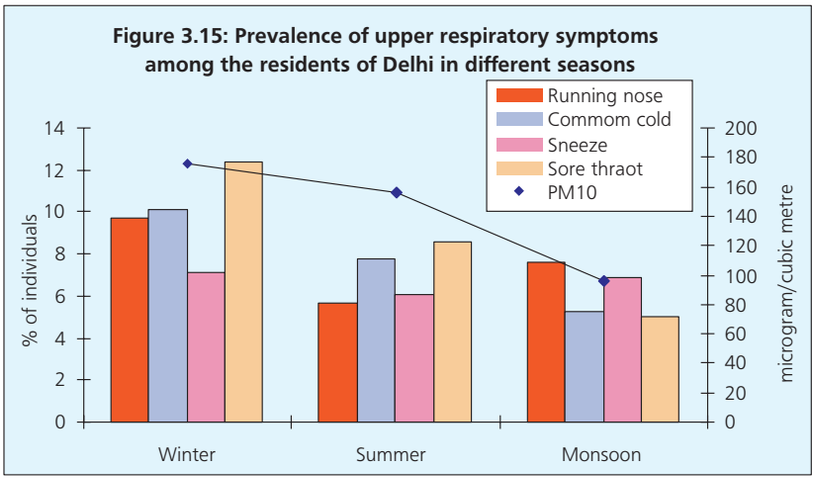
Seasonal variation

It is evident from Table 3.17 that the upper respiratory symptoms except sinusitis were most prevalent in Delhi during winter, followed by summer and monsoon. In case of sneezing, however, the prevalence was more in monsoon than in summer. Similarly sinusitis had highest prevalence during monsoon followed by summer and winter (Figure 3.15).

Table 3.17: Seasonal variation in the prevalence (%) of URS in Delhi

Symptoms	Summer		Monsoon		Winter	
	Control n=213	Delhi n=1515	Control n=489	Delhi n=2692	Control n=344	Delhi n=1798
Sinusitis	6.6	7.0	8.8	9.9	4.4	6.7
Runny/stuffy nose	4.7	5.7	6.1	7.6	3.5	9.7
Sneezing	5.2	6.1	3.7	6.9	3.2	7.1
Sore throat	5.6	8.6	3.3	5.0	6.1	12.4
Common cold	4.2	7.8	2.1	5.3	5.8	10.1

*More than one symptom was present in many subjects; *, p<0.05*



(b) Prevalence of lower respiratory symptoms (LRS)

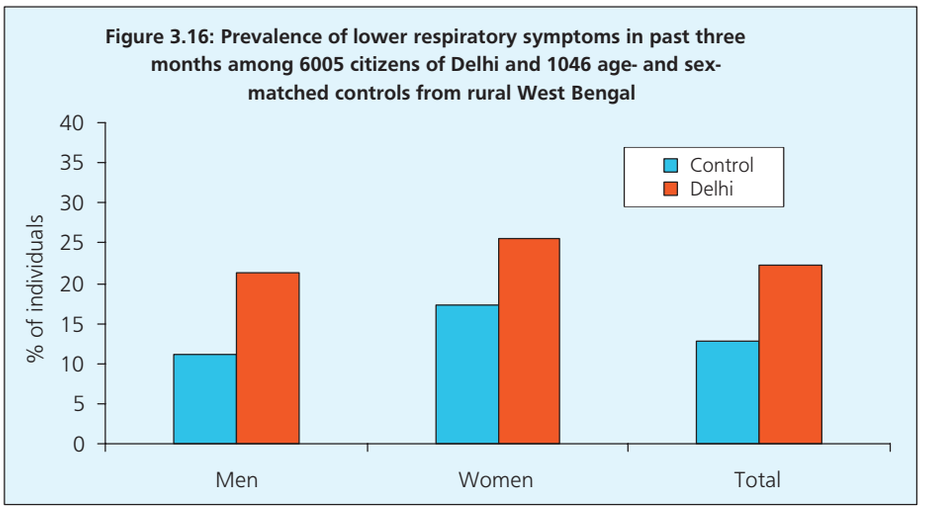
(i) Greater prevalence in Delhi

Recurrent dry cough, cough with phlegm (wet cough), wheeze, breathlessness on exertion and chest discomfort were the major lower respiratory symptoms (LRS) encountered. The prevalence of LRS was significantly higher ($p<0.001$) among the citizens of Delhi in comparison with age- and sex-matched rural controls (Table 3.18, Figure 3.16). For example, 22.3% residents of Delhi (1339 out of 6005) had one or multiple LRS in the past three months in contrast to 12.7% prevalence in controls (133 out of 1046). The relative risk (RR) of LRS among the residents of Delhi was 1.7, with OR=1.67 (95%CI 1.32-1.93) in univariate logistic regression analysis.

Table 3.18: Prevalence (%) of LRS in past three months

Group	Control (n=1046)	Delhi (n=6005)
Men	11.1	21.2*
Women	17.3	25.5*
Overall	12.7	22.3*

**P<0.001 compared with respective control in Chi-square test*



(ii) Women had more LRS than men

Delhi's women had higher prevalence of LRS than their male counterparts (25.5% vs. 21.2%). In control group also, women had higher prevalence of LRS than men (17.1% vs. 11.1%, Table 3.18, Figure 3.16). However, the higher prevalence of LRS among Delhi's women compared with their male counterparts was not significant in conditional logistic regression analysis (OR=1.02, 95% CI 0.90-1.15).

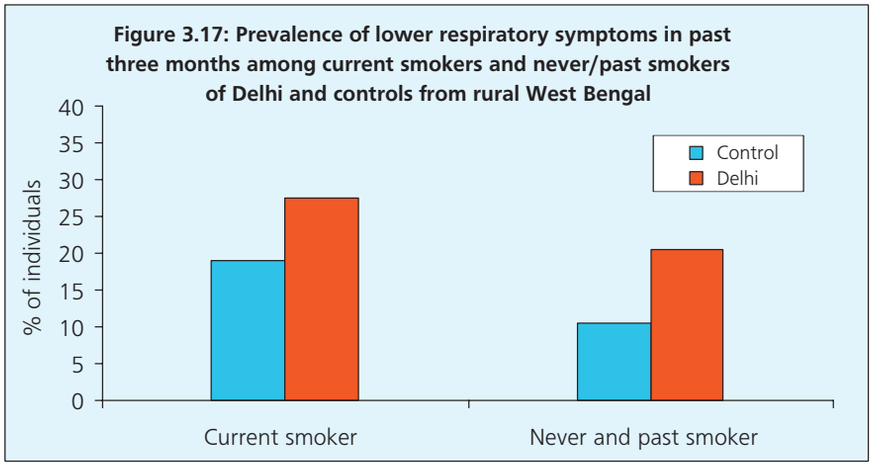
(iii) Smoking and LRS

Smoking was found to be associated with excess LRS, as the symptoms were more prevalent in current smokers than ex- and never-smokers. In Delhi, 402 out of 1420 current smokers (27.6%) had LRS. In contrast, 937 out of 4585 never smokers and past smokers of the city (20.6%) had LRS (Table 3.19, Figure 3.17). The difference in LRS prevalence between these two groups was statistically significant in Chi-square test ($p<0.05$). Similarly in control group, 50 out of 262 current smokers (19.1%) and 83 out of 784 ex- and never-smokers (10.6%) had LRS (Table 3.19, Figure 3.17), and the difference between these groups was highly significant ($p<0.001$). Compared with Delhi, the rise in LRS among rural smokers was remarkable probably because majority of them smoked bidi, which has greater adverse effects on respiratory health than cigarette smoking, a more common practice in Delhi. In essence, smokers had greater prevalence of LRS than non-smokers. More importantly, Delhi's non-smokers had LRS prevalence even greater than the rural smokers.

Table 3.19: Prevalence (%) of LRS in relation to smoking

Group	Control	Delhi
Current smoker	19.1	27.6*
Never smoker and past smoker	10.6	20.6*

** P<0.001 compared with respective control in Chi-square test*



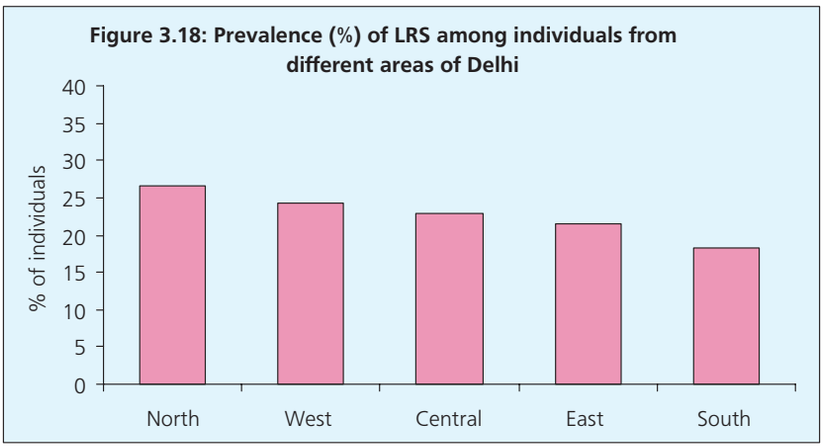
(iv) More symptoms in North Delhi

Air pollution level with respect to particulate pollution was higher in North, Central and West Delhi than East and South Delhi. A similar pattern was found in the prevalence of LRS. The residents of North Delhi had greatest prevalence of LRS (26.7%), followed by residents of West and Central Delhi (24.3% and 23.0%) and least prevalent in inhabitants of South Delhi (18.3%, Table 3.20, Figure 3.18). The difference in LRS prevalence between South and North (OR= 2.04, 95% CI 1.65-2.45), West (OR= 1.46, 95% CI 1.21-1.76), and Central Delhi (OR= 1.43, 95% CI 1.24-1.65) was significant in conditional logistic regression analysis.

Table 3.20: Prevalence (%) of LRS in different areas of Delhi

Symptoms	East (n= 1100)	West (n=765)	North (n=793)	South (n=1438)	Central (n=1909)
LRS	21.6	24.3*	26.7*	18.3	23.0*

** P<0.05 compared with South in Chi-square test*



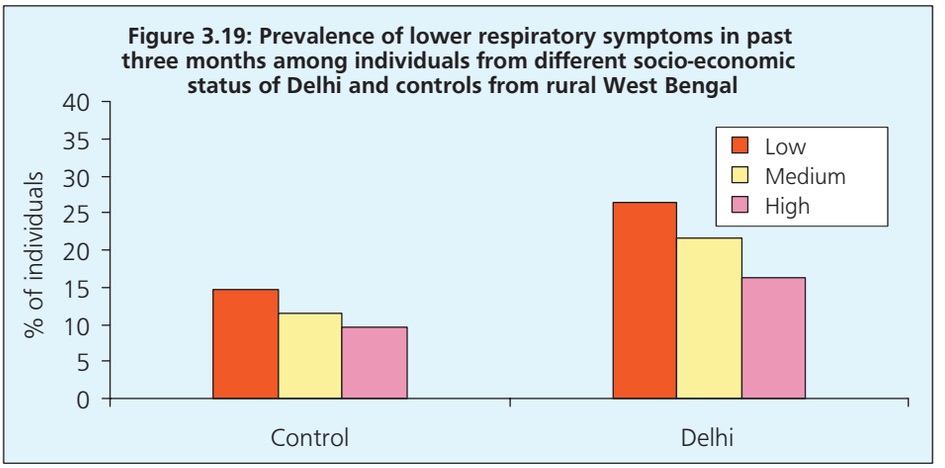
(v) People from low socio-economic status (SES) suffered most

LRS was most prevalent among the weaker section of the society. Both in rural and urban settings, highest prevalence of LRS were found in people belonging to low SES. In Delhi, LRS prevalence in low, medium and high SES was 26.3%, 21.7% and 16.3% respectively. In control group the prevalence was 14.6%, 11.6% and 9.6% in low, medium, and high SES respectively (Table 3.21, Figure 3.19). The impact of SES on LRS prevalence in Delhi was significant in conditional logistic regression analysis (OR= 1.75, 95% CI 1.55-1.98, high vs. low; and OR =1.64 95%CI 1.42-1.90 high vs. medium SES).

Table 3.21: Prevalence (%) of LRS in different socio-economic conditions

SES	Control	Delhi
Low	14.6	26.3*
Medium	11.6	21.7*
High	9.6	16.3*

** P<0.001 compared with respective control in Chi-square test*

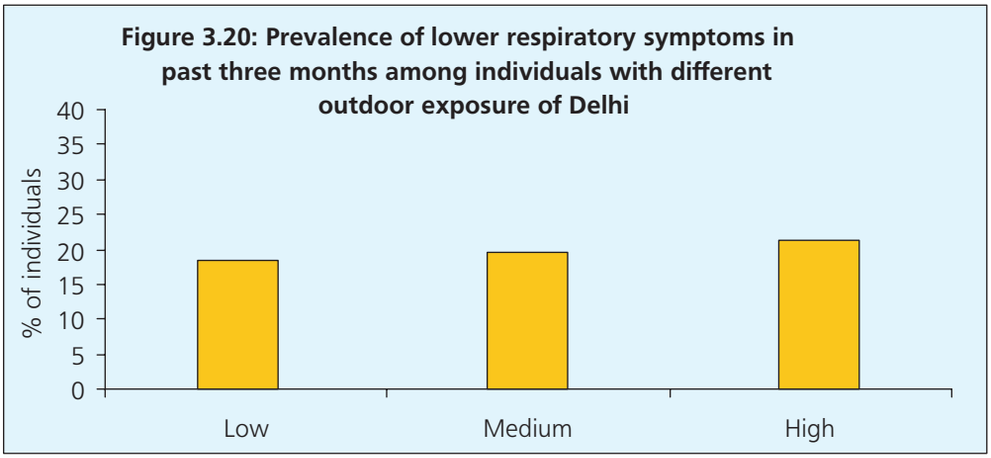


(vi) Individuals with higher outdoor exposure suffered more

In a total number of 4585 past and never smokers of Delhi who had participated in this study, 1155 had low average outdoor exposures (<2 hr/day), 475 had medium exposures (2-4 hr/day) and 2955 had high outdoor exposures (>4 hr/day). We found greater prevalence of LRS in persons with high outdoor exposures, and, conversely, lowest prevalence in persons having low outdoor exposures (Table 3.22, Figure 3.20).

Table 3.22: Prevalence (%) of LRS among non-smokers of Delhi in relation to outdoor exposures

Outdoor exposure	n	Prevalence (%) of LRS
Low (<2 hr/day)	1155	18.3
Medium (2-4 hr/day)	475	19.7
High (>4 hr/day)	2955	21.4



The positive association between outdoor activity and LRS was significant in conditional logistic regression analysis (OR=1.35, 95%CI 1.15-1.68 for high vs. low exposure, and OR=1.24, 95% CI 1.02-1.40 for high vs. medium exposure, p<0.01).

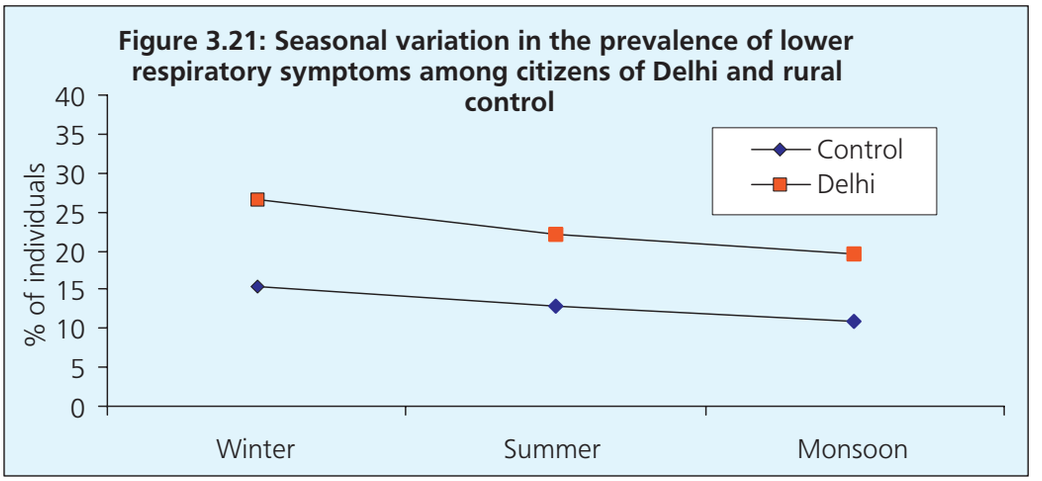
(vii) Greater prevalence during winter

Both in rural and urban settings, LRS was most prevalent during winter months when the pollution level in air was highest. Conversely, lowest prevalence was recorded in monsoon when the air was cleanest. In Delhi, LRS prevalence was 26.6% in winter, 22% in summer and 19.5% in monsoon. In control areas, the prevalence of LRS in winter, summer and monsoon was 15.45, 12.9% and 10.8% respectively (Table 3.23, Figure 3.21). The differences in LRS prevalence in Delhi between winter and monsoon (OR=1.50, 95% CI 1.30-1.73) and between winter and summer (OR=1.28, 95% CI 1.09-1.51) were statistically significant (p<0.01) in conditional regression analysis.

Table 3.23: Prevalence (%) of LRS in different seasons

Season	Control	Delhi
Summer	12.9	22.0*
Monsoon	10.8	19.5*
Winter	15.4	26.6*

** P<0.001 compared with respective control in Chi-square test*



(viii) Positive association between ambient PM_{10} level and LRS prevalence

In multivariate logistic regression analysis, the level of RSPM (PM_{10}) in ambient air was found to be positively associated with the prevalence of LRS even after controlling potential confounders like smoking, SES, outdoor exposure and season (OR=1.42, 95% CI 1.22-1.63). In conditional logistic regression analysis taking LRS prevalence during RSPM level of 50-75 µg/m³ as baseline (OR=1), odds ratio of 2.90 with 95% CI 2.05-4.16 was found when RSPM level in ambient air crosses the level of 150 µg/m³ (Figure 3.22).

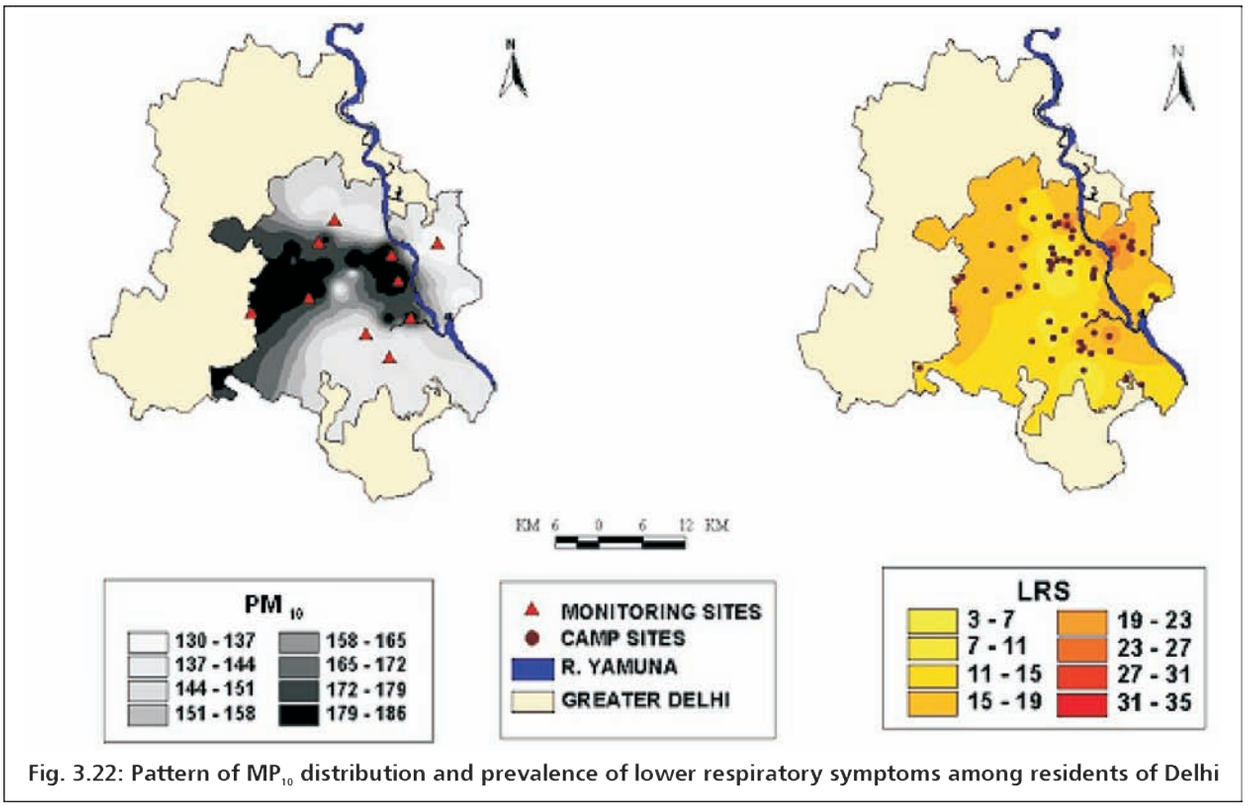


Fig. 3.22: Pattern of MP_{10} distribution and prevalence of lower respiratory symptoms among residents of Delhi

(ix) Prevalence of five individual symptoms under LRS

L1. Dry cough

Chronic dry cough was present in 7.3% adult individuals of Delhi (439/6005). In contrast, 4.2% of control subjects (44/1046; p<0.05, Table 3.24, Figure 3.33) enrolled in this study had this symptom. The relative risk of dry cough among the residents of Delhi was 1.7 with an OR= 1.80 (95% CI 1.29-2.50). The symptom was more prevalent among women than in men in Delhi (8.3% vs. 7%) as well as in control group (5.2% vs. 3.9%), but the gender difference in the prevalence of dry cough was not significant (p>0.05).

Table 3.24: Prevalence (%) of dry cough in past three months

Group	Men	Women	Total
Control	3.9	5.2	4.2
Delhi	7.0*	8.3*	7.3*

**, p<0.05 in Chi-square test compared with respective control group*

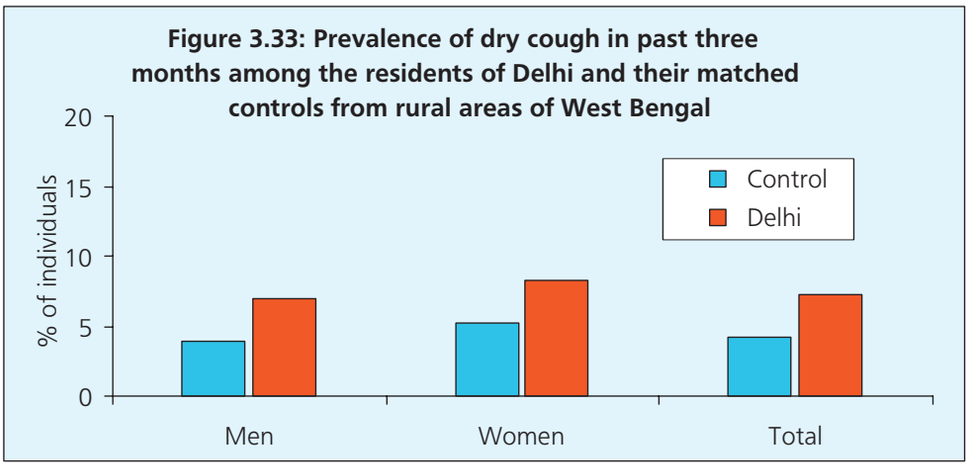


Figure 3.33: Prevalence of dry cough in past three months among the residents of Delhi and their matched controls from rural areas of West Bengal

Smoking was found to be positively associated with dry cough. In Delhi, dry cough was prevalent among 9.8% of current smokers compared with 6.3% of never-smokers and ex-smokers. Likewise, current smokers in control group had greater prevalence than never-smokers and ex-smokers (5% vs. 3.4%).

Highest prevalence of recurrent dry cough (8.7%) was recorded among the residents of North Delhi, and lowest in South Delhi (5.4%), suggesting a positive correlation with particulate air pollution exposure. Residents of West (7.8%) and Central Delhi (8%) had greater prevalence than the city's average, while East Delhi had a marginally lower than average prevalence (7.2%, Table 3.30). Dry cough among the residents of Delhi was inversely related to SES. The prevalence was highest (8.2%) in low and lowest in high SES (5.3%, Table 3.31).

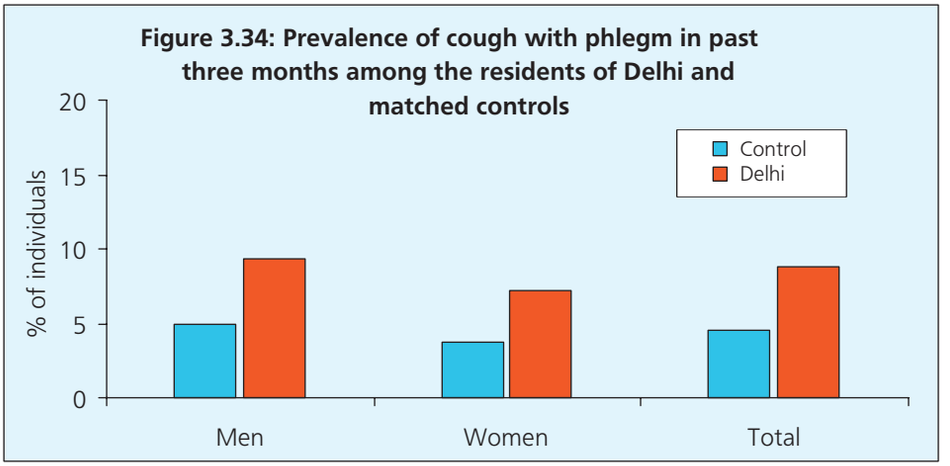
L2. Cough with phlegm

Recurrent cough with phlegm (sputum production) or wet cough was present in 8.8% (528 0f 6005) of Delhi's residents. In contrast, 4.6% of control subjects (48 out of 1046) had this symptom (Table 3.25; Figure 3.34). The relative risk of cough with phlegm among the residents of Delhi was 1.9, OR=2.00 (95% CI 1.47-2.75). Wet cough was more prevalent in men than in women both in Delhi (9.3 vs. 7.2%) and control group (4.9 vs. 3.7%).

Table 3.25: Prevalence (%) of cough with phlegm in past three months

Group	Men	Women	Total
Control	4.9	3.7	4.6
Delhi	9.3*	7.2*	8.8*

**, p<0.05 in Chi-square test compared with respective control group*



The prevalence of wet cough was significantly higher (p<0.05) among current smokers. For example, 12.6% and 6.0% of current smokers in Delhi and control group respectively had chronic wet cough compared with 7.7% and 4.3% never-smokers and ex-smokers of these to groups respectively.

Like dry cough, highest prevalence of wet cough (10.5%) was recorded among the residents of North Delhi, while the prevalence was lowest among residents of South Delhi (7.2%), suggesting once again a positive correlation with particulate pollution. Residents of Central (9.5%) and West Delhi (9.3%) had greater prevalence than the city's average, while East Delhi had lower than average prevalence (8%, Table 3.30).

The symptom was most prevalent in citizens of Delhi with low SES (11.4%), compared with medium (8.3%) and high (5.0%) SES (Table 3.31). The problem was more frequent in winter when the Pm_{10} level was highest compared with monsoon when the RSPM level was lowest (10.6 vs. 7.1%, Table 3.32).

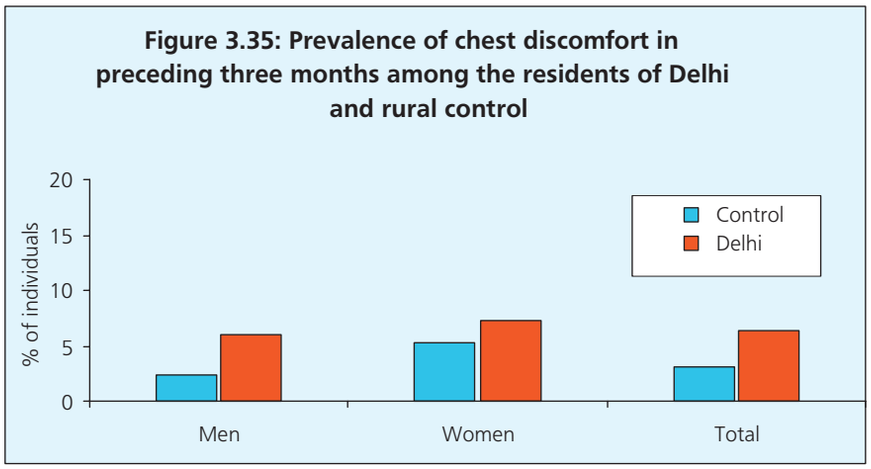
L3. Chest discomfort and pain

Six percent of men and 7.2% of women of Delhi who had participated in this study had frequent chest pain or chest discomfort. Overall, 6.3% citizens of Delhi (378/6005) had this symptom against 3.1% of controls (p<0.01)). In the control group, women had 2-fold greater prevalence than their male counterparts (5.2 vs. 2.3%, p<0.05). Thus, chest discomfort or pain was more prevalent in women than in men both in urban and rural settings (Table 3.26, Figure 3.35).

Table 3.26: Prevalence (%) of chest discomfort in past three months

Group	Men	Women	Total
Control	2.3	5.2	3.1
Delhi	6.0**	7.2*	6.3*

**, p<0.05, **p<0.001 in Chi-square test compared with respective control group*



Current smokers had a higher prevalence of chest discomfort than past and never-smokers (6.7% vs. 5.8% in Delhi and 3.0% vs. 2.0% in control). Chest discomfort was most prevalent among the residents of West and Central Delhi (6.8%), and least prevalent in the South (5.3%), while East and North Delhi had a prevalence of 6.4% and 6.3% respectively (Table 3.30). The prevalence of the symptom was highest (6.8%) in citizens of Delhi from low SES. Citizens from medium and high SES had a prevalence of 5.8% and 5.9% respectively (Table 3.31). The relative risk of chest tightness/discomfort among the residents of Delhi was 2.0, OR=2.13 (95% CI 1.46-3.13, Table 3.29).

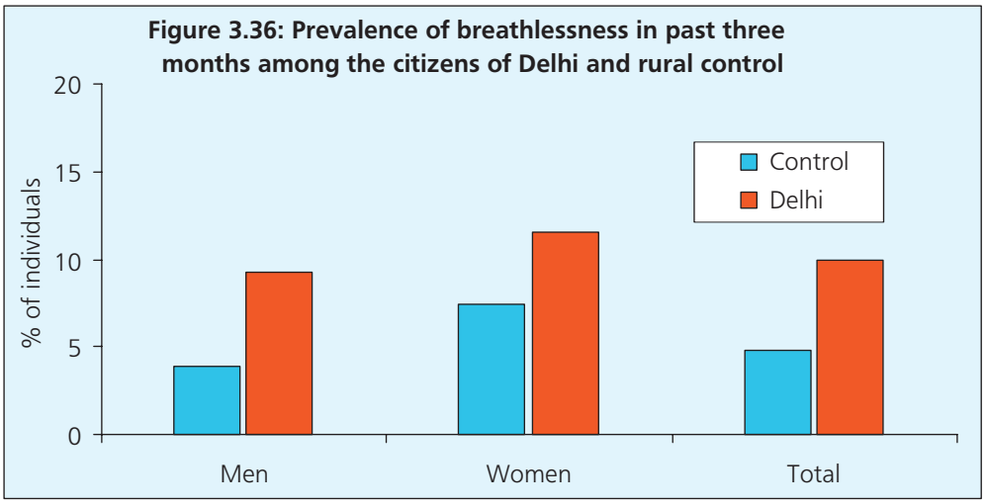
L4. Shortness of breath

Out of breathe or breathless on exertion was reported by nearly 9.9% participants from Delhi (595/6005) compared with 4.8% (50/1046) subjects in control group. Its prevalence was higher in women than in men both in Delhi (11.5% vs. 9.3%) and in control group (7.4% vs. 3.9%, p<0.01), especially the latter (Table 3.27, Figure 3.36).

Table 3.27: Prevalence (%) of breathless on exertion in past three months

Group	Men	Women	Total
Control	3.9	7.4	4.8
Delhi	9.3**	11.5*	9.9*

**, p<0.05, **p<0.001 in Chi-square test compared with respective control group*



Current smokers had higher prevalence than never-smokers and ex-smokers in Delhi (12.5 vs. 8.7%) as well as in control (5.2% vs. 3.2%).

The prevalence of breathlessness was more than city's average in North (11.2%), West (11%), East (10.2%), and Central Delhi (10.1%). As in case of other LRS symptoms, residents of South Delhi had lowest incidents of breathlessness (8.2%, Table 3.30). Likewise, the symptom was more prevalent in low (11%) and medium (9.9%) SES compared with high SES (8.1%, Table 3.31). The relative risk of breathlessness among the residents of Delhi was 2.1, OR=2.19 (95% CI 1.61-2.98, Table 3.29).

L5. Wheezing breath

Wheeze or whistling sound during breathing was present in 396 (6.6%) participants from Delhi compared with 30 subjects in control group (2.9%), and the difference was significant ($p<0.05$). The relative risk of wheezing breath was 2.3, OR= 2.39 (95%CI 1.62-3.55). Prevalence of wheeze was higher in men than in women both in urban and control group. For example, 6.7% of Delhi's men had wheeze compared with 6.2% in women. Similarly, 3% of control males had wheeze in contrast to 2.6% in females (Table 3.28, Figure 3.37). Smoking had marginal influence on wheeze as 7% and 3.3% of current smokers in Delhi and control group respectively had wheeze against 6.7% and 2.8% of ex- and never-smokers.

Table 3.28: Prevalence (%) of wheezing breath in past three months

Group	Men	Women	Total
Control	3.0	2.6	2.9
Delhi	6.7	6.2	6.6*

**, p<0.05 in Chi-square test compared with respective control group*

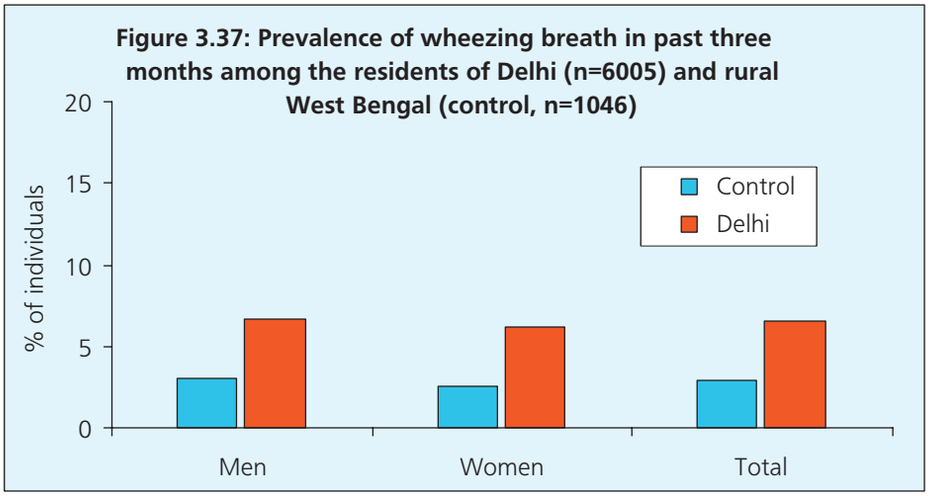


Figure 3.37: Prevalence of wheezing breath in past three months among the residents of Delhi (n=6005) and rural West Bengal (control, n=1046)

The prevalence of wheeze was more or less uniform throughout the city, except for a slightly higher prevalence among the residents of North (7.2%), Central (6.8%) and West Delhi (6.7%, Table 3.30). Incidents of wheeze were more in high and low than in medium SES (6.9%, 6.5% and 5.9% respectively, Table 3.31).

(x) Summary of the prevalence of LRS in Delhi

Prevalence of LRS was significantly elevated among the residents of Delhi. The symptoms were most prevalent during winter when the air pollution level was highest, and lowest in monsoon when the air was least polluted. Excepting wheeze, all the symptoms were most prevalent in low SES and least prevalent in high SES.

Most common LRS among the citizens of Delhi were breathlessness on exertion (9.95 vs. 4.8% in control), sputum-producing cough (8.8% vs. 4.6%) and dry cough (7.3 vs. 4.2%), while highest relative risk (2.3) was present for wheezing breath (Table 3.29).

Table 3.29: Prevalence (%) of lower respiratory symptoms in past 3 months

Symptom	Control (n=1046)	Delhi (n=6005)	Relative Risk	Odds Ratio (95% CI)
Dry cough	4.2	7.3	1.7	1.80 (1.29-2.50)*
Cough with phlegm	4.6	8.8	1.9	2.00 (1.47-2.75)*
Wheeze	2.9	6.6	2.3	2.39 (1.62-3.55)*
Breathlessness	4.8	9.9	2.1	2.19 (1.61-2.98)*
Chest discomfort	3.1	6.3	2.0	2.13 (1.46-3.13)*
LRS, total	**12.7**	**22.3**	**1.7**	**1.97 (1.62-2.40)***

*More than one symptom were present in many subjects; *, p<0.05*

Residents of North, west and Central Delhi suffered more from LRS than did residents of east and particularly South Delhi. The prevalence of all the LRS excepting chest discomfort was highest in North Delhi, while chest pain or chest discomfort was most prevalent in West and Central Delhi (Table 3.30).

Table 3.30: Prevalence (%) of LRS in different areas of Delhi

Symptoms	East (n= 1100)	West (n=765)	North (n=793)	South (n=1438)	Central (n=1909)
Dry cough	7.2	7.8	8.7	5.4	8.0
Cough with phlegm	8.0	9.3	10.5	7.2	9.5
Chest discomfort	6.4	6.8	6.3	5.3	6.8
Breathlessness	10.2	11.0	11.2	8.2	10.1
Wheezing breath	6.2	6.7	7.2	6.2	6.8
LRS	21.6	24.3	26.7	18.3	23.0

More than one symptom were present in many subjects

Poor people belonging low socioeconomic status were most susceptible to LRS. They had highest prevalence of all the symptoms excepting wheeze, which was most prevalent in people belonging to high social and economic class (Table 3.31).

Table 3.31: Prevalence (%) of LRS in different socio economic status in Delhi

Symptoms	Low (n=2660)	Medium (n=1754)	High (n=1591)
Dry cough	8.2	7.6	5.3
Cough with phlegm	11.4	8.3	5.0
Chest discomfort	6.8	5.8	5.9
Breathlessness	11.0	9.9	8.1
Wheezing breath	6.5	5.9	6.9
Total LRS	**16.3**	**21.7**	**26.3**

More than one symptom were present in many subjects

All the symptoms including wheeze were most prevalent during winter when the particulate pollution level in breathing air was highest. Conversely, lowest prevalence of the symptoms was found in monsoon when the air was least polluted (Table 3.32).

Table 3.32: Seasonal variation in the prevalence (%) of LRS in Delhi

Symptoms	Winter (n=1802)	Summer (n=1513)	Monsoon (n=2690)
Dry cough	8.7	8.4	5.6
Cough with phlegm	10.6	9.6	7.1
Chest discomfort	7.9	6.1	5.4
Breathlessness	12.4	9.5	8.5
Wheezing breath	8.9	6.1	5.3
Total LRS	**26.6**	**22.0**	**19.5**

More than one symptom were present in many subjects

(c) Prevalence of bronchial asthma

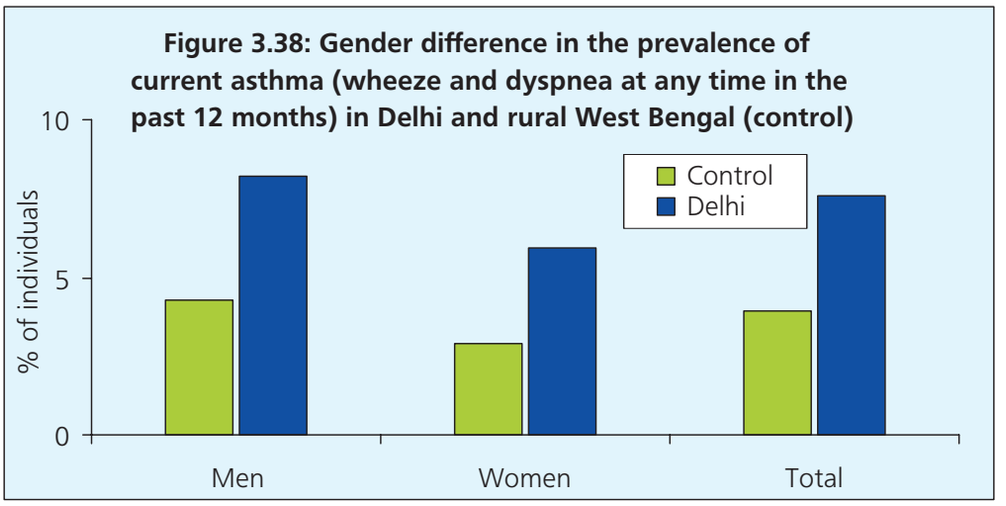
(i) More in Delhi than in rural areas

The prevalence of current asthma (dyspnea and wheeze at any time in the last twelve months) and physician-diagnosed asthma among the participants of Delhi were 7.6% and 3.6 % respectively (Table 3.33, Figure 3.38) which were significantly higher than the corresponding prevalences in control group- 3.9% and 2.1% respectively ($p<0.05$ in Chi-square test).

Table 3.33: Prevalence of bronchial asthma in control subjects and citizens of Delhi

Type of asthma	Control (n=1046)	Delhi (n=6005)	p
Current asthma			
Men	4.3	8.2	<0.05
Women	2.9	5.9	<0.05
Total	**3.9**	**7.6**	**<0.05**
Physician diagnosed asthma			
Men	2.1	3.5	<0.05
Women	2.2	3.9	<0.05
Total	**2.1**	**3.6**	**<0.05**

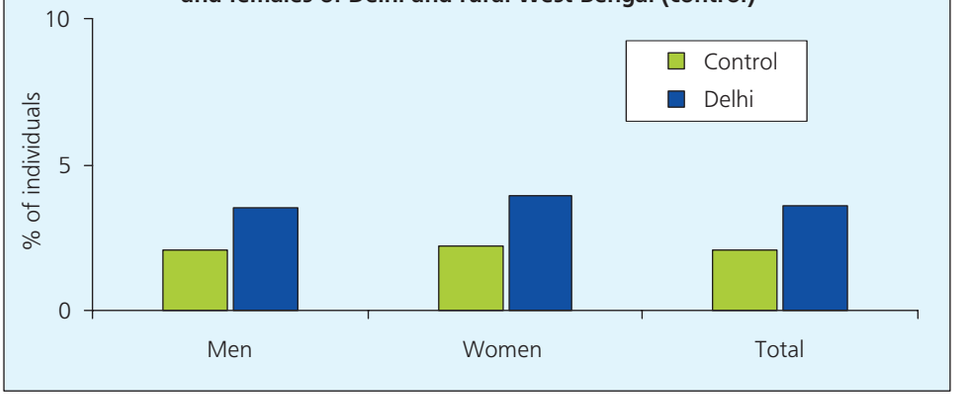
Results are expressed as number of affected persons with the percentage in parentheses



(ii) Current asthma more in men, doctor-diagnosed asthma more in women

Current asthma was more prevalent among men in Delhi (8.2% vs. 5.9%) as well as in rural West Bengal (control; 4.3 vs. 2.9%). However, physician-diagnosed asthma was more prevalent among women. In Delhi, doctor-diagnosed asthma was present in 3.9% of women compared with 3.5% of men, indicating 11% more asthma incidents in women. In the control group, women had a prevalence of 2.2% against 2.1% of men (Table 3.33, Figure 3.39), implying modest 5% excess asthma prevalence in rural women compared with rural men.

Figure 3.39: Prevalence of physician-diagnosed asthma in males and females of Delhi and rural West Bengal (control)



(iii) Smoking and asthma

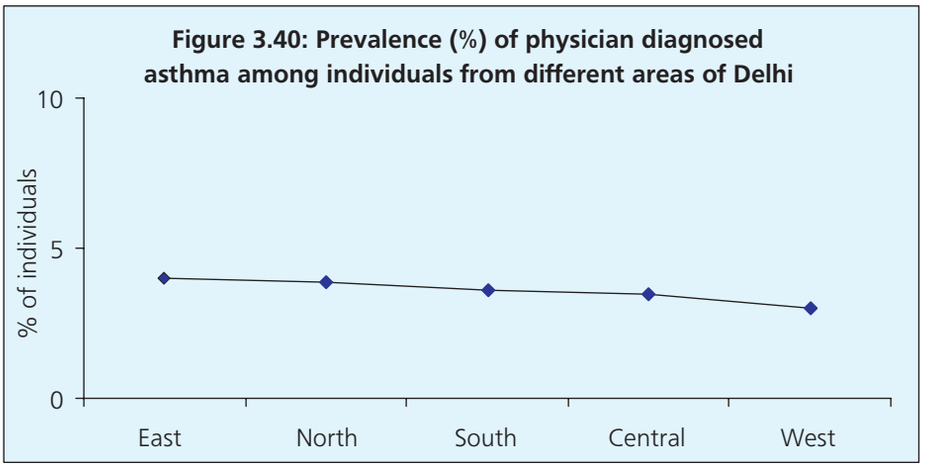
Out of the 1420 current smokers among the participants in Delhi, 53 (3.7%) had doctor-diagnosed asthma. In contrast, 163 of 4585 ex-smokers and never-smokers (3.6%) had asthma. The never-smokers and ex-smokers were clubbed together because there were only a few ex-smokers among the participants (n=40), and the difference in asthma prevalence between ex- and never-smokers was not statistically significant (5.0 vs. 3.5% $p>0.05$). Thus, it seems that smoking did not have significant influence on the prevalence of doctor-diagnosed asthma although it could have exacerbated the disease. Similarly, smoking marginally influenced the prevalence of current asthma. In Delhi, 8% of the smokers had current asthma against 7.5% of ex- and never smokers.

(iv) Prevalence of asthma in different areas of Delhi

The prevalence of doctor diagnosed asthma was more than the Delhi's average among the residents of the East (4.0%) and North (3.9%), it was equal to average in South, and lower than average in West (3.0%) and Central Delhi (3.5%; Table 3.34, Figure 3.40).

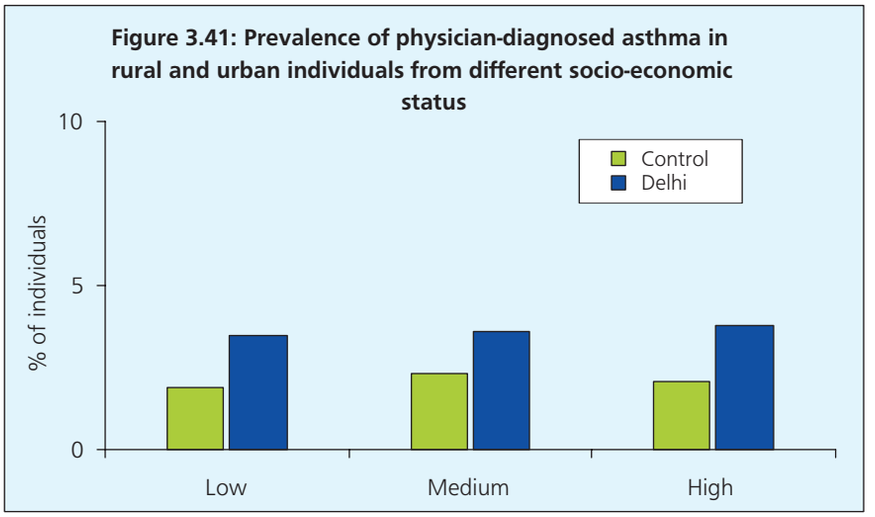
Table 3.34: Prevalence of doctor-diagnosed asthma in different areas of Delhi

Area	n	Persons with doctor-diagnosed asthma	% of total
North	793	31	3.9
South	1438	52	3.6
East	1100	44	4.0
West	765	23	3.0
Central	1909	66	3.5
Total	**6005**	**216**	**3.6**



(v) Asthma and SES

The prevalence of physician-diagnosed asthma in Delhi was more or less similar in people from all socioeconomic conditions, although a modest increase was found in high SES. The prevalence was 3.5%, 3.6% and 3.8% in low, medium and high SES respectively. In controls, subjects from medium SES has slightly higher prevalence (2.3%) than people belonging to low (1.9%) and high (2.1%) SES (Figure 3.41).



(d) Prevalence of other symptoms

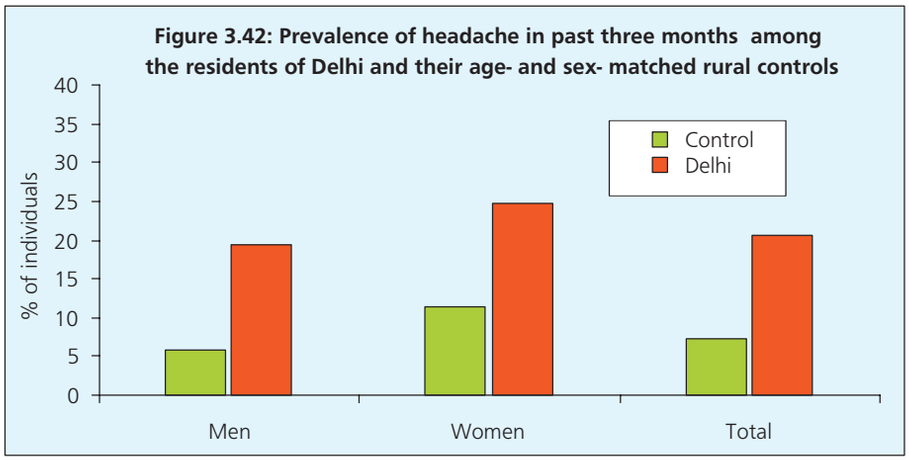
(i) Headache

Recurrent headache in past here months was present in 20.7% participants of Delhi. In contrast, only 7.3% of control subjects had chronic headache, and the difference was significant ($p<0.001$). Headache was more prevalent in women both in residents of Delhi and control group (Table 3.35, Figure 3.42). In the latter, women had nearly 2-times more prevalence than the men (11.4 vs. 5.8%, $p<0.05$). The prevalence of headache was highest among the residents of North Delhi (26.1%), followed by residents of Central (24.8%), West (21%) and East Delhi (18%). Inhabitants of South Delhi had the lowest prevalence of headache (14.3%). Headache was more prevalent during winter (26.2%) and summer months (22.6%) than in monsoon (16%).

Table 3.35: Prevalence (%) headache in past three months

Group	Men	Women	Total
Control	5.8	11.4	7.3
Delhi	19.3**	24.8*	20.7**

**, p<0.05 and **, p<0.001 in Chi-square test compared with respective control group*



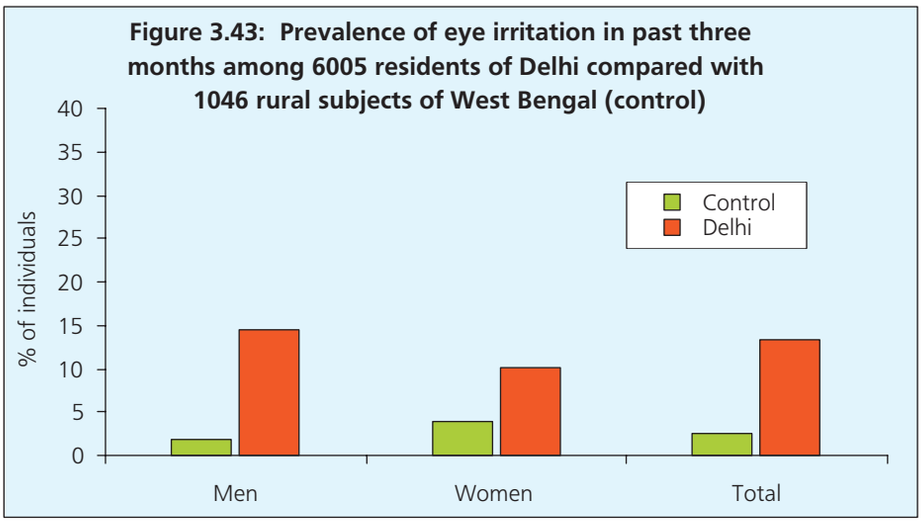
(ii) Eye irritation

Eye irritation including burning, dryness, itching, and eye watering in past three months was reported by 13.4% of the participants in Delhi. In contrast, only 2.5% of the control subjects had eye irritation, and the difference between urban and control group was highly significant (p<0.001). Eye irritation was more prevalent among women in control group (4.0% vs. 1.9%), whereas men had a higher prevalence in Delhi (14.5 vs. 10.1%) (Table 3.36, Figure 3.43).

Table 3.36: Prevalence (%) of eye irritation in past three months

Group	Men	Women	Total
Control	1.9	4.0	2.5
Delhi	14.5**	10.1**	13.4**

***, p<0.001 in Chi-square test compared with respective control group*



The problem was more frequent during winter, as 20.4% of participants in Delhi during this season complained of eye irritation, compared with 15.2% in summer and 7.6% during monsoon. Like headache, eye irritation was more prevalent among the residents of North (16.6%) and Central Delhi (15.8%) than in residents of West (14.4%), East (13.2%), and South Delhi (7.9%).

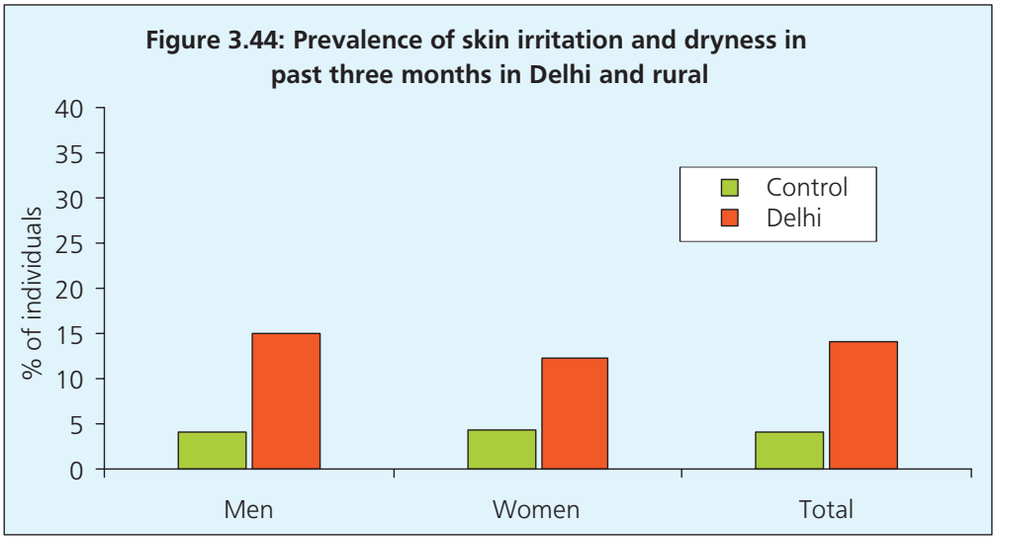
(iii) Skin irritation or dryness

Skin irritation like burning sensation and itching, and dryness was reported by 852 (14.2%) participants of Delhi. In contrast, 4.2% of the control subjects had skin problems, and the difference between urban and control group was highly significant ($p<0.001$). Skin irritation was prevalent in men than in women of Delhi, while control women had a higher prevalence than men (Table 3.37, Figure 3.44). Skin irritation was most prevalent during winter, and least in monsoon. Nearly 19% of Delhi's residents had skin irritation during winter compared with 14.3% in summer and 11.1% in monsoon. The problem was most prevalent among the residents of North Delhi (15.9%), and least among residents of East Delhi (12.0%). Residents of West, South and Central Delhi had 15.0%, 14.7% and 14.0% prevalence of skin irritation respectively.

Table 3.37: Prevalence (%) of skin irritation and dryness in past three months in Delhi and rural West Bengal (control)

Group	Men	Women	Total
Control	4.1	4.4	4.2
Delhi	14.9*	12.2*	14.2*

**, p<0.05 in Chi-square test compared with respective control group*



3.4 FINDINGS

1. The prevalence of respiratory and associated symptoms among the citizens of Delhi who were living in the city for the last ten years or more was investigated in a case-control study conducted during 2002-2005. A total number of 6005 apparently healthy individuals of Delhi (male 4467 and female 1538) and 1046 from rural West Bengal (male 775 and female 271) as control were enrolled. The age of the participants was between 21 and 67 years.
2. Data on respiratory symptoms in past three months were collected through structured questionnaire, personal interview and clinical examination. One or more respiratory symptoms were present in

33.2% residents of Delhi and 19.6% of control subjects, indicating that respiratory symptoms were 1.7-times more prevalent in Delhi.

3. Respiratory symptoms were grossly divided into two: Upper respiratory symptoms (URS), such as sinusitis, runny or stuffy nose, sneezing, sore throat and common cold with fever, and lower respiratory symptoms (LRS), such as chronic dry cough, recurrent sputum-producing cough, wheezing breath, breathlessness on exertion, and chest pain or tightness. URS was present in 21.5% residents of Delhi compared with 14.7% control subjects. Therefore, Delhiites had 1.5-times greater prevalence of URS which was statistically significant ($p<0.05$) in logistic regression analysis after controlling potential confounders like current smoking, environmental tobacco smoke and socio-economic conditions (OR= 1.24, 95% CI 1.08-1.54).
4. Barring sinusitis, the prevalence of all symptoms under URS was significantly higher ($p<0.05$) among the residents of Delhi when compared with age- and sex-matched rural controls. The risk factors associated with URS were female gender, smoking, low SES, winter months, and elevated RSPM level.
5. Like URS, the prevalence of LRS in past three months was 1.8-times higher among the residents of Delhi in comparison with matched controls (22.3 % vs. 12.7%, $p<0.001$). All the individual symptoms under LRS were significantly more prevalent in Delhi. The risk factors for LRS were smoking, low SES, winter months, and elevated RSPM level. After controlling potential confounders, logistic regression analysis showed that RSPM level was positively associated with LRS (OR= 1.66, 95% CI 1.21-2.34).
6. The prevalence of current asthma (dyspnea and wheeze at any time in the last twelve months) was 7.6% in Delhi and 3.9% in controls ($p<0.05$). Physician-diagnosed asthma was recorded in 3.6% individuals of Delhi against 2.1% in controls ($p<0.05$). Thus, asthma was significantly more prevalent among the residents of Delhi. Males had higher prevalence of current asthma both in Delhi (8.2% vs. 5.9%) and in control (4.3% vs. 2.9%), whereas women had a slight edge over men for the prevalence of physician-diagnosed asthma in Delhi (3.9% vs. 3.5%) as well as in control areas (2.2 vs. 2.1 %).
7. Compared with matched controls, residents of Delhi had significantly higher prevalence of headache (20.7 vs. 7.3%), eye irritation (13.4 vs. 2.5%), and skin irritation (14.2 vs. 4.2%).

CHAPTER-4.0

ASSESSMENT OF LUNG FUNCTION BY SPIROMETRY

4.1 INTRODUCTION

Lungs are the primary target organ of all the inhaled pollutants. It is reasonable to assume therefore that the function of this organ could be adversely affected following interactions with air toxics. In view of this, cellular and functional lung activity is generally assessed to get an estimate of the health impact of air pollution.

Lung function could be quantitatively measured using a broad array of tests that measure lung volume, airflow and gas diffusion. The most convenient test procedure is spirometry that measures how well the lung functions in exhaling air. Spirometric maneuvers are used to assess forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), maximum mi-expiratory flow (MMEF), and peak expiratory flow rate (PEFR). The information gathered using spirometry is useful in assessing airway obstruction and functional lung capacity.

Rapid lung growth begins *in utero* and continues until the late teens in girls and early 20s in boys. Lung function reaches a maximum by 18-20 years of age in females and 22-25 years in males (Tager et al., 1988). Some males may show a small increment in lung function even in their mid '20s. After that, lung function declines at a slow but steady rate. A non-smoker loses about 1% of lung function per year, whereas a smoker loses 1.5% per year (Dockery et al., 1988). Lung function varies widely among adults. The big difference in lung function in adults are due to attained lung function at maturity, which can differ by a factor of two for individuals of the same age, sex, height, weight and race (Dockery et al., 2005). Thus, factors that can affect growth of lung function in childhood are important in determining the level of lung function in adulthood. Chronic exposure to air pollution is one such factor that can reduce lung function growth. Against this background, lung function was measured in a representative group of urban and rural subjects to unveil the impact of Delhi's air pollution on lung function of the residents.

4.2 MATERIALS AND METHODS

(a) Subjects

A total number of 3616 subjects aged between 21 and 67 years participated in spirometric measurements of lung function. In Delhi, the number of participants was 2833 of which 2165 (76.4%) were men and 668 (23.6%) were women. In the control group, 783 subjects participated in spirometry – 584 (74.6%) were men and 199(25.4%) were women.

(b) Pulmonary function tests by spirometry

Lung function tests by spirometry were performed with informed consent of the participant. The tests were performed according to the methods suggested by the American Thoracic Society (1995) using a portable, electronic spirometer (Spirovit SP-1, Switzerland). The spirometers were calibrated daily in the morning using a 2.0 liter syringe. Before performing the pulmonary function test, the height and weight of the subject was measured with shoes removed. Each subject performed at least three forced expiratory maneuvers while sitting with free mobility and nose closed with a nose clip to prevent passage of air through the nose to ensure reproducibility of results (Fig.4.1). Using a computer assisted quantitative assessment the best maneuver for acceptance was determined. The data were compared with predictive values based on age, sex, height and ethnic group. Flow volume loops provided a graphic illustration of a subject's spirometric efforts. Flow is plotted against volume to display a continuous loop from inspiration to expiration, the overall shape of the flow volume loop is important in interpreting spirometric results. The following spirometric parameters were recorded for analysis:

- Forced vital capacity (FVC), i.e. the volume of air in liters that can be maximally forcefully exhaled
- Forced expiratory volume at 1 second (FEV_1), i.e. volume of air (in liter) that is forcefully exhaled in one second.
- Ratio of FEV_1 to FVC (FEV_1/FVC), expressed as percentage
- Forced expiratory flow at 25-75% ($FEF_{25-75\%}$) or maximal mid-expiratory flow rate (MMEF), which is the average expiration flow rate during the middle 50% of the FVC
- Peak expiratory flow rate (PEFR) – the peak flow rate during expiration

The abnormalities that could be detected by spirometry tests are obstruction, restriction and combined type of lung function deficits.

In ***obstructive lung diseases*** such as emphysema or chronic bronchitis the FEV_1 is reduced disproportionately more than the FVC resulting in an FEV_1/FVC ratio less than 70%. This reduced ratio is the primary criteria for diagnosing obstructive lung disease by spirometry. The appearance of the flow volume curve changes when there is an obstruction, as with a normal curve there is a rapid peak expiratory flow but the curve descends more quickly than normal and takes on a concave shape, reflected by a marked decrease in the $FEF_{25-75\%.}$ With more severe obstruction the peak becomes sharper and the expiratory flow rate drops precipitously. This results from dynamic airway collapse, which occurs as diseased conducting airways are more readily compressed during forced expiratory efforts. The following scale was used to grade the severity of obstruction:

In restrictive lung disease, the FVC is reduced to less than 80% of predicted value based on height, weight, gender, age and ethnicity. The shape of the flow volume loop is relatively unaffected in restrictive disease, but the overall size of the curve appears smaller when compared to normal on the same scale. The following scale was used to grade the severity of restriction:

In combined lung disease both FVC and FEV_1/FVC ratio are decreased. FVC less than 80% and FEV_1/FVC less than 70% are considered to be combined lung function.

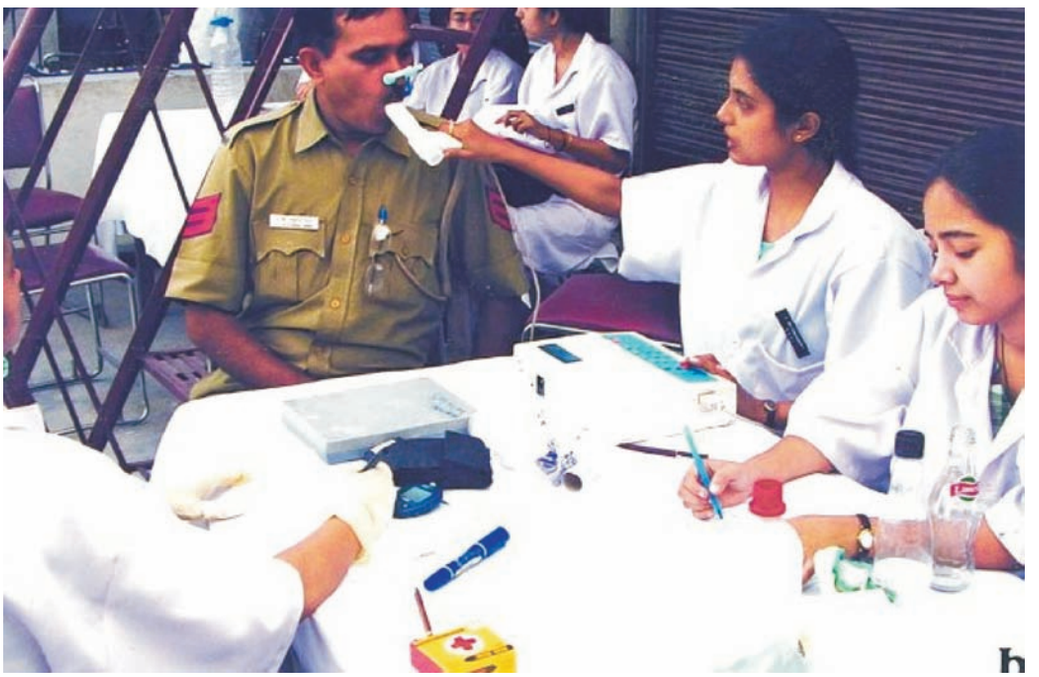


Figure 4.1: Police offiers at a health check-up camp organized at a roadside camp at Sabzi Mandi, Janakpuri, New Delhi

(c) Diagnosis of chronic obstructive pulmonary disease (COPD)

Field surveys were conducted in different parts of Delhi with the help of a structured and validated questionnaire. COPD was initially diagnosed on the basis of symptoms of chronic bronchitis (presence of cough and expectorations on most of the days for at least three months in a year for two consecutive years or more). Confirmation of diagnosis and further classification of COPD were based on spirometric measurements following the criteria of Global Initiative for Chronic Obstructive Lung Diseases (GOLD) which are as follows (Pauwels et al., 2001), (Table 4.1):

Table 4.1: GOLD diagnosis of COPD

Stage of COPD	Severity	Spirometric value	Symptom
I	Mild	FEV_1/FVC <70% FEV_1 70-79% of predicted	With or without chronic symptoms like cough, sputum expectoration, dyspnea
II a	Moderate	FEV_1/FVC <70% FEV_1 51-69% of predicted	"
II b	Severe	FEV_1 /FVC <70% FEV_1 30-50% of predicted	"
III	Very Severe	FEV_1 /FVC <70% FEV_1 <30% of predicted	Chronic respiratory failure

(d) BMI calculation

Body mass index (BMI) was calculated following the procedure of National Heart, Lung and Blood Institute, Department of Health and Human Services, Bethesda, USA. In essence, BMI was calculated by dividing the body weight in kilogram by the square of standing height in meter. BMI is expressed as kg/m^2. Relative body weight was calculated as follows (Bray and Gray, 1988, Table 4.2):

Table 4.2: Significance of body mass index values

BMI (kg/m^2)	Significance
<18.5	Underweight
18.5-24.9	Normal weight
25.0-30.0	Overweight
>30.0	Obese

The World Health Organization has categorized BMI values between 18.5-24.9 kg/m^2 as normal; 25-29.9 kg/m^2 as grade I overweight; 30-39.9 kg/m^2 as grade II overweight; and 40 kg/m^2 or higher as grade III overweight (WHO, 1986).

(e) Statistical analysis

All data are expressed as mean± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between lung function and possible confounders such as RSPM levels. Spearman's rank correlation test

for continuous variables and Chi-square test for categorical variables were done. P<0.05 was considered as significant.

4.3 RESULTS

4.3.1 Successful pulmonary function tests (PFT): 2816 in Delhi and 780 in controls

Seventeen participants in Delhi (0.6%), male 12 and female 5, failed to do pulmonary function tests satisfactorily after repeated attempts. In the control group 3 participants (0.4%), 1 male and 2 females failed to do the tests. Hence, these 20 individuals were excluded from the study. Therefore, lung function was successfully measured in 3596 participants-2816 in Delhi and 780 in control group. Out of these 2816 individuals of Delhi who successfully performed pulmonary function test, 2153 (76.4%) were men and 663 (23.6%) were women. In the control group of 780 successful performers, 583 (74.7%) were men and 197 (25.3%) were women (Table 4.3).

Table 4.3: Participants in pulmonary function test by spirometry

Pulmonary function test	Male	Female	Total
No. of participants	2749	867	3616
Delhi	2165	668	2833
Control	584	199	783
Unsuccessful performers	13	7	20
Delhi	12	5	17
Control	1	2	3
Successful performers	2736	860	3596
Delhi	2153	663	2816
Control	583	197	780

(a) Decrease in overall lung function in Delhi

Compared with rural controls, the residents of Delhi had diminished levels of all spirometric measurements (Table 4.4, Figure 4.2, 4.3). Their mean FVC, FEV_1, $FEF_{25-75\%}$ and PEFR values were decreased by 9.4%, 13.3%, 10.4%, and 9.3% respectively. All these changes were statistically significant (p<0.05).

Table 4.4: Comparison of spirometric lung function measurements between control subjects and participants in Delhi

Spirometric measurements	Control (n=780)	Delhi (n=2816)	p value
FVC (L)	3.38 ± 0.52	3.06 ± 0.43	<0.05
FEV_1(L)	2.94 ± 0.54	2.55 ± 0.53	<0.05
$FEF_{25-75\%}$ (L/s)	3.27±0.65	2.93±0.47	<0.05
PEFR (L/s)	3.65±0.83	3.31±0.66	<0.05

Figure 4.2: Degree of deviation in measured (i) FVC (L) and (ii) FEV1 (L/s) values from the predicted value in some residents of Delhi

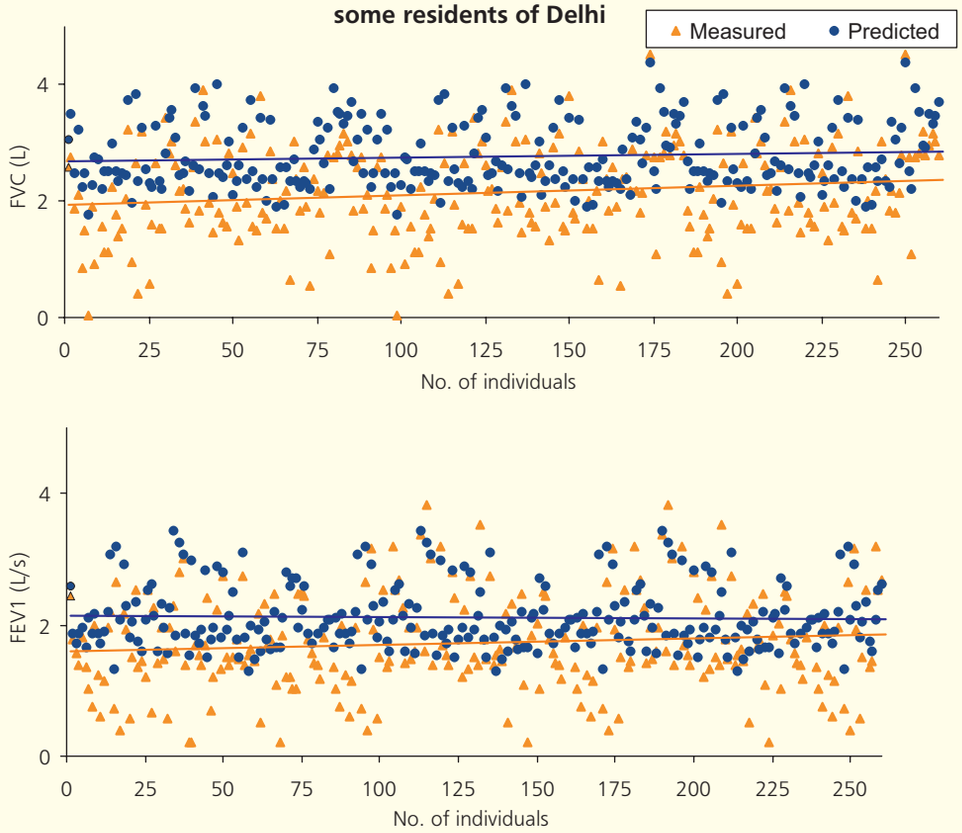
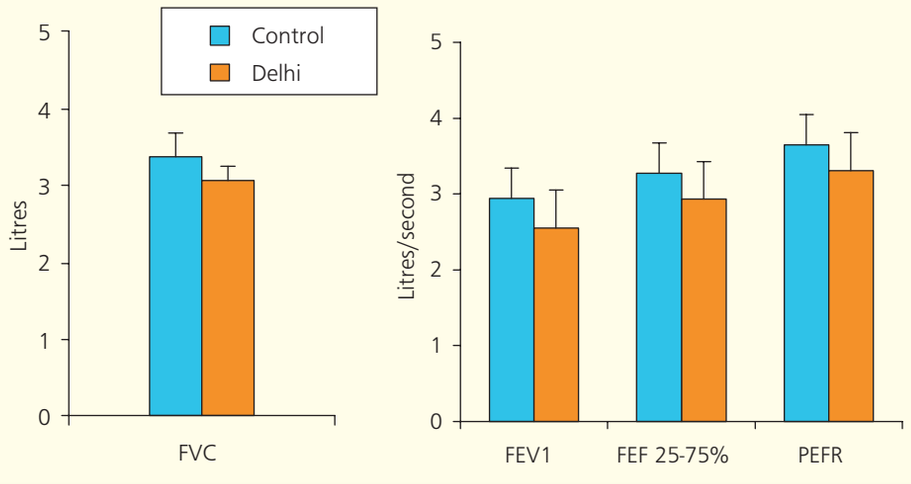


Figure 4.3: Comparison of spir ometric lung function measurements between control subjects and participants of Delhi



(b) Restrictive type of impairment more prevalent than obstructive type

Reduction in lung function could be restrictive, obstructive, or combined (both obstructive and restrictive) type. The hallmark of restrictive type of lung function deficits is reduction of forced vital capacity (FVC) to less than 80% of predicted value which was present in 29.6% (834 out of 2816) participants of Delhi in contrast to 13.5% (105/780) of control subjects. Thus, 29.6% citizens of Delhi and 13.5 % of control subjects had restrictive type of lung function decrement, and the difference between these two groups in this regard was highly significant ($p<0.001$). However, 200 residents of Delhi (7.1% of total) among these 834 subjects with FVC<80% predicted had <70% FEV_1/FVC which is the characteristic of obstructive type of lung function deficit. Therefore, they had 'combined' type of lung function deficits. Hence, 834-200 = 634 subjects (22.5% of total) had only restrictive type of lung function decrement. Similarly, 16 control subjects (2.0%) had combined lung function deficits. Hence, 106-16 = 89 control subjects (11.4%) had only restrictive type of lung function deficits (Table 4.5, Figure 4.4).

Obstructive type of lung function deficit ($FEV_1/FVC<70\%$), on the other hand, was found in 17.8% (501/2816) of Delhi's residents against 8.7% (68/780) of controls ($p<0.05$). Since 7.1% of urban and 2% of control subjects had combined type of lung function deficits, only obstructive type of decrement in lung function was present in 301 urban (10.7%) and 52 control (6.6%) subjects (Table 4.5, Figure 4.4).

Table 4.5: Prevalence (%) of reduced lung function

Type of lung function deficits	Men	Women	Total
Restrictive			
Control	10.8	13.2	11.4
Delhi	22.7*	21.9*	22.5*
Obstructive			
Control	6.2	8.1	6.6
Delhi	10.3*	12.1*	10.7*
Combined			
Control	1.5	3.5	2.0
Delhi	6.8*	8.0*	7.1
Overall			
Control	18.5	24.9	20.1
Delhi	39.8*	41.9*	40.3*

**, p<0.05 compared with respective control*

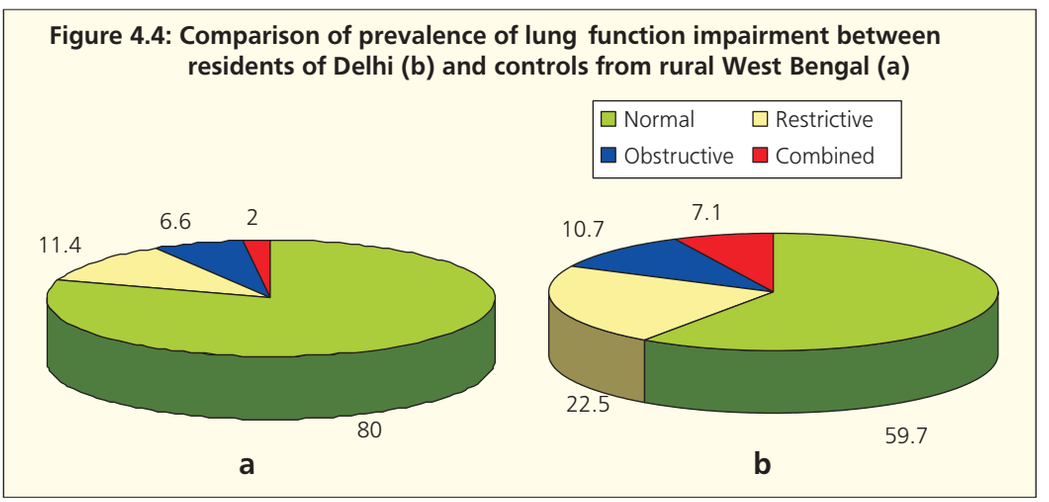


Figure 4.4: Comparison of prevalence of lung function impairment between residents of Delhi (b) and controls from rural West Bengal (a)

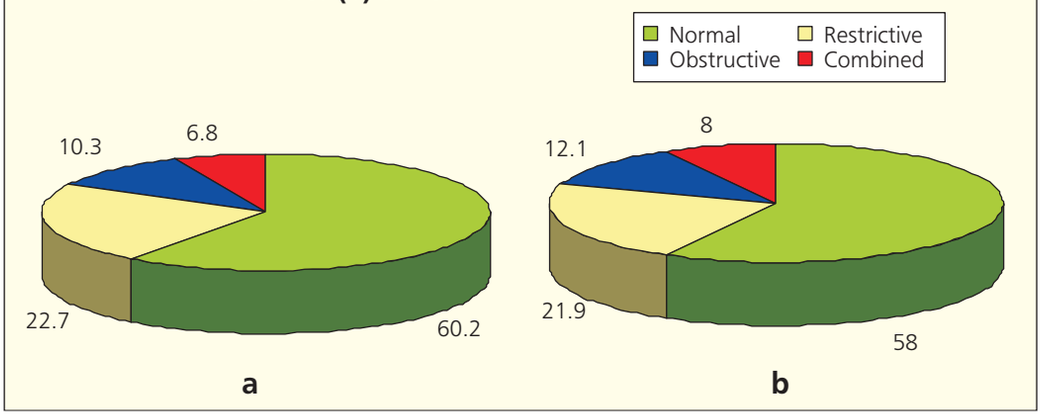
(c) Reduced lung function in 40.3% residents of Delhi compared with 20.1% of controls

Overall, lung function was reduced in 1135 residents of Delhi (40.3%) compared with 157 of control subjects (20.1%). The difference in the prevalence of lung function deficits between control and Delhi's residents was highly significant ($p<0.001$, Table 4.5).

(d) Women suffered more than men

Females had higher prevalence of lung function decrement than males both in Delhi as well as in control group. In Delhi, 41.9% women had decreased lung function compared with 39.8% of men ($p<0.05$). In control, 24.9% of women had reduced lung function compared with 18.5% of men ($p<0.05$). Women had greater prevalence of obstructive and combined type of lung function deficits than men in Delhi, whereas control women had greater prevalence of all three types of lung function deficits. For example, 12.1% and 8% of Delhi's women had obstructive and combined lung deficits against 10.3% and 6.8% of city's men. In the control group, 8.1% women had obstructive, 13.2% had restrictive and 3.5% had combined type of lung deficits against 6.2%, 10.8%, and 1.5% of men respectively (Table 4.5, Figure 4.5).

Figure 4.5: Comparison of prevalence of lung functio n impairment in males (a) and females (b) of Delhi



(e) Smokers had poorer lung function

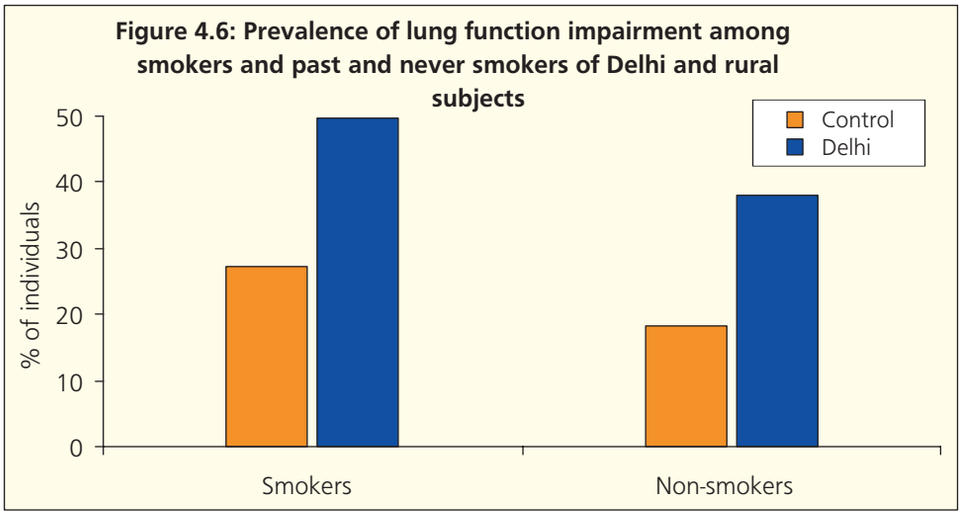
There were 568 current smokers (564/2153 male, 4/ female) among the participants in Delhi who successfully completed spirometry. In control group, 162 individuals (all male) were current smokers. Comparison of lung function between smokers and non-smokers (never smokers and past smokers) revealed marked reduction in spirometric values in the latter group (Table 4.6, Figure 4.6). Greatest reduction was recorded in $FEF_{25-75\%}$ value (32%), and PEFR (30%).

Lung function was decreased in 282 out of 568 current smokers (49.6%) of Delhi, compared with 853 out of 2248 non-smokers (37.9%; Figure 4.6). The difference was highly significant in Chi-square test ($p<0.001$), with an OR of 1.46 and 95% CI 1.21-1.76, after controlling possible confounders. Similarly in the control group, lung function decrement was observed in 44/162 (27.2%) current smokers compared with 113 of 618 non-smokers (18.3%).

Table 4.6: Comparison of lung function between smokers and non-smokers of Delhi

	Non-smoker, total (male + female) [n=2248]	Non-smoker, male (n=1589)	Current smoker (n=568)
FVC (L)	3.1 ± 0.7	3.3 ± 0.5	2.9 ± 0.7*
FEV_1(L)	2.6 ± 0.7	2.8 ± 0.5	2.4 ± 0.6*
FEV_1/FVC (%)	83.9	84.8	82.7
$FEF_{25-75\%}$ (L/s)	3.1 ±0.4	3.4 ±0.7	2.3±0.5*
PEFR (L/s)	3.4 ±0.8	3.9 ±0.6	3.0±0.7*

**, p<0.05 compared with both non-smoker groups*



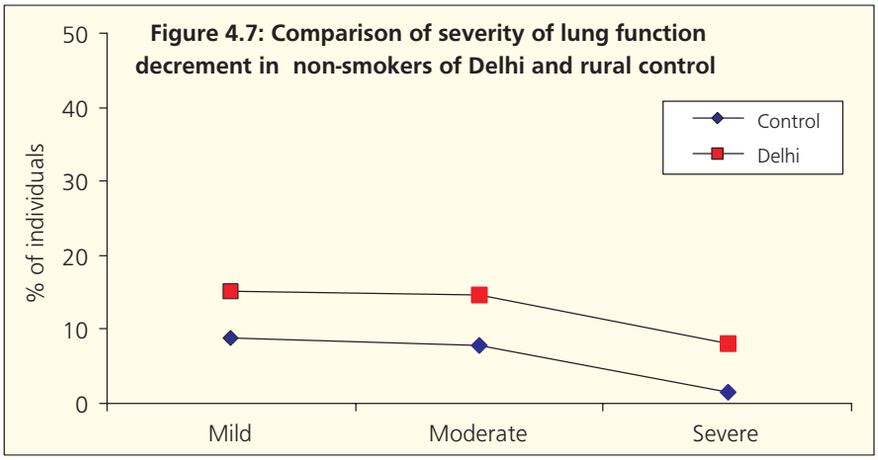
Smokers had increased prevalence of obstructive, restrictive as well as combined type of lung function deficits. For instance, 15% smokers in Delhi had obstructive lung compared with 9.6% of the non-smokers (p<0.05). Likewise, 24.1% and 10.5% smokers had restrictive and combined type of lung function deficits compared with 22.1% and 6.2% respectively of non-smokers. Thus, the results indicate that smoking was associated with significant increase in the prevalence of lung function deficits, particularly an excess of obstructive and combined type of lung function deficits.

(f) Magnitude of lung function reduction in never-smokers and ex-smokers: more in Delhi

The severity of lung function impairment was subdivided into three categories based on FVC and FEV_1/FVC values: mild (FVC 60-79% and/or FEV_1/FVC 50-69%), moderate (FVC 40-59% and/or FEV_1/FVC 30-49%), and severe (FVC <40% predicted and/or FEV_1/FVC <30%). It is evident that 15.1% of Delhi's non-smokers had mild, 14.6% had moderate and 8.2% had severe decrement in lung function. In contrast, 8.9%, 7.8% and 1.6% of control subjects had mild, moderate and severe type of lung function decrement respectively (Table 4.7, Figure 4.7).

Table 4.7: Percentage of never-smokers and ex-smokers with different grades of lung function decrement

Magnitude of lung function decrement	Control (n=618)	Delhi (n=2248)	p
Mild	8.9	15.1	<0.05
Moderate	7.8	14.6	<0.001
Severe	1.6	8.2	<0.001



(g) Marked reduction in FEF25-75% in non-smokers of Delhi, suggesting small airway obstruction

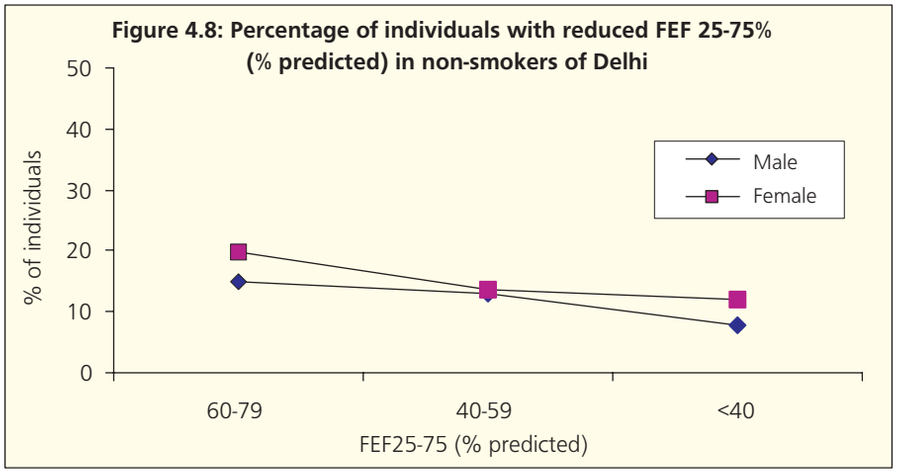
$FEF_{25-75\%}$ was declined below 80% of predicted value in 867/2248 (38.6%) nonsmokers of Delhi. In the control group, 133/618 (21.5%) participants had $FEF_{25-75\%}$ value less than 80% predicted, suggesting obstruction in the small airways. Therefore, spirometric measurements indicate 1.8-times greater prevalence of small airway obstruction among the non-smokers of Delhi compared with age-matched non-smokers of control group ($p<0.001$).

Reduction in $FEF_{25-75\%}$ was more prevalent among women. For instance, 45.3% of Delhi's women had $FEF_{25-75\%}$ value less than 80% of predicted compared with 35.9% of men. Similarly, 27.9% (55/197) of control women had reduced $FEF_{25-75\%}$ than 18.5% (78/421) of nonsmokers in control (Table 4.8, Figure 4.8). Thus, women suffered more from small airway obstruction than men both in urban and rural settings.

Table 4.8: Reduction in $FEF_{25-75\%}$ in never-smokers and ex-smokers of Delhi

FEF_{25-75} (% predicted)	Male (n=1589)	Female (n=659)	Total (n=2248)
60-79	238 (15.0)	130 (19.8)	368 (16.4)
40-59	206 (13.0)	90 (13.6)	296 (13.2)
<40	125 (7.9)	78 (11.9)	203 (9.0)
$FEF_{25-75\%}$ <80% predicted	569 (35.8)	298 (45.2)	867 (38.6)

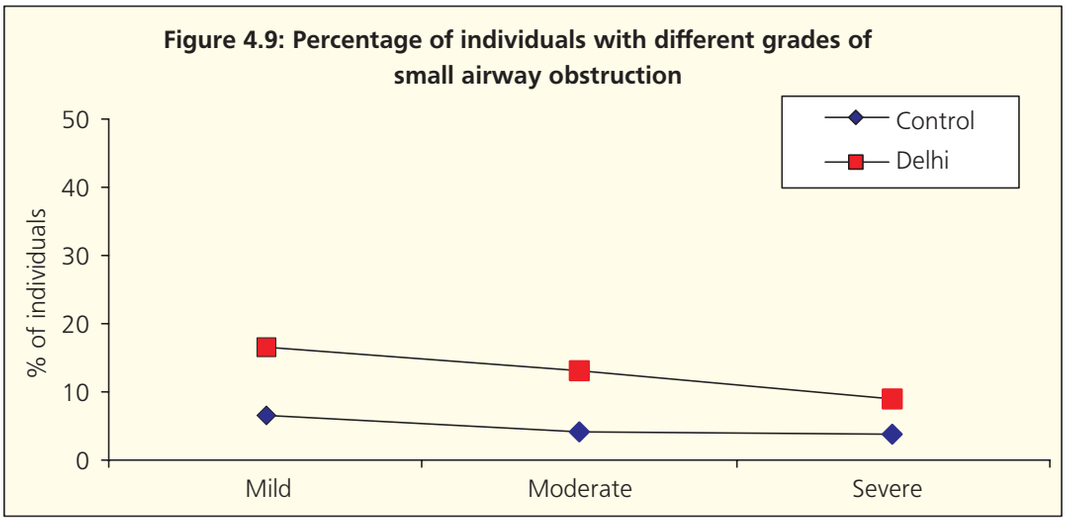
Results are expressed as number of individuals with percentage in parentheses



The magnitude of the reduction in FEF $_{25-75\%}$ was mild (60-79% of predicted value) in 16.4% of city's inhabitants, moderate (40-59% predicted) in 13.2% subjects and severe (FEF $_{25-75\%}$ fell below 40% of predicted value) in 9.2% participants. In contrast, in the control group the magnitude of the reduction in FEF $_{25-75\%}$ was mild in 6.6 %, moderate in 4.0% and severe in 3.7% cases (Table 4.9, Figure 4.9).

Table 4.9: Percentage of individuals with different grades of small airway obstruction

Severity of obstruction	Control (n=618)	Delhi (n=2248)	p
Mild (FEF $_{25-75\%}$ 60-79% predicted)	6.6	16.4	<0.001
Moderate (FEF $_{25-75\%}$ 40-59% predicted)	4.0	13.2	<0.001
Severe (FEF $_{25-75\%}$ <40% predicted)	3.7	9.0	<0.001

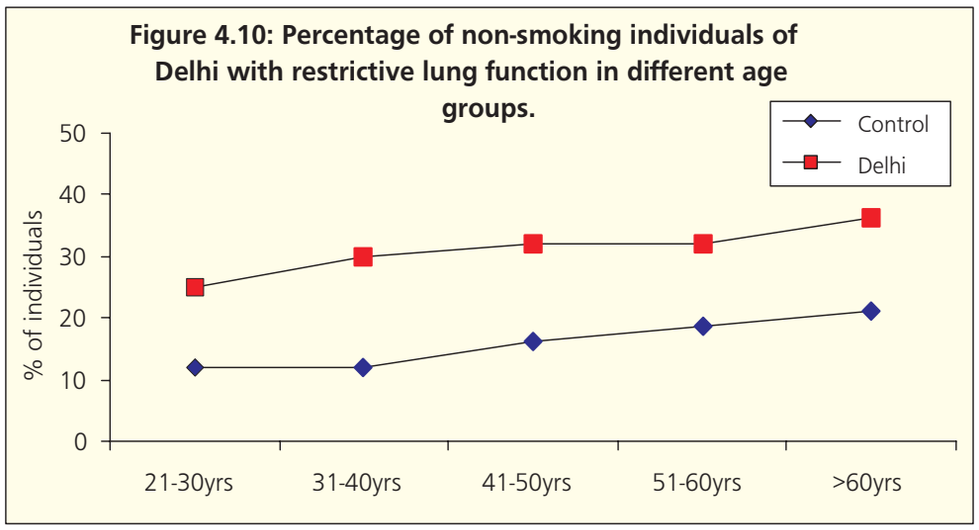


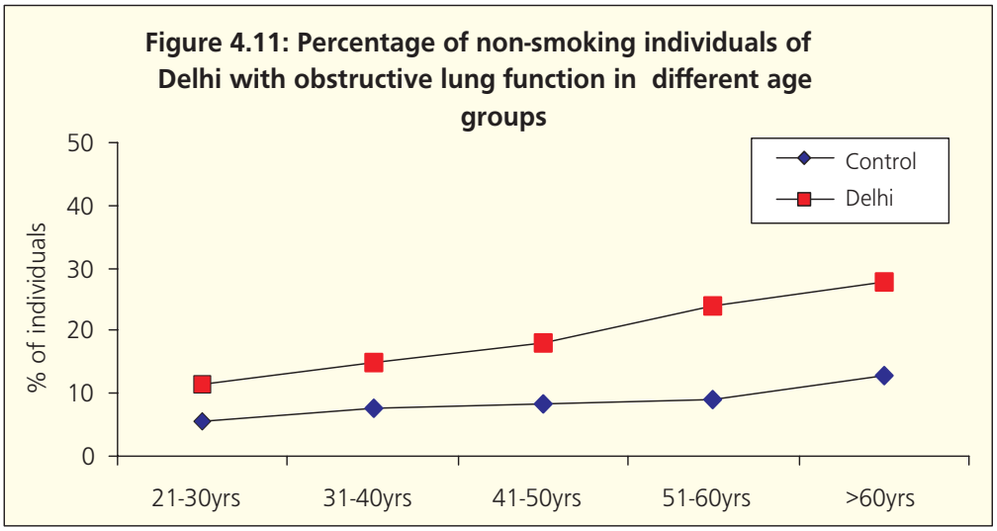
(h) Lung function decreases with age with age

The prevalence of restrictive and obstructive type of lung function deficit increased steadily with age both in Delhi and control group (Table 4.10, Figure 4.10, 4.11).

Table 4.10: Percentage non-smoking individuals of Delhi (with control value in parentheses) with reduced lung function in different age groups

Age group	n	Restrictive lung (FVC<80%)	Obstructive lung (FEV1/FVC<70%)
21-30 yr	783 (215)	24.9 (12.1)	11.5 (5.6)
31-40 yr	629 (173)	29.9 (12.1)	14.9 (7.6)
41-50 yr	430 (118)	31.9 (16.1)	17.9 (8.5)
51-60 yr	237 (65)	32.1 (18.5)	23.8 (9.2)
>60 yr	169 (47)	36.1 (21.3)	27.8 (12.8)
Overall	2248 (618)	29.3 (14.2)	16.2 (7.6)





(i) Reduction in PEFR: more in Delhi

Decline in PEFR below 80% of predicted value based on height, weight, age, gender and ethnicity was found in 52.5% participants of Delhi. Men and women suffered equally: 52.6% of women had reduced PEFR against 52.5% of men (Table 4.11, 4.12; Figure 4.12, 4.13). In the control group, 168 subjects (35.7%) had PEFR less than 80% predicted. Like urban subjects, men and women had similar prevalence. The difference in the prevalence of deficits in PEFR between control subjects and the participants of Delhi was significant ($p<0.001$).

Table 4.11: Percentage of individuals with different grades of PEFR reduction

Severity of reduction in PEFR	Control	Delhi	p
Mild 60-79% predicted)	5.5	9.6	<0.5
Moderate (40-59% predicted)	11.7	19.5	<0.05
Severe (<40% predicted)	18.5	23.4	<0.05

Table 4.12: Reduction in PEFR in different age groups

PEFR <60% predicted	Male (n=1245)	Female (n=581)	Total (1826)
21-30 yr	54.7	46.6	52.4
31-40 yr	52.3	49.0	51.4
41-50 yr	42.8	50.0	45.1
51-60 yr	48.1	48.2	48.1
>60 yr	52.5	72.9	60.6
All ages	50.5%	50.6%	50.5%

Figure 4.12: Percentage of individuals with different grades of PEFR (% predicted) reduction

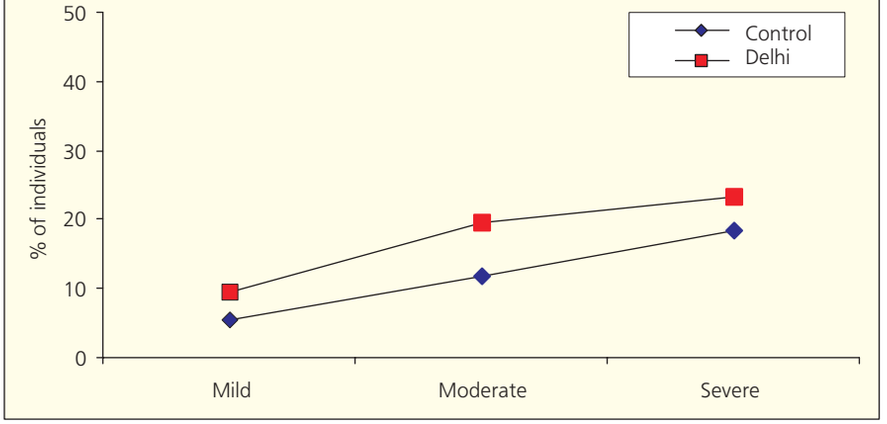
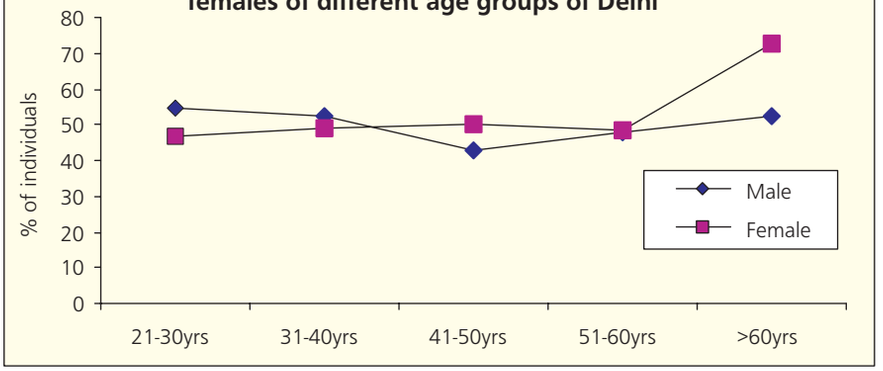


Figure 4.13: Reduction in PEFR among males and females of different age groups of Delhi



The severity of PEFR reduction was mild (60-79% predicted) in 9.6%, moderate (PEFR 40-59% predicted) in 19.5% and severe (PEFR <40% predicted) in 23.4% participants of Delhi. In the control group, 5.5% had mild, 11.7% had moderate, and 18.5% had severe reduction in PEFR (Table 4.11, Figure 4.12).

4.3.2 Prevalence of obesity: 9.8% in Delhi against 1.2% in controls

Obesity was diagnosed on the basis of body mass index (BMI). BMI was calculated as kg/m^2 from body weight (in kg) and standing height (in meter) of a total number of 2812 individuals (male 2099, female 713) of Delhi and 827 control subjects (male 622, female 205). A total of 9.8% Delhiites (276 of 2812) were obese (BMI of >30.0 kg/m^2). In contrast, only 1.2% of rural controls were obese, and difference

was highly significant (p<0.001; Figure 4.14). Ladies predominate the obese persons of Delhi, as 15.4% women in Delhi were obese compared with 7.9% obese men (Table 4.13, Figure 4.15). The gender difference in the prevalence of obesity in Delhi was highly significant (p<0.001). In the control group, obesity prevalence was slightly more in men than in women (1.3 vs. 1%), but the difference was not significant (p>0.05, Table 72).

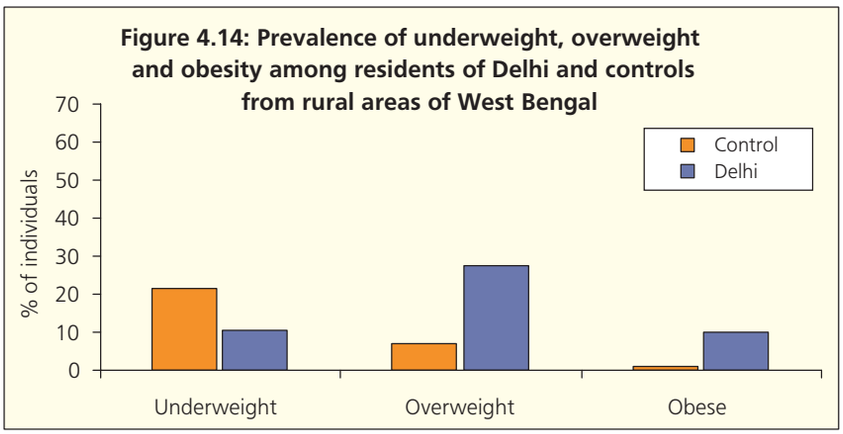
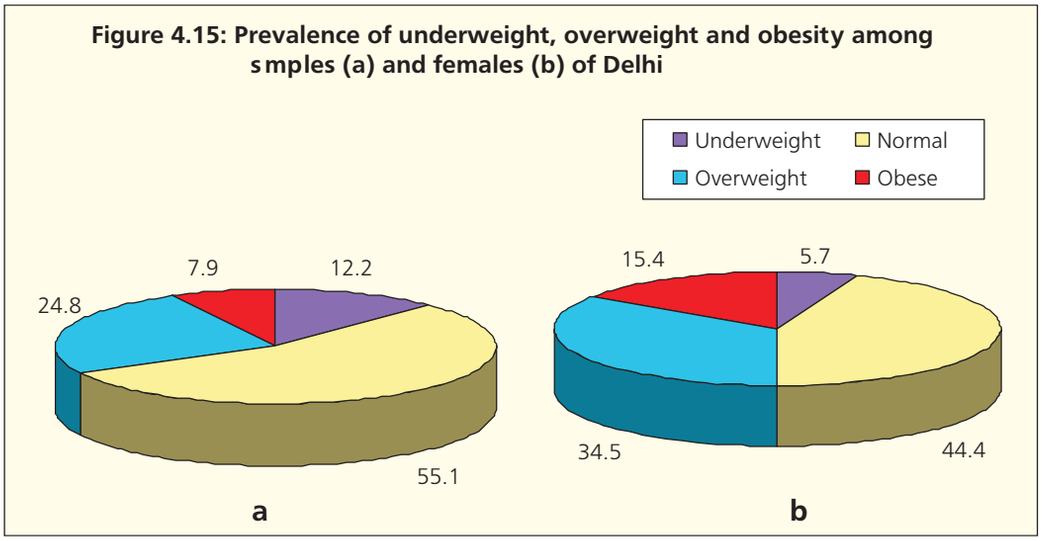


Table 4.13: Prevalence (%) of obesity in Delhi

BMI (kg/m²)	Status	Male		Female		Total	
		Control n=622	Delhi n=2099	Control n=205	Delhi n=713	Control n=827	Delhi n=2812
<18.5	Underweight	18.0	12.2*	31.7	5.7*	21.4	10.6*
18.5-24.9	Normal	73.6	55.0*	60.0	44.3*	70.3	52.3*
25.0-30.0	Overweight	7.1	24.8*	7.3	34.5*	7.1	27.3*
>30.0	Obese	1.3	7.9*	1.0	15.4*	1.2	9.8*

**, p<0.05 compared with respective control*



(a) Prevalence of overweight: 27.3% in Delhi against 7.1% in controls

Besides obesity, 27.3% residents of Delhi were overweight (BMI 25.0-30.0 kg/m²) in contrast to only 7.1% overweight persons among controls (p<0.001). As in case of obesity, the problem of overweight

was more among Delhi's women as 34.5% of city women enrolled in this study were overweight compared with 24.8% of overweight men ($p<0.05$). In contrast, no such gender difference in overweight was found in controls (Table 4.13, Figure 4.14, 4.15).

(b) Prevalence of underweight: 21.4% in controls against 10.6% in Delhi

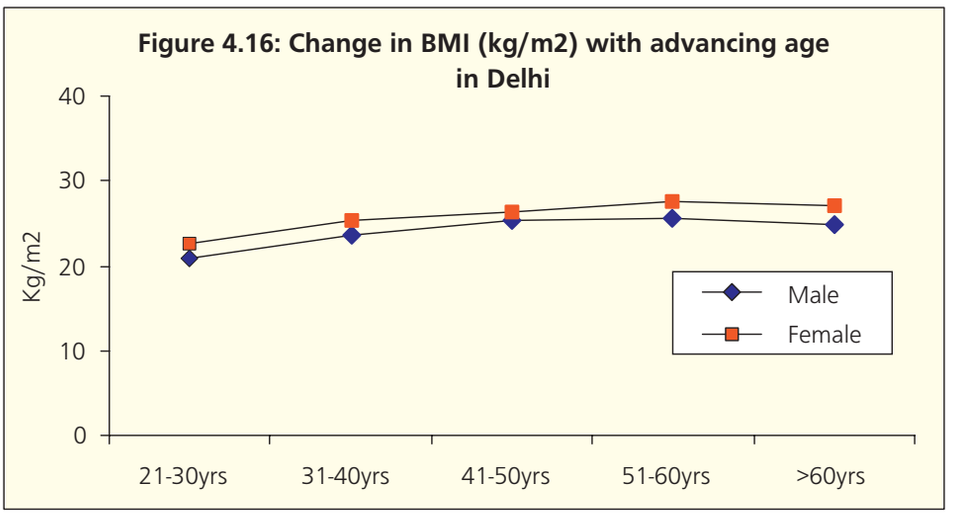
The prevalence of underweight (BMI <18.5 kg/m^2), however, was significantly higher among control subjects (21.4 vs. 10.6% in Delhi, $p<0.001$). The problem was more acute in women (31.7 vs. 18% of control men), while a greater percentage of males in Delhi were underweight than females (12.2 vs. 5.7%, $p<0.05$, Table 4.13, Figure 4.14, 4.15).

(c) Physiological increase in body weight with advancing age

A progressive rise in BMI was observed with advancing age. BMI stared increasing slowly but steadily from the age of 31 years till 60 years. Thereafter, a modest decline in BMI was recorded (Table 4.14, Figure 4.16). For the same age group, BMI was more in females than the males.

Table 4.14: Change in BMI (kg/m^2) with advancing age in Delhi

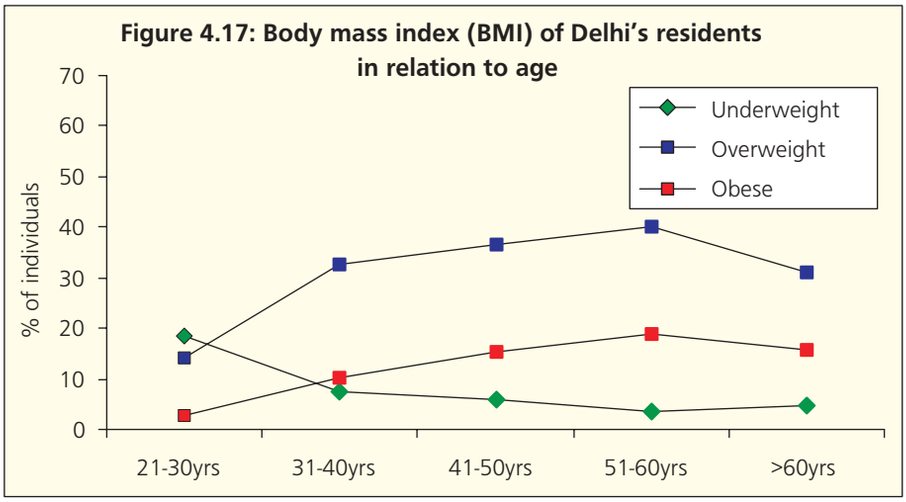
Age (yr)	BMI (kg/m^2)		
	Male	Female	Total
21-30	20.9	22.5	21.1
31-40	23.7	25.3	24.2
41-50	25.3	26.3	25.4
51-60	25.6	27.6	26.2
>60	24.8	27.0	25.5



The percentage of overweight and obese persons increased steadily with increasing age. There was a quantum jump when an individual reaches the 30+ age. Thereafter it increases progressively, reaching a peak at 51-60 years. A slight decline then follows. Overall, persons aged between 51 and 60 years had greatest prevalence of overweight and obesity (Table 4.15, Figure 4.17).

Table 4.15: BMI of Delhi's residents in relation to age

BMI (kg/m²)	Percentage of individuals				
	21-30 yr	31-40 yr	41-50 yr	51-60 yr	>60 yr
<18.5	18.5	7.6	6.0	3.5	4.9
18.5-24.9	63.9	49.4	42.2	37.2	47.9
25.0-29.9	14.3	32.6	36.7	40.3	31.2
>30.0	2.8	10.3	15.3	18.8	15.9



(d) Overweight and obesity among Delhi's residents in relation to age

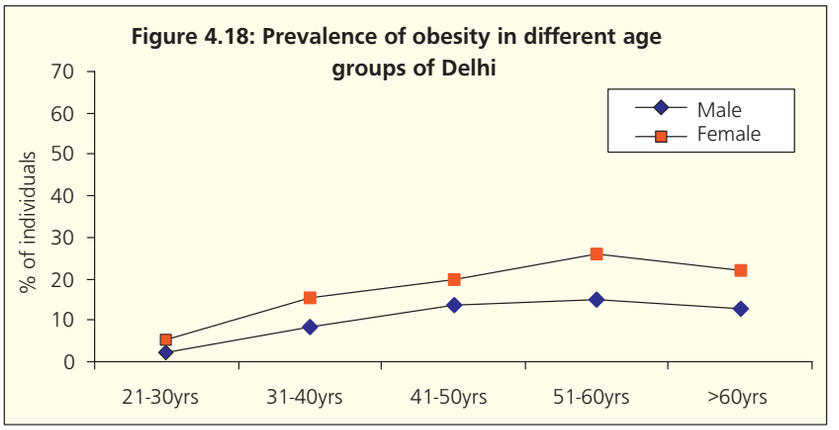
The problem of overweight and obesity was more in females than in males in all the age groups. As many as 45.0% women aged 51-60 years were overweight and another 26.0% were obese leaving only 29.0% women having normal weight. In contrast, 47.0% men of similar age had normal weight, 37.9% were overweight and 15.1% were obese (Table 4.16, 4.17; Figure 4.18, 4.19).

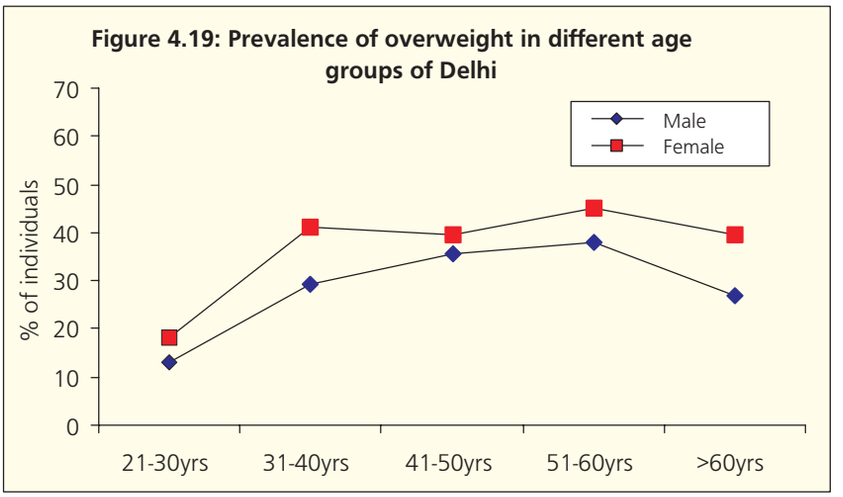
Table 4.16: Prevalence (%) of obesity in different age groups of Delhi

Age (year)	Male	Female	Total
21-30	2.2	5.3	2.8
31-40	8.5	15.3	10.3
41-50	13.7	19.6	15.3
51-60	15.1	26.0	18.8
60+	12.7	22.2	15.9

Table 4.17: Prevalence of overweight in different age groups

Age (year)	Male	Female	Total
21-30	13.1	18.0	14.1
31-40	29.3	41.3	32.4
41-50	35.7	39.7	36.6
51-60	37.9	45.0	40.3
60+	27.0	39.7	31.2





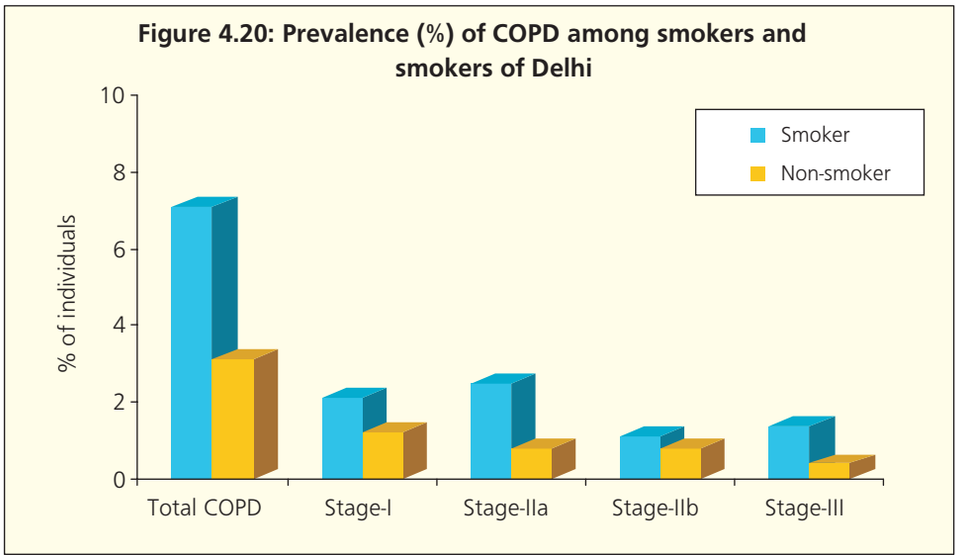
4.3.3 Prevalence of COPD: 3.9% in Delhi against 0.8% in control

Chronic obstructive pulmonary disease (COPD) was present in 110 individuals out of 2816 residents of Delhi who successfully completed spirometry procedures, with an overall prevalence of 3.9%. In contrast, only 6 individuals out of 780 control subjects (0.8%) had COPD ($p<0.001$). Males had an increased COPD prevalence than the females. Out of the 2153 male participants, 86 (4.0%) had COPD compared with 24 out of 663 (3.6%) females. Thus, the male: female ratio of COPD prevalence in Delhi was 1.1:1, compared with 1.6:1 in controls (Table 4.18).

Table 4.18: Prevalence (%) of chronic obstructive pulmonary disease (COPD)

Group	Control (n=780)	Delhi (n=2816)
Male	0.8	4.0
Female	0.5	3.6
Overall	0.8	3.9

Smoking of tobacco is a known risk factor for COPD development. In this study also smokers had a significantly higher ($p<0.05$) prevalence of COPD than the non-smokers. Overall, 7.0% of smokers had COPD against 3.1% of ex-, and never-smokers, with a smoker: non-smoker ratio of 2.3:1. In control, 3% of smokers and 0.2% of non-smokers had COPD. The severity of COPD was also greater among smokers (Figure 4.20).

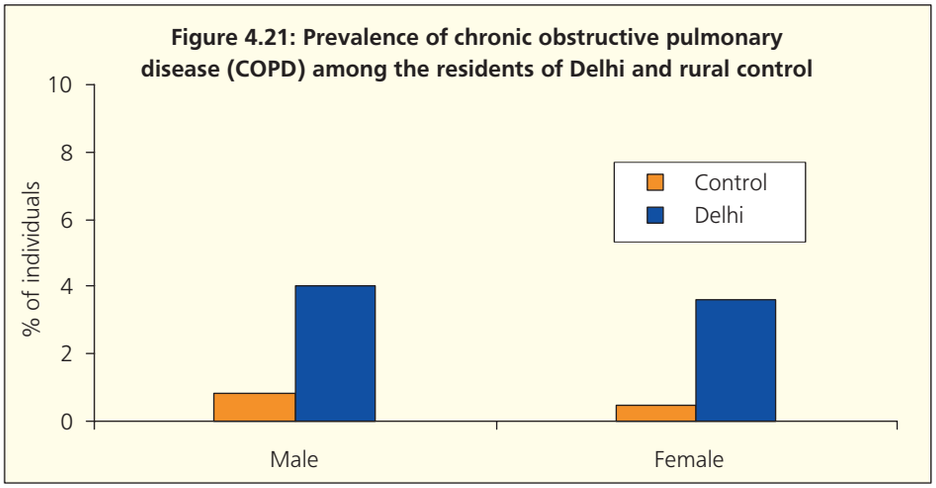


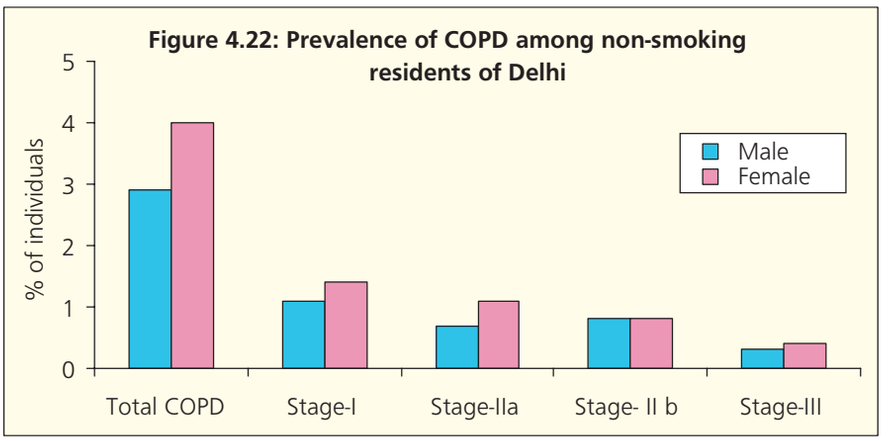
COPD among non-smokers

An important finding of this study is the presence of COPD in 3.1% of non-smokers of Delhi. More importantly, non-smoking women of the city illustrated a higher prevalence (4.0%) of COPD than their male counterparts (2.9%). Mild to moderate form of the disease (stage-I/IIa) was present in 1.8% of male (29/1589) and 2.5% of female non-smokers (16/659). However, the prevalence of severe and very severe form of the disease (stage IIb/III) was more or less similar in women (1.2%; 8 out of 603) and men (1.1%; 17 out of 1589; Table 4.19, Figure 4.21, 4.22).

Table 4.19: Prevalence (%) of COPD among non-smokers of Delhi

Category	Male (n=1589)	Female (n=659)	Total (2248)
Stage-I	1.1	1.4	1.2
Stage-IIa	0.7	1.1	0.8
Stage –IIb	0.8	0.8	0.8
Stage-III	0.3	0.4	0.4
COPD, total	2.9	4.0	3.1





4.3.4 Possible confounding factors for lung function decrement in non-smokers

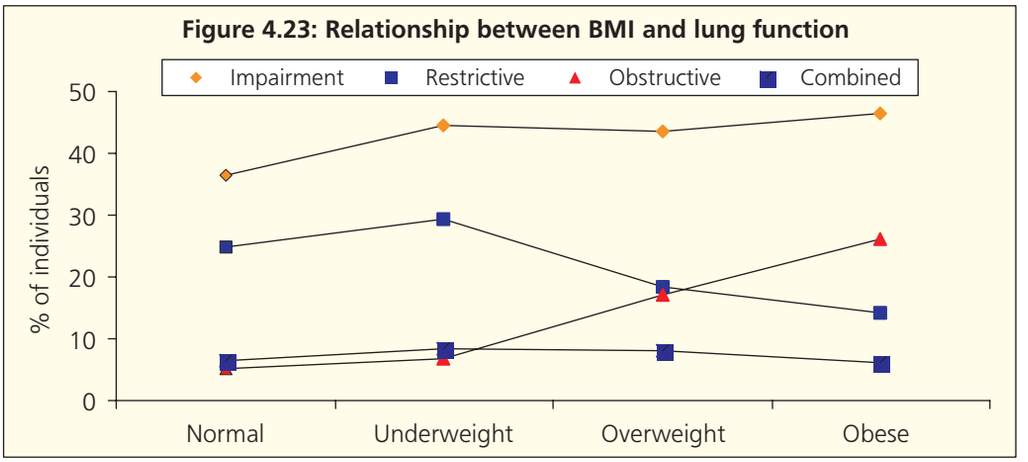
(a) Body mass index (BMI)

Reduced lung function in underweight and overweight/obese persons

Highest prevalence of reduced lung function was recorded in obese subjects as 46.5% of obese subjects of Delhi had decreased lung function compared with 36.6% of individual with BMI in the normal range. Overweight and underweight persons also showed greater frequency of lung function decrement (43.5 and 44.6% respectively) than persons with normal body weight (Table 4.20, Figure 4.23). The result showed that obese, underweight and overweight persons had increased prevalence of lung function deficits than persons having normal body weight. Conditional logistic regression analysis showed that deviation in BMI from the normal range was associated with significant rise in the prevalence of reduced lung function (Table 4.20).

Table 4.20: Relationship between BMI and lung function in Delhi

Lung function	Underweight	Normal BMI	Overweight	Obese
Reduced (%)	44.6	36.6	43.5	46.5
Restrictive (%)	29.5	24.8	18.3	14.1
Obstructive (%)	6.7	5.3	17.0	26.1
Combined (%)	8.4	6.5	8.1	6.2



Although higher prevalence of lung function deficits was recorded in both underweight and overweight/ obese subjects there was a marked difference in the type of impairment. Overweight and obese subjects had increased prevalence of obstructive type of lung, while underweight persons had predominantly restrictive type of lung function deficits (Table 4.21).

Table 4.21: Conditional logistic regression analysis of the association between BMI and lung function deficits

Body weight	OR (95% CI) for reduction in lung function		
	Restrictive type	Obstructive type	Overall
Normal	1	1	1
Underweight	1.71 (1.28-2.28)*	1.18 (0.82-1.66)	1.72 (1.30-2.30)*
Overweight	0.83 (0.68-1.03)	1.37 (1.17-1.63)*	1.38 (1.12-1.55)*
Obese	0.97 (0.71-1.32)	1.44(1.21-1.83)*	1.39(1.11-1.64)*

**, p<0.05*

(b) Socio-economic status (SES)

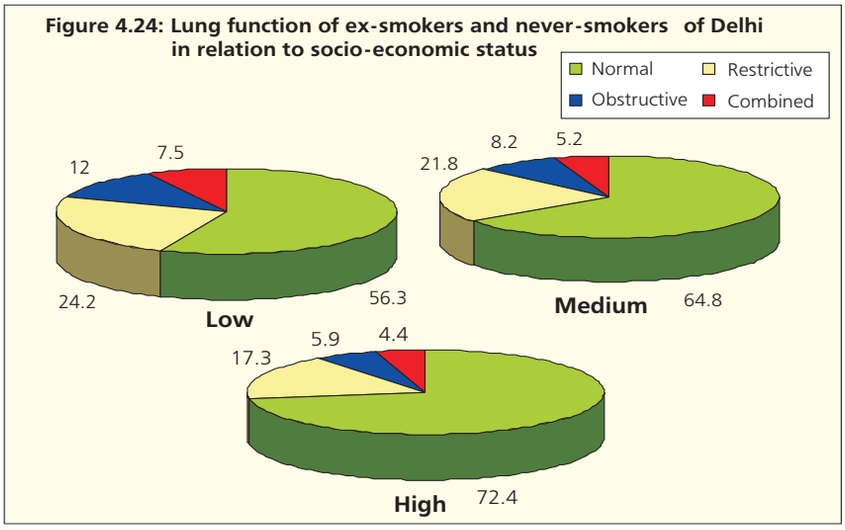
Highest prevalence of lung function deficits in low SES

Persons from low SES had greatest prevalence of lung function decrement, as 43.7% individuals of this group had reduced lung function compared with 35.2% and 27.8% citizens from medium and high SES (Table 4.22, Figure 4.24). Persons belonging to low SES had greatest prevalence of all types of lung function deficits- restrictive (24.2% vs. 17.3% in high SES, p<0.05), obstructive (12.0 vs. 5.9, p<0.05), as well as combined type (7.5 vs. 4.4%, p<0.05) of lung function deficits.

Table 4.22: Lung function of ex-smokers and never-smokers of Delhi in relation to socio-economic status (SES)

Type of lung function deficits	Low SES (n=1065)	Medium SES (n=780)	High SES (n=403)
Restrictive	24.2*	21.8	17.3
Obstructive	12.0*	8.2	5.9
Combined	7.5*	5.2	4.4
Overall	43.7*	35.2	27.8

*Results are expressed as percentage of individuals; *, p<0.001 compared with high SES*

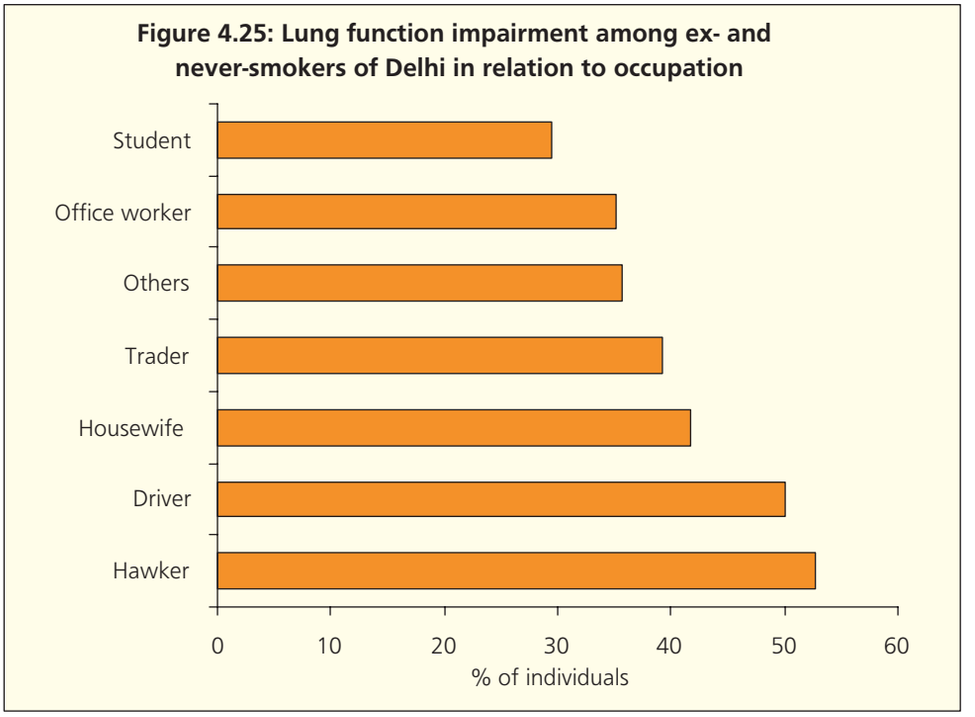


(c) Occupation

Decline in lung function among the ex- and never-smoking residents of Delhi in relation to occupation of the subjects showed highest prevalence in roadside hawkers who were engaged in selling of various items such as foodstuff, books and magazines, garments, toys etc. on the pavement or in stalls within 30 feet of the busy thoroughfares. About 53% of these hawkers had reduced lung function compared with Delhi' overall prevalence of 37.9% for non-smokers. Half of the auto rickshaw and taxi drivers of the city had reduced lung function (Table 4.23, Figure 4.25). Chronic exposure to vehicular exhausts could be a major contributor to higher prevalence of lung function deficits, because the roadside hawkers usually spend 8-10 hours per day, 6 days per week in the vicinity of high traffic load. Similarly, the drivers spend 10-14 hours per day, 6-7 days per week on city roads exposing themselves to automobile exhausts for hours every day.

Table 4.23: Lung function of ex-smokers and never-smokers of Delhi in relation to occupation

Area	n	Persons with decreased lung function	%
Office job	832	292	35.1
Driver	82	41	50.0
Roadside hawker	142	75	52.8
Housewife	412	172	41.7
Student	210	62	29.5
Trader	206	81	39.3
Others	364	130	35.7
Delhi, total	2248	853	37.9
Rural control	618	113	18.3



Housewives of the city also had greater-than-average prevalence of reduced lung function (41.7%). Since most of them were engaged in daily household cooking they were additionally exposed to cooking

fuel and cooking oil emissions. That could be a reason for higher prevalence of lung function deficits in housewives. Lowest prevalence of reduced lung function (29.5%) was observed in non-smoking students, and the finding could be related to their younger age, since lung function in adults reduces progressively with age. In summary, among the non-smokers best lung activity was found in students while poorest lung function was found in roadside hawkers of the city.

(d) Particulate air pollution

Of all the potential confounders, particulate air pollution was most intimately associated with lung function decrement. The correlation was strongest for FVC (rho value 0.74, $p<0.0005$), followed by PEFR (rho=0.66, $p<0.0005$), and FEV_1 (rho=0.62, $p<0.0005$, Table 4.24).

Table 4.24: Spearman's rank correlation (r_s value) between RSPM and spirometric values

	FVC	FEV_1	$FEF_{25-75\%}$	PEFR
PM_{10}	-0.74 (p<0.0005)	-0.62 (p<0.0005)	-0.34 (p<0.05)	-0.66 (p<0.0005)
FVC	-	0.865 (p<0.0005)	0.323 (p<0.0025)	0.759 (p<0.0005)
FEV_1	-	-	0.531 (p<0.0005)	0.835 (p<0.0005)
$FEF_{25-75\%}$	-	-	-	0.572 (p<0.0005)

All spirometric values are measured values

After controlling potential confounders like age, gender, BMI and SES, logistic regression analysis confirmed that particulate pollution (PM_{10}) was positively associated with both restrictive (OR= 1.33, 95% CI 1.17-1.58) and obstructive (OR=1.42, 95% CI 1.22-1.89) type of lung function deficits. Conditional regression analysis reaffirmed this finding (Table 4.25). Thus, it appears that Delhi' particulate air pollution is a strong, significant contributor to lung function reductions observed in a large number of residents of the city.

Table 4.25: Conditional logistic regression analysis of the association between particulate pollution and lung function decrement

PM_{10} ($\mu g/m^3$)	OR (95% CI)		
	Restrictive	Obstructive	Overall
50-100	1	1	1
101-150	1.32 (1.11-1.57)	1.45 (1.24-1.84)	1.39 (1.15-1.77)
>150	1.63 (1.31-2.29)	1.83 (1.42-2.67)	1.70 (1.37-2.41)

(e) 'Hot spots' of lung impairment in Delhi

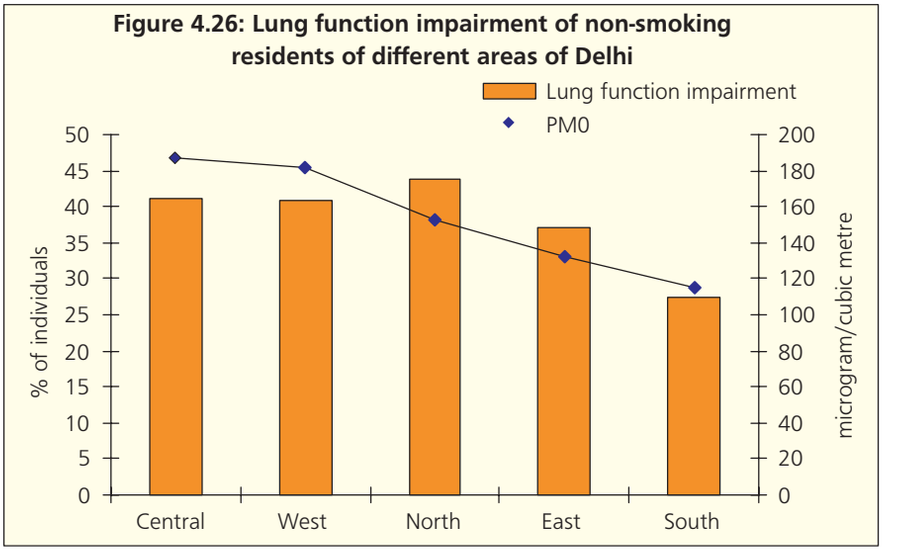
Prevalence of lung function impairment varied among residents of different areas of Delhi who were either ex-smokers of never-smokers, mostly the latter. Prevalence was highest in those residing at North Delhi (43.8%), and lowest (27.4%) among the residents of South Delhi. Residents of Central (41.1%) and West Delhi (40.9%) also had higher than Delhi's average prevalence (37.9%) of reduced lung function among non-smokers (Table 4.26, Figure 4.26). The finding was in agreement with the air pollution scenario of the city- more particulate pollution in northern, central and western Delhi and less in eastern and southern Delhi.

Table 4.26: Lung function of ex-and never-smoking residents of different areas of Delhi

Area	n	Persons with decreased lung function	%
East	378	140	37.0*
West	403	165	40.9*
North	468	205	43.8*
South	496	136	27.4*
Central	503	207	41.1*
Delhi, total	2248	853	37.9*
Rural control	618	113	18.3

**, p<0.05 compared with control in Chi-square test*

Figure 4.26: Lung function impairment of non-smoking residents of different areas of Delhi



Also, the prevalence of lung function impairment varied between localities within a particular region. For example, 42.2% residents of Gandhi Nagar had lung function decrement compared with 21.7% in Vasundhara Enclave ($p<0.05$), although both these localities are in same (eastern) area. For this reason, prevalence of lung function decrement of different localities has been tabulated separately (Table 4.27 a, b, c, d, e).

Table 4.27: Prevalence (%) of lung function decrement in never-smokers and ex-smokers of different localities of Delhi

Table 4.27a: EAST DELHI

Area	n	Persons with decreased lung function	%
East Arjun Nagar	178	62	34.8
Shahdara	94	38	40.4
Gandhi Nagar	83	35	42.2
Vasundhara Enclave	23	5	21.7
Total	**378**	**140**	**37.0**

Table 4.27b: CENTRAL DELHI

Area	n	Persons with decreased lung function	%
ITO	44	22	50.0
Ajmeri Gate	58	28	48.3
Nizamuddin	29	13	44.8
Hailey Road	42	14	33.3
Old Rajinder Nagar	47	15	31.9
New Rajinder Nagar	44	17	38.6
Pusa Road	70	22	31.4
Karol Bagh	152	68	44.7
Hari Nagar	17	8	47.0
Total	**503**	**207**	**41.1**

Table 4.27c: NORTH DELHI

Area	n	Persons with decreased lung function	%
Chandni Chowk	55	28	50.9
Civil Lines	27	8	29.6
Darya Ganj	21	9	42.8
Kamla Nagar	54	20	37.0
Virendra Nagar	45	20	44.4
Kalyan Vihar	13	6	46.1
Ashok Vihar	37	17	45.9
Rohini	52	19	36.5
Shalimar Bagh	43	16	37.2
Sangam Park Extension	20	8	40.0
Shahzada Bagh	101	54	53.5
Total	**468**	**205**	**43.8**

Table 4.27d: WEST DELHI

Area	n	Persons with decreased lung function	%
Paschim Vihar	50	20	40.0
Janak Puri	117	51	43.6
Tilak Nagar	50	22	44.0
Jaidev Park	18	8	44.4
Kangan Heri	26	9	34.6
Inder Puri	77	30	38.9
Virendra Nagar	65	27	41.5
Total	**403**	**165**	**40.9**

Table 4.7e: SOUTH DELHI

Area	n	Persons with decreased lung function	%
R.K.Puram	16	5	31.2
Sarojini Nagar	17	6	35.3
Vasant Place	37	4	10.8
Safdarjung Enclave	17	6	35.3
Lajpat Nagar	23	9	39.1
Green Park	48	16	33.3
Yusuf Sarai	68	21	30.9
Tughlakabad Industrial Area	58	11	18.9
Nehru Place	162	44	27.2
Lodhi Road	50	14	28.0
Total	**496**	**136**	**27.4**

4.4 FINDINGS

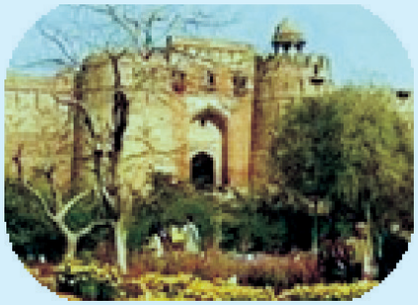
1. Lung function test was successfully carried out in 3596 subjects (2816 in Delhi and 780 in control) aged between 21 and 67 years by portable spirometer following the protocol of American Thoracic Society.
2. Compared with rural controls, the residents of Delhi had diminished levels of all spirometric measurements. Their mean FVC, FEV_1, $FEF_{25-75\%}$ and PEFR values were decreased by 9.4%, 13.3%, 10.4%, and 9.3% respectively. All these changes were statistically significant ($p<0.05$). The prevalence of lung function deficits increased with age.

3. Overall, lung function was reduced in 40.3% individuals of Delhi compared with 20.1% in control group.
4. Residents of Delhi showed statistically significant ($p<0.05$) increased prevalence of restrictive (22.5% vs. 11.4% in control), obstructive (10.7% vs. 6.6%), as well as combined (both obstructive and restrictive) type of lung functions deficits (7.1% vs. 2.0%).
5. Lung function reduction was more prevalent in women than in men both in rural and urban settings. In Delhi, 41.9% women had lung function deficits against 39.8% of men. In control group, 24.9% women had lung function deficit than 18.5% of men.
6. Besides greater prevalence, the magnitude of lung function reduction was much more in Delhi. For instance, 6.7% individuals of Delhi had severe lung restriction (FVC <40%) against 1.3% of control ($p<0.001$), and 2.7% citizens of Delhi had severe obstructive lung (FEV_1/FVC <30%) against 0.8% of control subjects ($p<0.001$).
7. In Delhi, 26.2% of men and 0.6% of women were current smokers. Similarly, 27.8% of men in control group were current smokers, but none of the control women was a smoker, either current or past.
8. Smoking had adverse effects on lung function. In Delhi 49.6% of current smokers had reduced lung function compared with 37.9% of never-smokers and ex-smokers. In control group, 27.2% of current smokers and 18.3% of never-smokers and ex-smokers had reduced lung function respectively.
9. Chronic obstructive pulmonary disease (COPD) was detected in 3.9% residents of Delhi against 0.8% of controls. Current smokers had significantly higher prevalence of COPD than ex-smokers and never-smokers. For example, 7% and 3% of current smokers in Delhi and control respectively had COPD against 3.1% and 0.2% of non-smokers. Thus, 3.1% of non-smokers of Delhi who participated in this study had COPD compared with only 0.2% of non-smokers with COPD in control group.
10. Nearly 10% adult individuals of Delhi were obese, against 1.2% of controls. Another 27.3% Delhiites were overweight against 7.1% of controls. Obesity in Delhi was more prevalent in women than in men (15.4% vs. 7.9% $p<0.001$). On the other hand, underweight prevalence was much more in controls (21.4% vs. 10.6% in Delhi, $p<0.001$).
11. Greatest prevalence of reduced lung function was recorded in obese subjects, as 46.4% of obese and 43.4% of overweight citizens of Delhi had reduced lung function against 36.6% of citizens with normal body weight. Underweight individuals also had poor lung function as 44.6% of them had reduced lung function in Delhi. Overweight and obese subjects had increased prevalence of obstructive type of lung function deficits, while underweight persons had predominantly restrictive type of lung function reduction.
12. Socio-economic status (SES) of the people had significant influence on their lung function. People from lower SES had greater prevalence of lung function deficits both in urban and areas. For instance, 43.7% non-smokers of Delhi belonging to low SES had lung function decrement compared with 35.2% and 27.8% citizens from medium and high SES respectively. Persons belonging to low

SES had greatest prevalence of all types of lung function deficits- restrictive (24.2% vs. 17.3% in high SES, $p<0.05$), obstructive (12.0 vs. 5.9, $p<0.05$), as well as combined type (7.5 vs. 4.4%, $p<0.05$) of lung function deficits.

13. Besides gender, smoking habit, BMI and SES, particulate air pollution was positively associated with lung function deficits. A strong negative correlation was found in Spearman's correlation test between PM_{10} level and all lung function measurements. The correlation was strongest for FVC (rho = 0.74, $p<0.0005$), followed by PEFR (rho=0.66, $p<0.0005$), and FEV_1 (rho=0.62, $p<0.0005$). After controlling potential confounders like age, gender, BMI and SES, conditional logistic regression analysis showed positive association between PM_{10} level in ambient air with restrictive (OR= 1.33, 95% CI 1.17-1.58) as well as obstructive (OR=1.42, 95% CI 1.22-1.89) type of lung function deficits.

14. The impact of particulate air pollution was further evidenced from the regional distribution of the prevalence of lung function deficits among the non-smoking residents of Delhi. Decrement in lung function was more prevalent in North (43.8%), Central (41.1%) and West Delhi (40.9%), where the RSPM level was relative high compared with relatively cleaner East (37.0%) and South Delhi (27.4%).



CHAPTER-5.0

ASSESSMENT OF CELLULAR LUNG REACTION TO DELHI'S AIR POLLUTION

5.1 INTRODUCTION

In the previous chapter adverse effects of Delhi's air pollution on lung function of the residents was demonstrated. A few studies have been conducted in Delhi (Chhabra et al., 2001, Natarajan et al., 2003, Ray et al., 2004, 2005) and elsewhere in India (Rao et al., 1992; Behera et al., 1998; Vijayan et al., 2000; Chattopadhyay et al., 2003; Singh et al., 2003; Sharma et al., 2004; Ingle et al., 2005) on impact of chronic urban air pollution on lung function. Like the present study, all these reports have demonstrated poor lung function in subject occupationally or environmentally exposed to high level of ambient air pollution.

Functional impairment is the ultimate manifestations of a complex pattern of cellular changes that took place for a long time inside the lung. Therefore, chronic exposures to city's air pollution are probably affecting the lung at the cellular level. To test this assumption, cellular lung response to Delhi's air pollution has been evaluated via sputum cytology and cytochemistry.

Objective

The specific objective of this study was to examine whether sustained exposures to ambient air pollution of the city are causing:

- Changes in cell growth and differentiation leading to the pathway of carcinogenesis
- Downregulation of pulmonary defense with special reference to the activity of alveolar macrophages, the first line of cellular defense in the lung,
- Inflammatory and allergic changes in the lung,
- Damage to the alveolar wall via upregulaton of tissue-degrading enzyme elastase, and
- Covert hemorrhages inside the lungs and airways that may impair lung activity

Importance and significance

Since most of the airway diseases including cancer take a long latent period to develop, the cellular changes are immensely helpful to identify the persons at risk so that the disease can be diagnosed at an early stage and medical intervention can be initiated for better therapeutic response. Secondly, the study is important from the point of biomonitoring of health effects of air pollution. In essence, it will help us to safeguard public health from the adverse effects of air pollution. For this reason this study has been undertaken. As a source of airway cells, spontaneously expectorated sputum was used, because it is a simple, sensitive and cost-effective technique ideally suited for such type of study in the developing countries.

5.2 MATERIALS AND METHODS

(a) Assessment of cellular lung reaction to air pollution: sputum cytology

Sputum collection

Sputum samples were collected from 550 control (male 357 and female 193) and 1050 residents of Delhi (male 706, female 344). All of them were never-smokers. This was done in order to eliminate the known modulatory influence of smoking on sputum cytology. The participants were instructed to wash their mouth with saline water and to cough vigorously to expectorate sputum. The samples were collected in a sterile plastic container. Four smears were made on clean glass slides from the non-transparent high viscosity part of each sample.

Fixation

The slides were semi-dried in air, and fixed in appropriate fixatives immediately at the site of collection and brought to the laboratory at Kolkata for staining. The smears were fixed for 30 minute in ethyl alcohol for Papanicolaou staining, 20 min in buffered formalin (40% formaldehyde in 0.1M phosphate buffer, pH 7.4, 3:1, v/v) for non-specific esterase, 10 min in 10% formalin for Perl's Prussian blue reaction, and 10 min in buffered formalin for elastase.

(i) Papanicolaou (Pap) staining for cytology

Papanicolaou is a multichromatic stain used principally on exfoliated cytologic specimens to get information about the cellular integrity, differentiation and functional state of different cell types. The staining was done following the procedure of Hughes and Dodds (1966; Figure 5.1).

Preparation of Harris' hematoxylin (stock solution)

Hematoxylin dissolved in absolute alcohol and
Potassium alum dissolved in distilled water
↓
The solutions are mixed and heated to boiling point
↓
Mercuric oxide added and stirred till color changes to purple
↓
Solution cooled in cold water bath and acetic acid added

Staining procedure

Fixed slides passed through graded alcohol and brought to water
↓
Stained with hematoxylin (1-2 min)
↓
Bluing under running tap water (10 min)
↓
Dehydrated in graded ethanol
↓
Stained with Orange-G (20 min)
↓
Washed in 95% ethanol (2- 3 dips)
↓
Stained with EA-50 (20 min)
↓
Washed in 95% ethanol (2- 3 dips)
↓
Dehydrated in absolute alcohol, cleared in xylene and mounted in DPX
↓
Observed under light microscope

Figure 5.1: Sputum cytology by Papanicolaou (PAP) staining

Reagents used

Harris' hematoxylin
Orange G 6
EA 50
Dehydrated alcohol
Distrene Plasticiser Xylene (DPX)

Preparation of stock solutions

Harris' hematoxylin (Sigma Chemicals, USA)	
Hematoxylin (Sigma Chemicals, USA)	1.0 g
Absolute alcohol (Bengal Chemical, India)	10.0 ml
Potassium alum	20.0 g
Distilled water	200 ml
Mercuric oxide (SRL, India)	0.5 g
Glacial acetic acid (Merck, India)	8.0 ml
Orange G 6	
Orange G crystals (Gurr, Germany)	10.0 mg
Distilled water	100 ml
Absolute alcohol	1 lit
Phosphotungstic acid (Sigma Chemical, USA)	0.15 g
EA 50	
Light green (Gurr, Germany) SF solution (0.5% in 95% alcohol)	45 ml
Bismarck brown (Gurr, Germany) solution (0.5% in 95% alcohol)	10 ml
Eosin yellow (Sigma Chemical, USA) solution (0.5% in 95% alcohol)	45 ml
Phosphotungstic acid	0.2 g
Lithium carbonate (Sigma Chem, USA) (saturated aqueous solution)	0.05 ml

Fixation and staining procedure

The semi dried smears were fixed in ethanol for 30 min. The fixed slides were then brought to 95% ethyl alcohol for 20 min, and ultimately to water through graded ethanol. The slides were stained with Harris' hematoxylin for 30 sec, subsequently rinsed in distilled water and placed in Scott's tap substitute for bluing. Thereafter, the slides were washed in running tap water, dehydrated in 70% and 90% ethanol, stained with Orange-G6 for 4 min, differentiated in 95% ethanol and subsequent staining with EA-50 solution for 4 min. Differentiation with absolute ethanol followed it. Finally, the slides were dehydrated in ethanol, cleared in xylene, mounted in DPX and observed under light microscope (Leitz, Germany).

Observation and scoring

The cell nucleus stained violet while cytoplasm and cytoplasmic granules stained green and/or orange depending on the differentiation stages of the cells. Under light microscope at least 20-50 high power

fields at 400x magnification were observed and the total and differential sputum cell count were scored. Frequency (%) of neutrophil, eosinophil, lymphocyte and epithelial cells present in the sputum was recorded. Emphasis was given on the identification of epithelial cell metaplasia and dysplasia, Curschmann's spiral, and indications of bacterial, viral and fungal infections.

(ii) Cytochemical localization of non-specific esterase

Staining for non-specific esterase, a marker enzyme for macrophages, was done by Fast Blue B method (Oliver et al., 1991; Figure 5.2).

Staining procedure

Fixed slides are incubated in a mixture of α- naphthyl acetate, acetone, 0.1M phosphate buffer (pH 7.4) and Fast Blue B for 30 min at room temperature

↓

Washed in H2O, dehydrated in graded alcohol

↓

Cleared in xylene and mounted in DPX

↓

Observed under light microscope

Figure 5.2: Alveolar Macrophage (Non-specific esterase)

Reagents used

Buffered formalin (fixative) – 0.1M phosphate buffer (pH 7.4) and formalin (40% formaldehyde, Merck, India) mixed in ratio 3:1, v/v.	50 ml
α-naphthyl acetate (Sigma Chem, USA)	10 mg
Acetone (S.D. Fine Chemicals, India)	0.25ml
Phosphate buffer (pH 7.4)	20 ml
Fast Blue B (BDH, England)	100 mg
Dehydrated alcohol (Bengal Chemical, India)	
Distrene Plasticiser Xylene (DPX, Qualigens, India)	

Fixation and staining procedure

The semi dried slides were fixed in buffered formalin for 20 min. The substrate solution was prepared by dissolving 10 mg of α-naphthyl acetate in 0.25 ml of acetone and then adding 20 ml of 0.1M phosphate buffer (pH 7.4), mixed well shaken well until most of the initial cloudiness disappeared. Thereafter 100 mg Fast Blue B was added, stirred and the mixture was filtered directly on to the smears. The slides were left undisturbed for 30 min at room temperature, then washed in running water, dehydrated in graded ethanol, cleared in xylene, mounted in DPX and observed under light microscope.

Observation and scoring

Non-specific esterase enzyme (NSE) activity was indicated by golden-brown staining in macrophages. At least 10 high power fields (hpf) at 400x magnification under microscope were observed and the average number of alveolar macrophages (AM) was recorded. The examined samples were graded on the basis of number of AM per hpf into four categories: Low, up to 5 AM/hpf; Moderate, 5.1 – 10 AM/hpf; High, 10.1 – 40 AM/hpf; and alarming, >40 AM/hpf. Moreover, the slides were also classified into different subgroups, depending upon the morphology, particle load and number of nuclei in AM.

(iii) Detection of tissue degrading enzyme, elastase

The elastase activity in AM and neutrophils was studied by Simultaneous Azo-dye coupling method following the procedure of Lojda et al., (1991, Figure 5.3).

Staining procedure

Fixed slides are incubated in a mixture of naphthol AS-D chloroacetate, N,N dimethylformamide, phosphate buffer (pH 6.8) and Fast Blue B (60 min, 37°C)

↓

Washed in distilled water

↓

Dehydrated in graded ethanol

↓

Cleared in xylene and mounted in DPX

↓

Observed under light microscope

Figure 5.3: Detection of tissue degrading enzyme- elastase

Reagents used

Naphthol AS-D chloroacetate (Sigma Chemical, USA)	3 mg
N,N-dimethylformamide (Sisco, India)	0.5 ml
0.1 M Phosphate buffer (pH 6.8)	10 ml
Fast Blue B (BDH, England)	10 mg

Fixation and staining

The slides were fixed in buffered formalin for 10 min. The incubation mixture was prepared by dissolving 3 mg of naphthol AS-D chloroacetate in 0.5 ml of N,N-dimethylformamide and 10 ml of 0.1M phosphate buffer (pH 6.8). Thereafter, 10 mg of Fast Blue B was added, mixed well and filtered. The slides were incubated in this mixture for 60 min at room temperature, dehydrated in ethanol, cleared in xylene, mounted in DPX and observed under light microscope.

Observation and scoring

Elastase enzyme activity in neutrophils and macrophages was indicated by blue color of the cytoplasm. Background blue staining of the slides indicates extracellular elastase enzyme presumably liberated by the neutrophils and macrophages. The enzyme activity was graded subjectively as low, moderate and high on the basis of intensity of color reaction.

(iv) Detection of iron deposition in lung: Perl's Prussian blue staining for siderophages

Perl's Prussian blue reaction was done to identify deposition of ferric iron (hemosiderin) in airway and inflammatory cells, especially the AM, by the method of Pearse (1985, Figure 5.4).

Staining procedure

Fixed slides are rinsed in distilled water
↓
Incubated in 2% potassium ferrocyanide & 2% HCl (60 min)
↓
Washed in distilled water
↓
Dehydrated in graded ethanol
↓
Cleared in xylene and mounted in DPX
↓
Observed under light microscope

Figure 5.4: Diagnosis of hemorrhage in lung (Perl's Prussian blue reaction)

Reagents used

10% formalin (fixative; Merck, India)	
2% HCl (Merck, India) in iron-free distilled water	25 ml
2% Potassium ferrocyanide (SD Fine Chem, India) in iron-free distilled water	25 ml

Fixation and staining

The staining was done in 205 sputum samples from control subjects and 411samples from the residents of Delhi. The slides were fixed in 10% formalin for 10 min at room temperature. Then the slides were brought to water and were exposed to a fresh mixture of equal parts of 2% potassium ferrocyanide and 2% HCl for 45 minutes. The slides were then washed in distilled water, dehydrated through graded ethanol, cleared in xylene and mounted in DPX.

Observation and scoring

Iron-containing AMs are known as siderophages. Presence of ferric iron in AM gives deep blue color reaction. Using a light microscope a minimum of 10 high power fields at 400x magnification were counted and the average frequency of siderophages was scored. The magnitude of iron

deposition in macrophages was graded subjectively into 5 categories: 0, no stainable iron deposits, 1+, iron deposits visible in 1–25% of AM, 2+, iron deposits visible in 26–76% of AM; 3+, iron visible in 77–100% of AM and 4+, ferric iron deposits are visible by naked eye inspection (Kolberg et al., 1975).

Golde score

The Golde score was used to assess alveolar hemorrhage (Golde et al., 1975). In slides stained for Perls Prussian blue reaction, an average of 100 AM were graded for hemosiderin on a scale of 0-4, 0 being the minimum and 400 the maximum score for 100 macrophages. The mean score of 100 AM was calculated. Hemosiderin resorption was considered normal if the Golde score ranged from 0-20, medium from 20-70, and high when the score was more than 70 (Golde et al., 1975). The scoring was done in a blinded fashion in all cases.

(b) Statistical analysis

All data are expressed as mean± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between cellular lung and possible confounders such as RSPM levels. Spearman's rank test for continuous variables and Chi-square test for categorical variables were done. $P<0.05$ was considered as significant.

5.3 RESULTS

Percentage of representative samples

Altogether, 180 samples (70 from Delhi and 110 from control) were discarded, as they were not representative of the lower airways because of the absence of alveolar macrophages (AM). The findings of the 1420-satifactory sputum samples (980 from Delhi and 440 from control subjects) are presented in Table 5.1.

Table 5.1: Sputum cytology of non-smoker adults of Delhi

Cell type	Control (n=440)	Delhi (n=980)
Cells/hpf	46.2± 11.9	76.2± 24.9*
Neutrophil (%)	64.2± 11.6	62.7±8.6
Neutrophil/hpf	29.8± 5.6	48.1±7.6*
Eosinophil (%)	1.2± 0.5	4.3±2.5*
Eosinophil/hpf	0.6±0.2	3.3±1.6*
Lymphocyte (%)	6.2±2.5	6.2±2.5
Lymphocyte/hpf	2.9±1.3	4.7±2.5*
AM (%)	15.2±3.5	17.2±3.5
AM/hpf	6.9±1.6	12.9±2.6*
Epithelial cells (%)	8.7±0.6	9.5±3.6
Epithelial cells/hpf	4.1± 2.6	7.3±2.8*

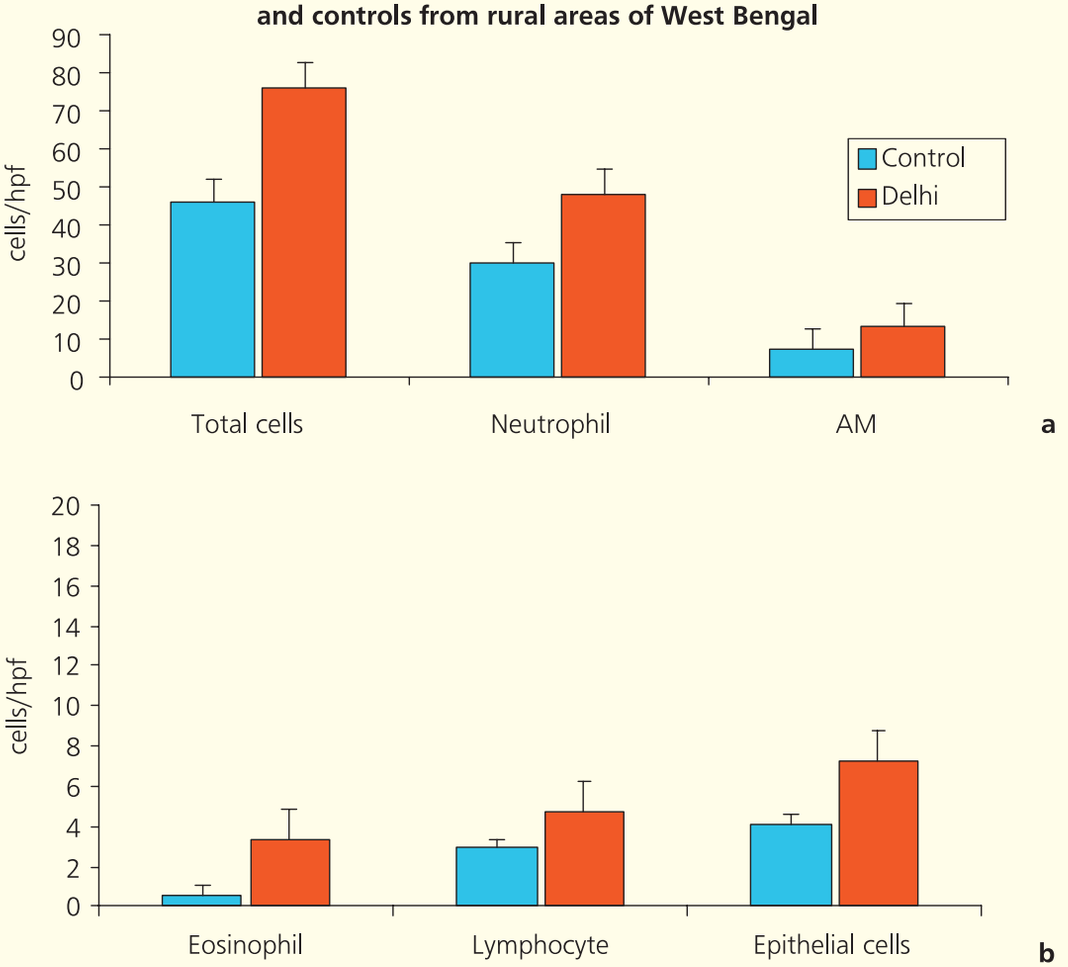
The results are expressed as mean ±SD; hpf, high power field of microscope (x400);
**, p<0.05 compared with control*

5.3.1 Sputum cytology

(a) Rise in inflammatory cell numbers in sputum of the residents of Delhi

Compared with controls, Pap-stained smears of sputum samples of the residents of Delhi were 1.6-times more cellular and contained significantly increased number ($p<0.05$) of all cell types. For example, the absolute number of neutrophils and lymphocytes were increased by 1.6-fold each, eosinophils number by 3.6-fold, AM by 1.9-fold and epithelial cells by 1.8-fold (Table 5.1, Figure 5.5 a, b). Remarkable increases in the number of these cells engaged in lung defense suggest inflammatory changes in the lung and airways of the residents of Delhi (Figure 5.6).

Figure 5.5 a, b. Sputum cytology of non smokers of Delhi and controls from rural areas of West Bengal



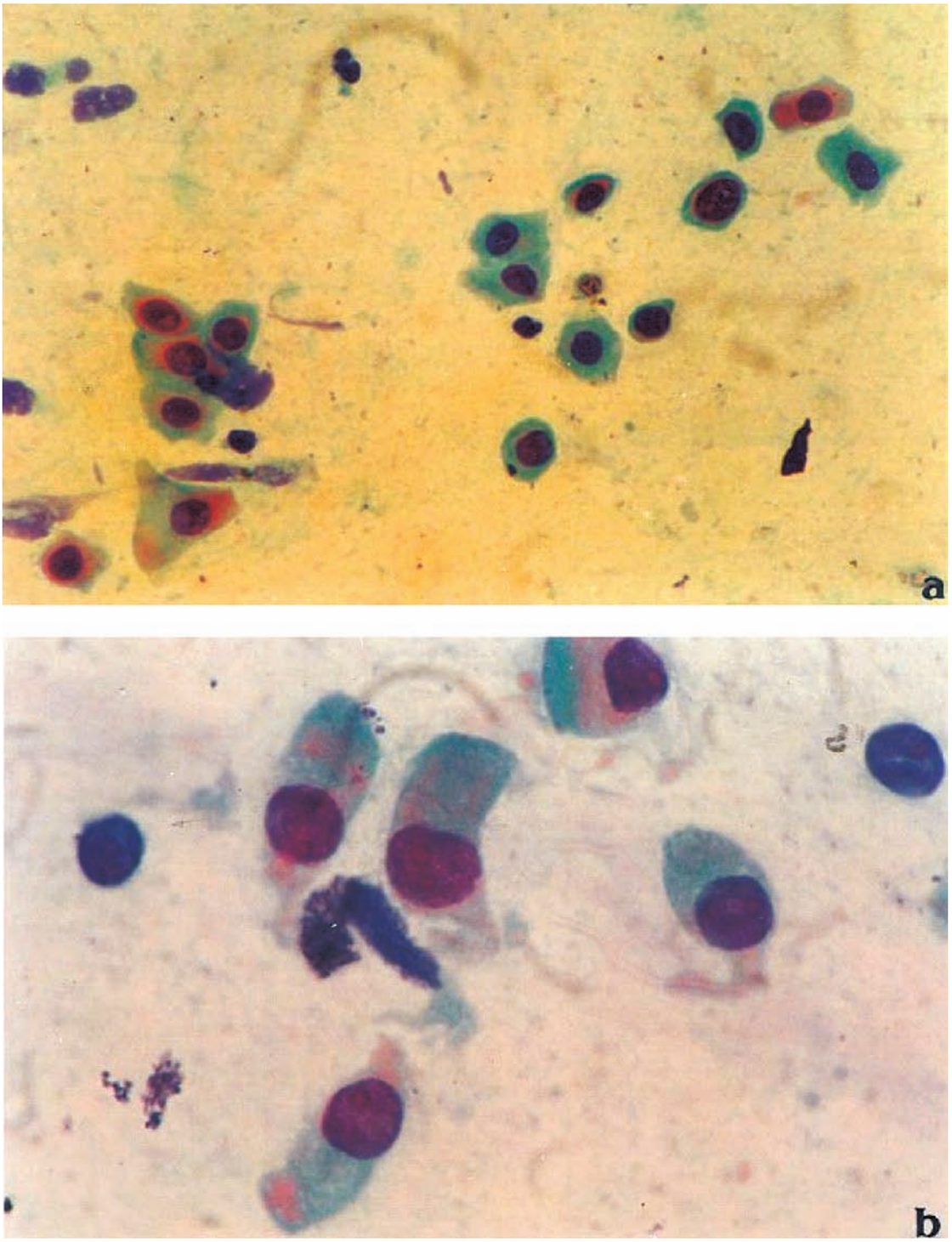


Figure 5.6: Sputum samples of a roadside hawker (a) and a trader (b) of Delhi showing sheets airway epithelial cells that may suggest airway injury. Papanicolaou-stained, x 400.

(b) Alveolar macrophage (AM) count: remarkably increased in Delhi

The residents of Delhi showed remarkable increase in AM number in sputum. NSE-positive AM per high power field (hpf) of light microscope (10x eye piece and 40x objective) was 12.9±2.6 in Delhi in contrast to 6.9±1.6 in controls ($p<0.001$; Figure 80a). Moreover, the AMs of the citizens of Delhi were heavily loaded with phagocytosed particles, and were larger in size (Figure 5.7, 5.8). For instance, the mean diameter of AM in rural controls was 16.2 µm. In contrast, mean diameter of AM in residents of Delhi was 27.8 µm (Table 5.2). Thus, the residents of Delhi who participated in this study had increased number of larger, particle-laden AM in their sputum compared with subjects from less polluted rural areas of West Bengal (Figure 5.9).

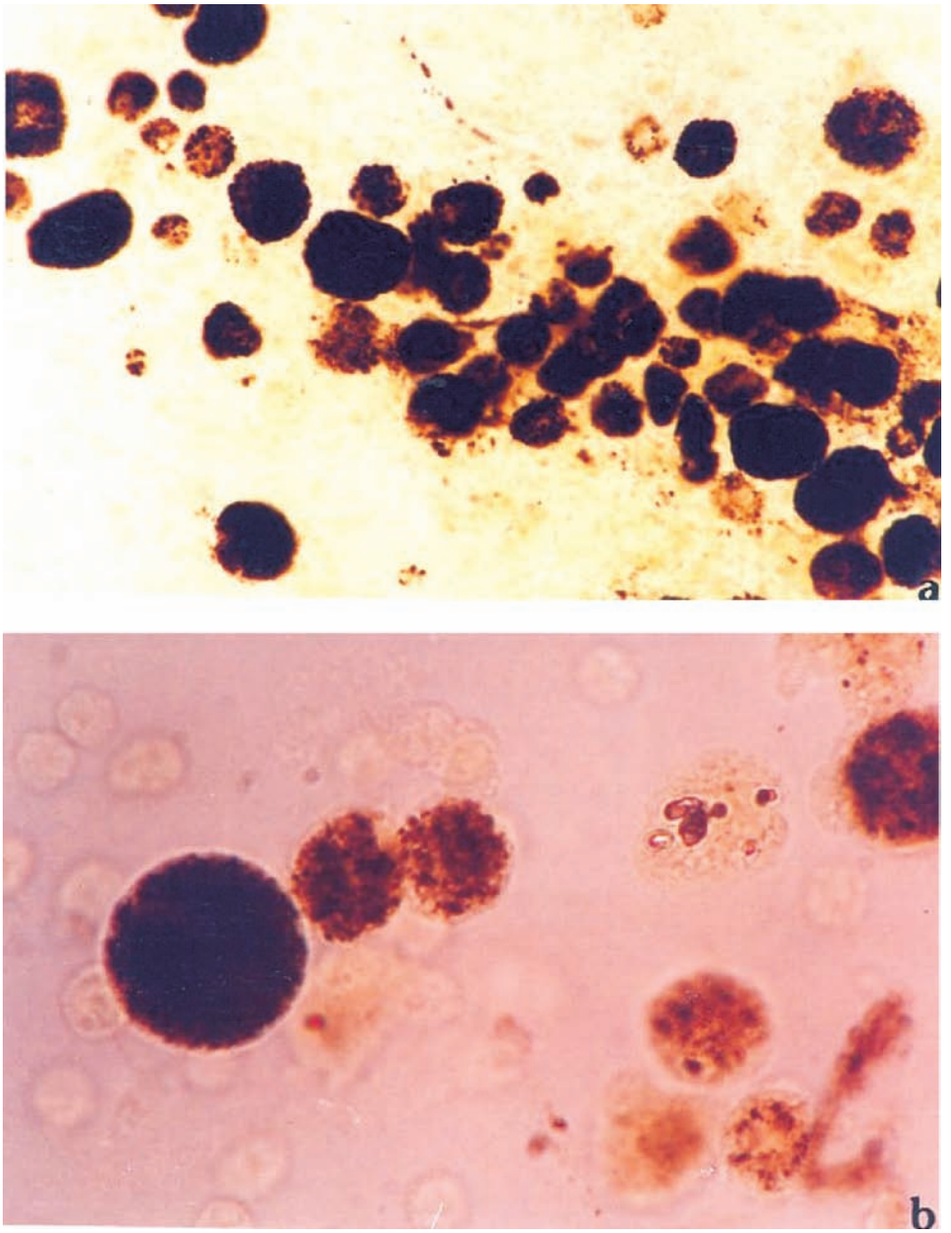


Figure 5.7: Photomicrograph of non-specific esterase stained slide of sputum of a traffic policeman of Delhi showing heavy deposition of carbonaceous materials inside the alveolar macrophages that has lead to massive increase in size of some cells (a, b). x 1000.

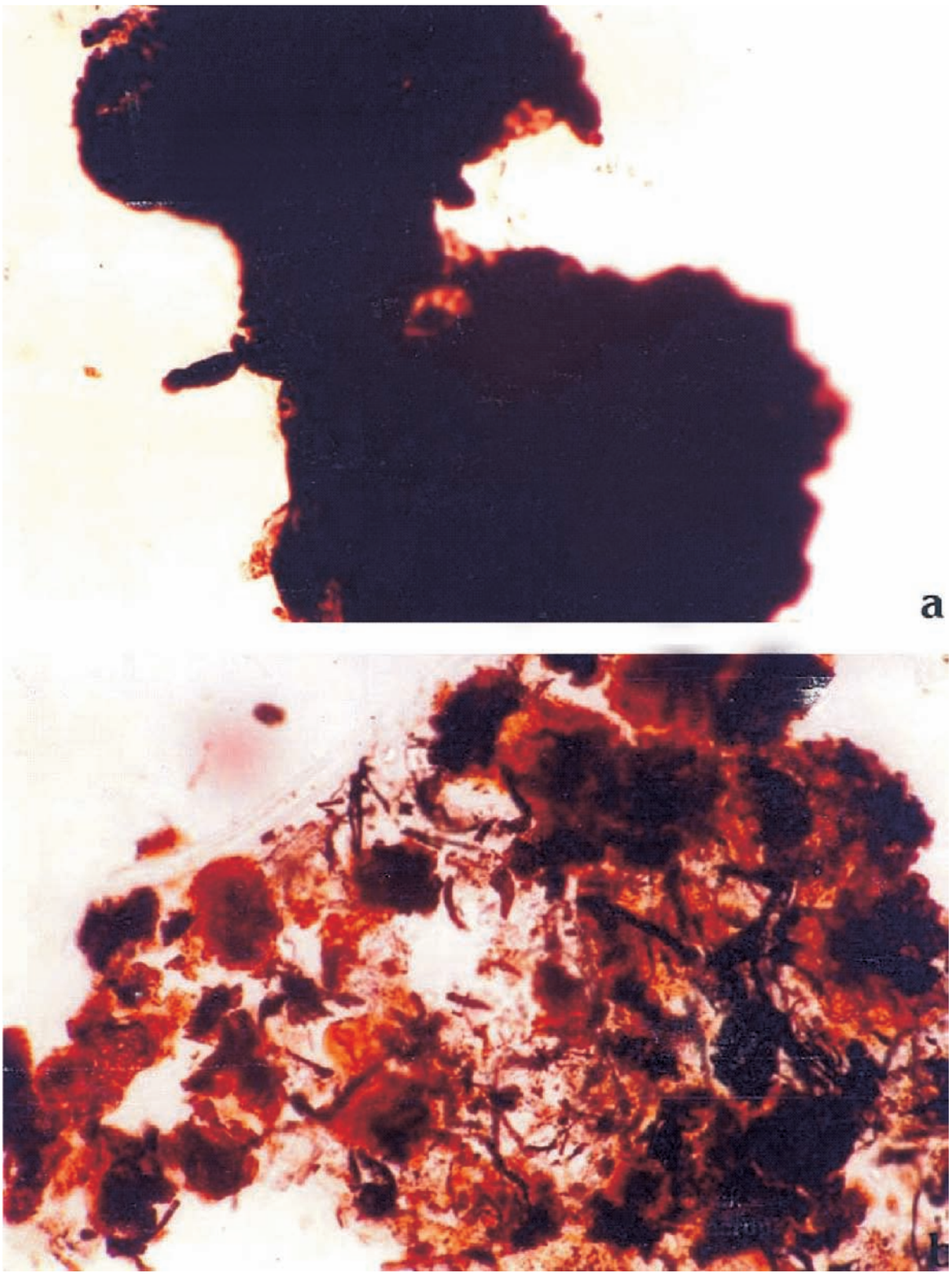


Figure 5.8: Sputum samples of Delhi's residents showing heavy deposition of carbonaceous (a) and fibrous materials (b) inside the lungs as a result of which cellular details are almost obliterated. Non-specific esterase-stained, x 1000.

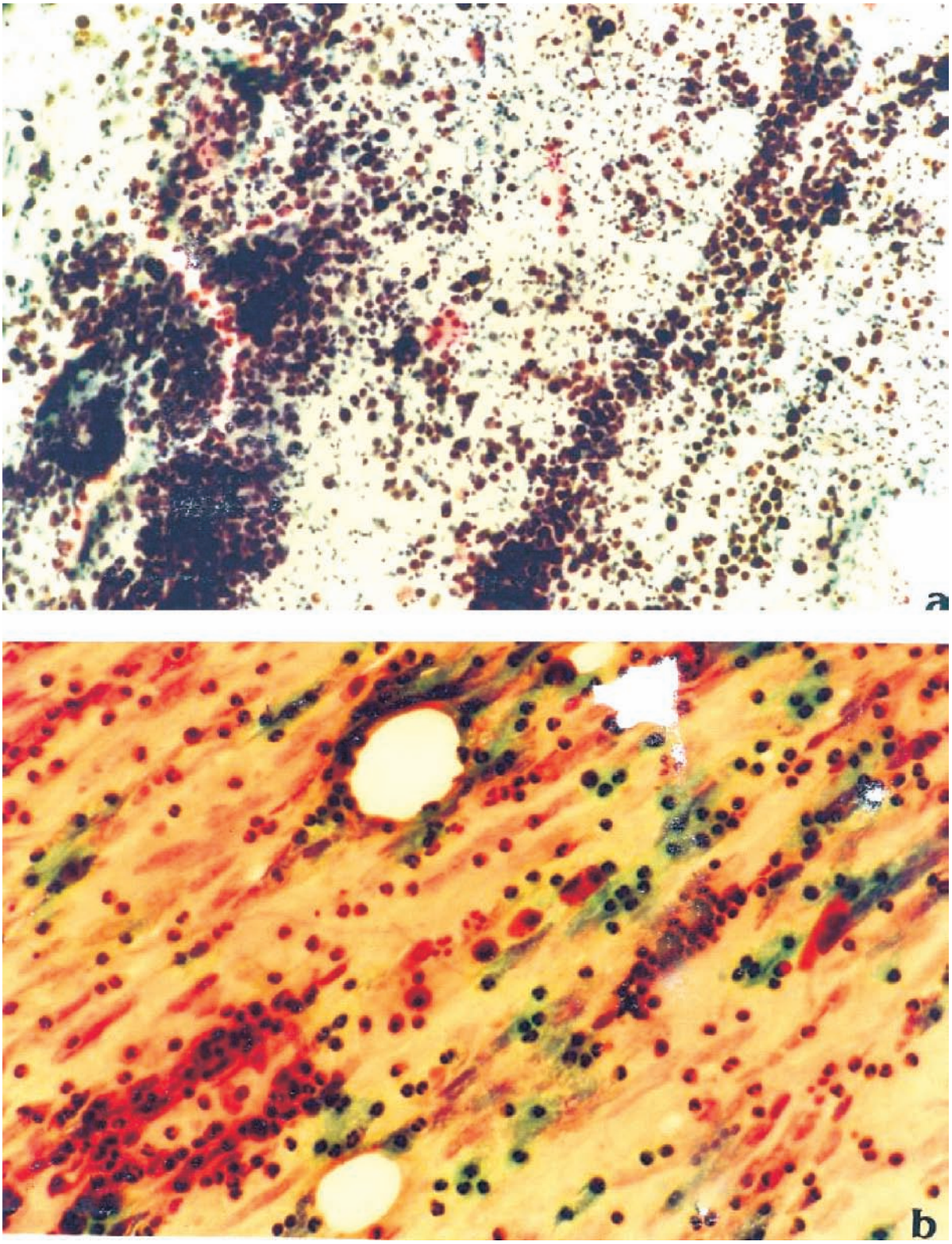


Figure 5.9: Photomicrographs of sputum showing marked increase in carbon-laden alveolar macrophages in an auto-rickshaw driver (a) and inflammatory cells in a student (b), both non smokers, of Delhi. Papanicolaou-stained, x 100 (a), x 200 (b)

Table 5.2: Diameter of alveolar macrophages in sputum

AM diameter (µm)	Control (440)	Delhi (980)
Mean ±SD	16.2 ±1.4	27.8 ± 2.6*
Range	13 - 22	16 - 45

*Results are mean ±SD; * p<0.05 compared with rural subjects*

AMs in general contain single, eccentric nucleus per cell. In some cases, particularly in case of sustained exposure to high level of air toxics, 2 or more nuclei are seen in a single AM. Abundance of multinucleated AM in sputum of Delhiites was found, while such change was rare in controls. For example, only 4% and 1% of AM of controls were bi- and trinucleated respectively. In contrast, 20% of AM in citizens of Delhi had two or more nuclei, of which 2, 3, and 3+ nuclei were found in 14%, 4%, and 2% macrophages respectively (Table 5.3, Figure 5.10, 5.11).

Table 5.3: Percentage of multinucleated alveolar macrophages in sputum

Number of nucleus per cell	Percentage of AM in sputum	
	Control	Delhi
One	95 ± 1	80 ± 7*
Two	4 ± 1	14 ± 4*
Three	1 ± 1	4 ± 1*
More than three	0	2 ± 1*

*Results are mean ±SD; * p<0.05 compared with rural subjects*

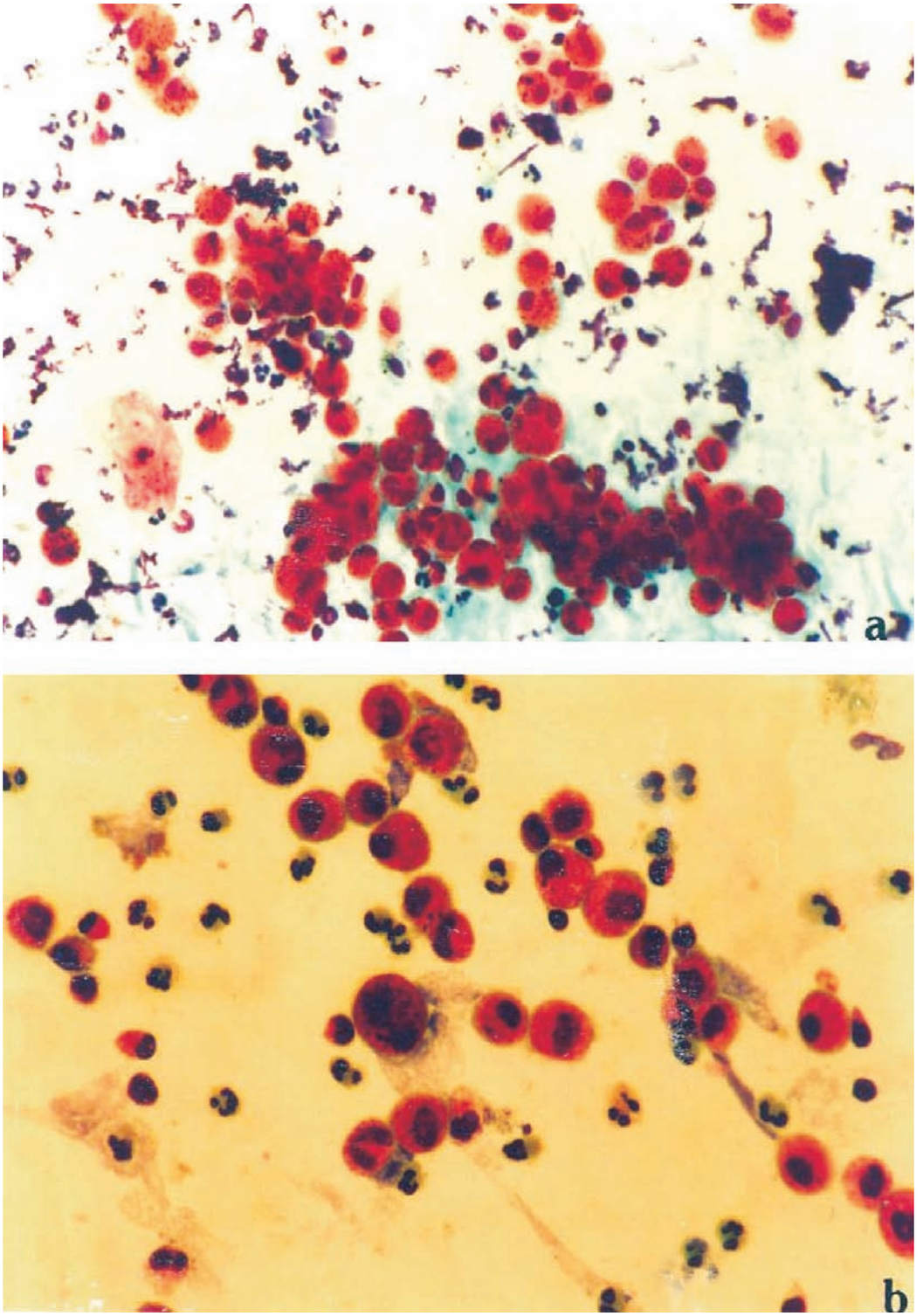


Figure 5.10: Photomicrographs showing aggregates of highly keratinized alveolar macrophages interspersed with inflammatory cells like neutrophils and eosinophils in sputum of citizens of Delhi who were lifelong non smokers. Note the presence of bi- and trinucleated alveolar macrophages which are generally absent in non smokers.
Papanicolaou-stained, x 200 (a), x 400 (b)

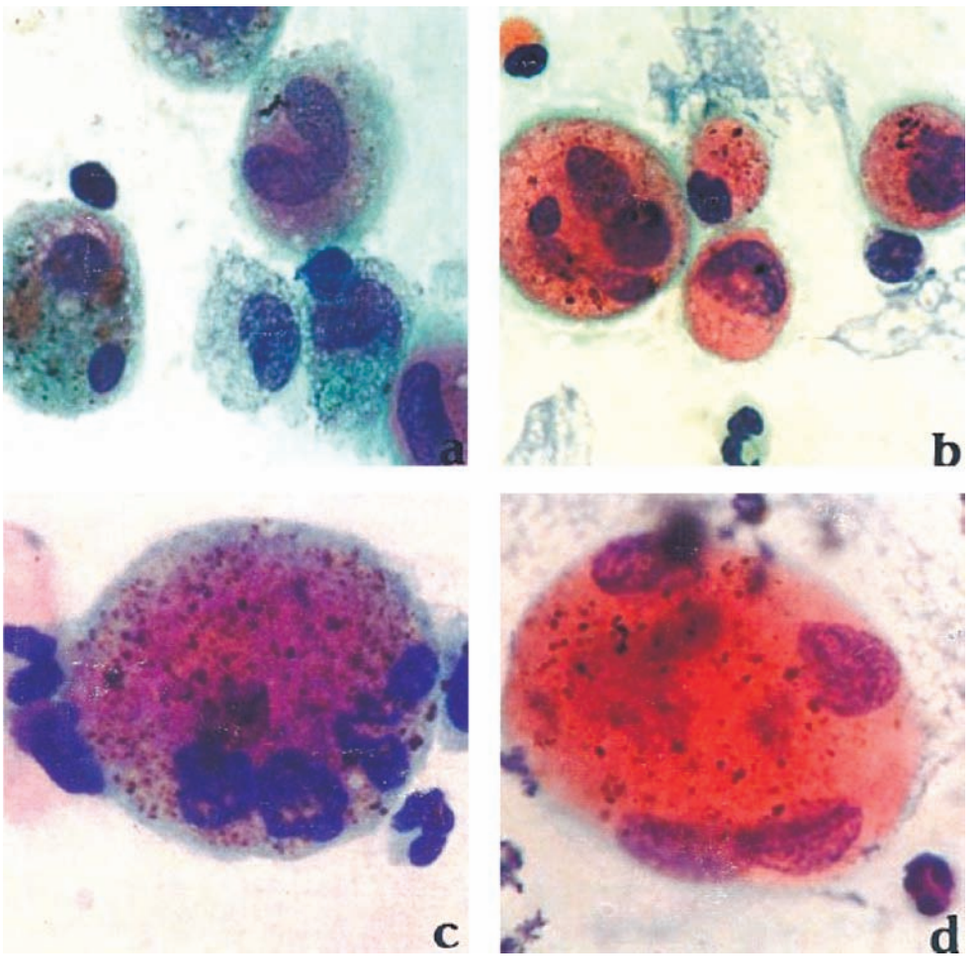


Figure 5.11: Photomicrographs of sputum of never-smoking citizens of Delhi showing nuclear heterogeneity in size and number of nucleus in the macrophages of a housewife (a), a roadside hawker at ITO (b), a taxi driver at Ajmeri Gate (c) and a traffic policeman at ITO (d). Papanicolaou-stained, x 1000.

Correlation with RSPM

The rise in AM number in spontaneously expectorated sputum and the percentage of individuals with high AM count (>10 AM/hpf) in Delhi were significantly correlated with the city's particulate pollution level (Table 5.4, Figure 5.12, 5.13, 5.14).

Table 5.4: Alveolar macrophage count in relation to air pollution level

Area	Average PM_{10} ($\mu g/m^3$)	Mean AM/hpf	% individual with high (>10/ hpf) AM count
North	140.1	11.8	17
North East	160.5	13.2	24
North West	158.2	12.8	22
Central	186.9	16.7	32
East	132.1	11.9	14
West	181.4	16.8	30
South	140.5	11.9	16
South East	104.2	7.2	10
South West	101.0	6.8	9

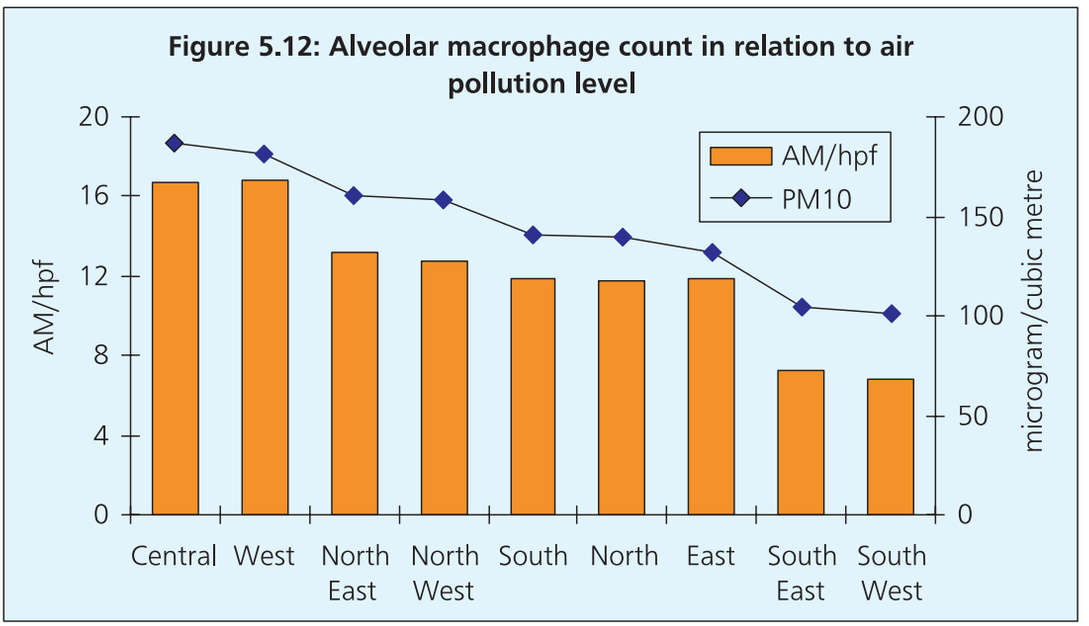


Figure 5.12: Alveolar macrophage count in relation to air pollution level

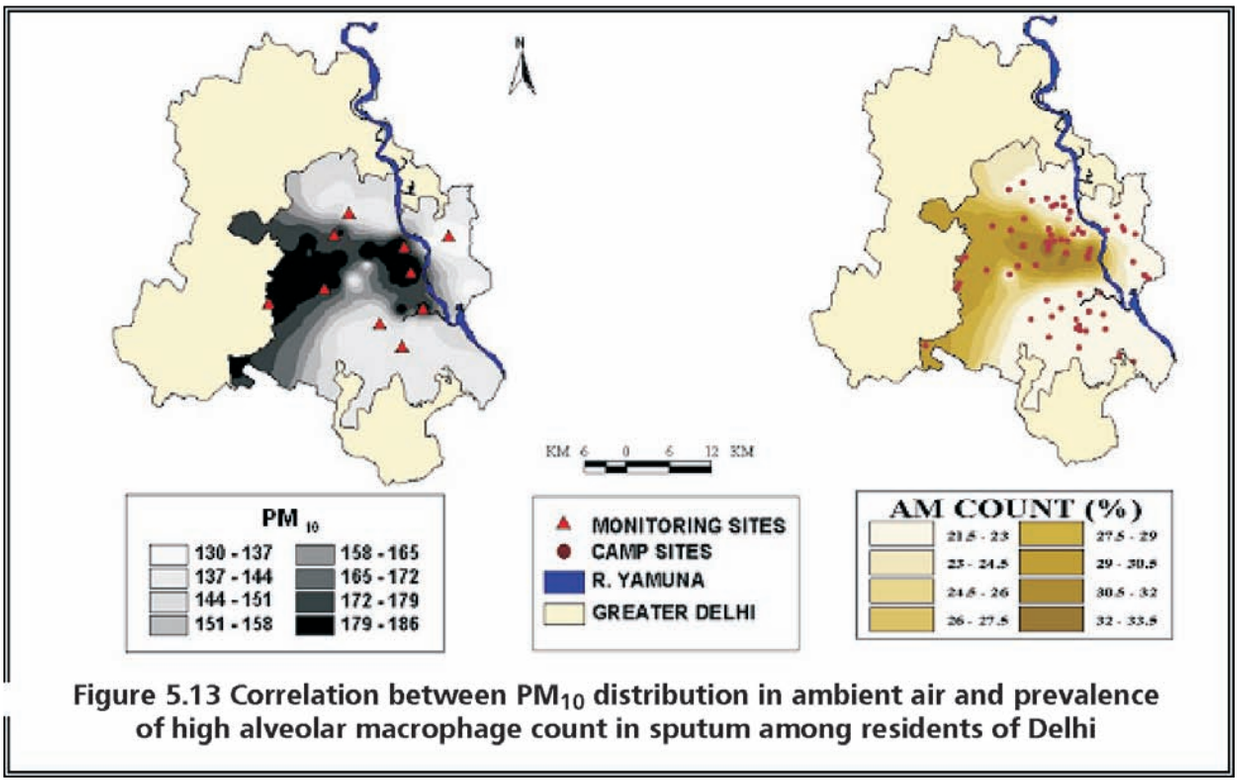


Figure 5.13 Correlation between PM_{10} distribution in ambient air and prevalence of high alveolar macrophage count in sputum among residents of Delhi

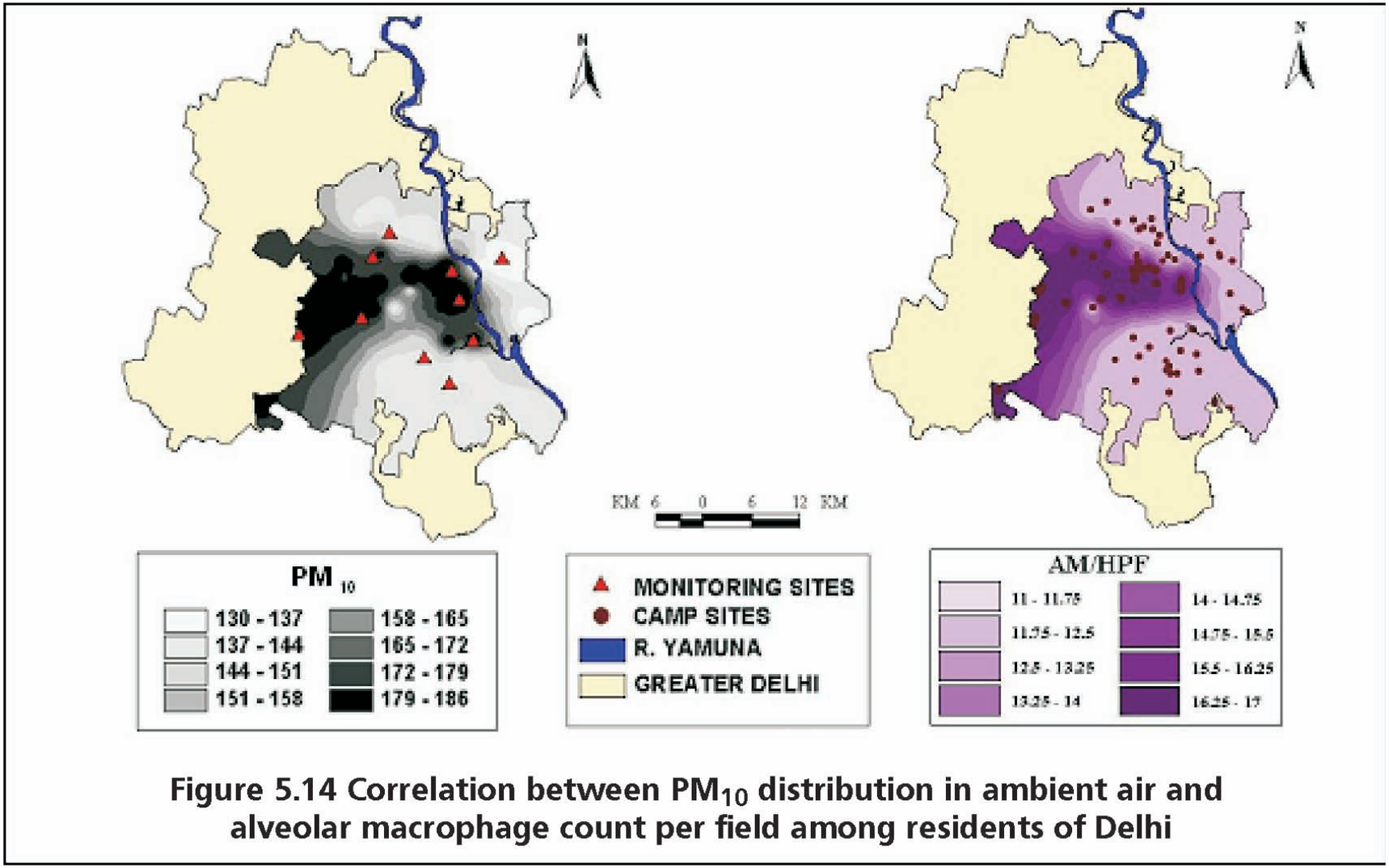


Figure 5.14 Correlation between PM_{10} distribution in ambient air and alveolar macrophage count per field among residents of Delhi

(c) Rise in the number of inflammatory cells in sputum

Besides AM, the number of neutrophils, eosinophils and lymphocytes in sputum was significantly increased ($p<0.05$) among the residents of Delhi. Compared to rural controls, eosinophil percentage was increased significantly in sputum of the citizens of Delhi (4.3% in vs. 1.2% in control, $p<0.001$, Table 5.1). The rise in absolute number of sputum eosinophils was even more - 5-fold over control (Table 5.1, Figure 5.5a, b, 5.15).

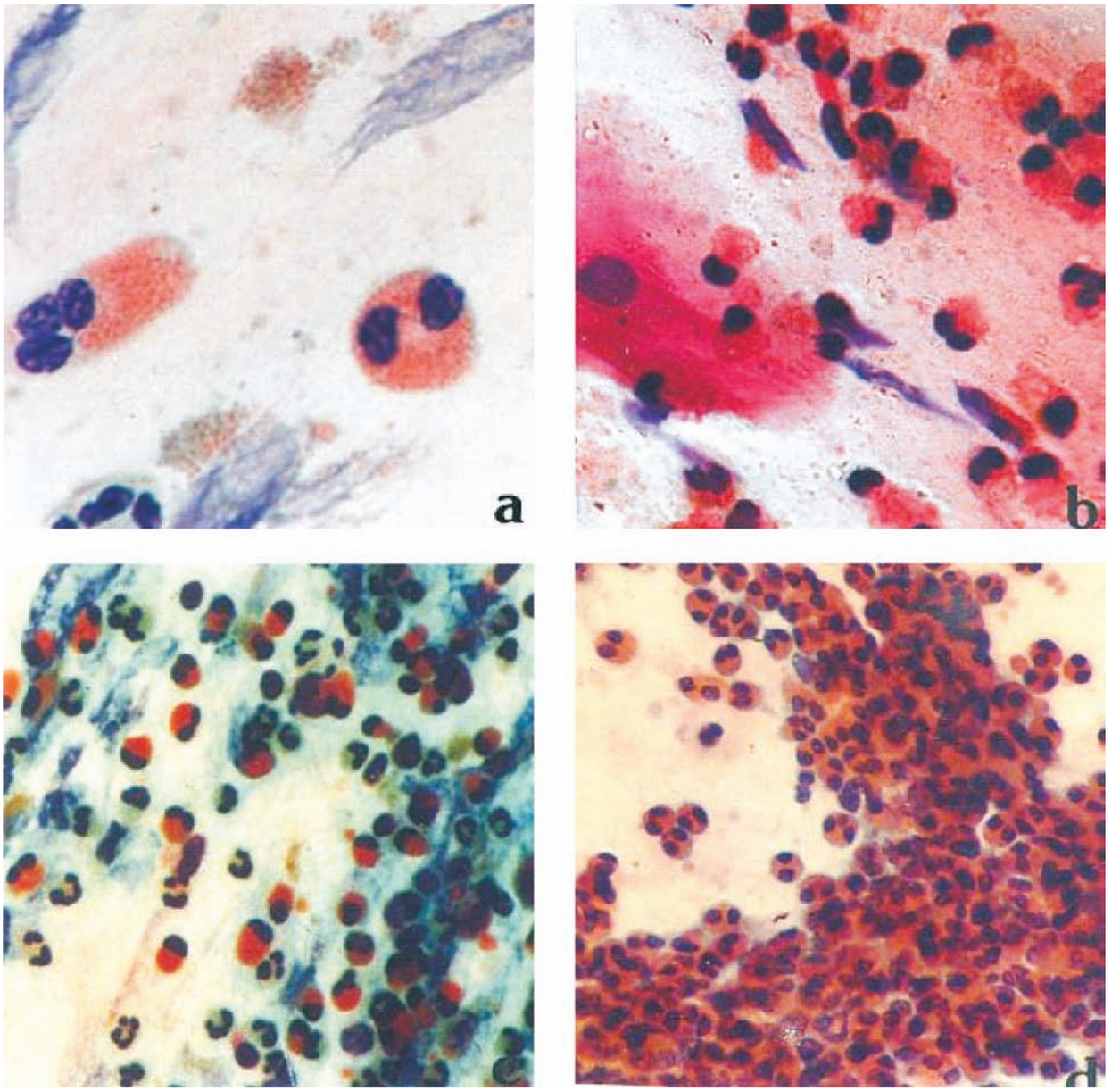


Figure 5.15: Photomicroraphs showing moderate to severe eosinophilia among a section of the residents of Delhi suggesting brobchial allergy, (a) a student, (b) housewife, (c) an office employee who was an asthmatic and (d) a taxi driver. Papanicolaou-stained, x 200 (a), x 400 (b,c), x 1000 (d)

Rise in the prevalence of sputum neutrophilia

The percentage of neutrophils in sputum of rural controls and citizens of Delhi was not significantly different (64.2% in control vs. 62.7% in Delhi). However, the absolute number of neutrophils per hpf was increased in Delhi by 61% ($p<0.01$, Table 5.1, Figure 5.5a, 5.16).

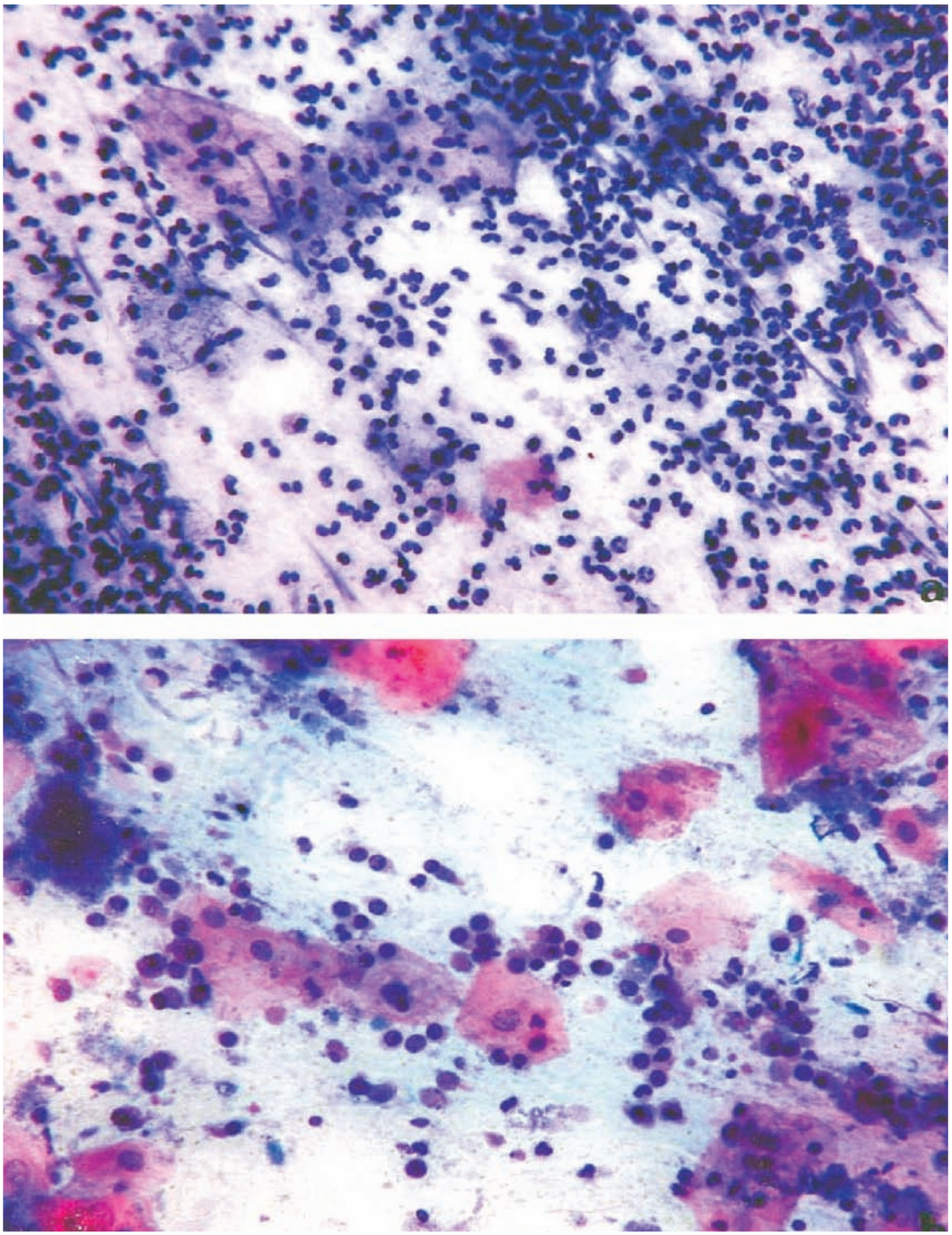


Figure 5.16: Sputum sample of a housewife (a) and a college student (b0 of Delhi showing sputum neutrophilia (a) and lymphocytosis (b) suggesting bacterial and viral infections respectively. Papanicolaou-stained, x 400

Change in sputum lymphocyte count

The absolute number of lymphocytes in sputum was significantly increased among the residents of Delhi, although the relative percentage of these cells in sputum was exactly similar in control and urban subjects (Table 5.1, Figure 5.5b, 5.16, 5.17).

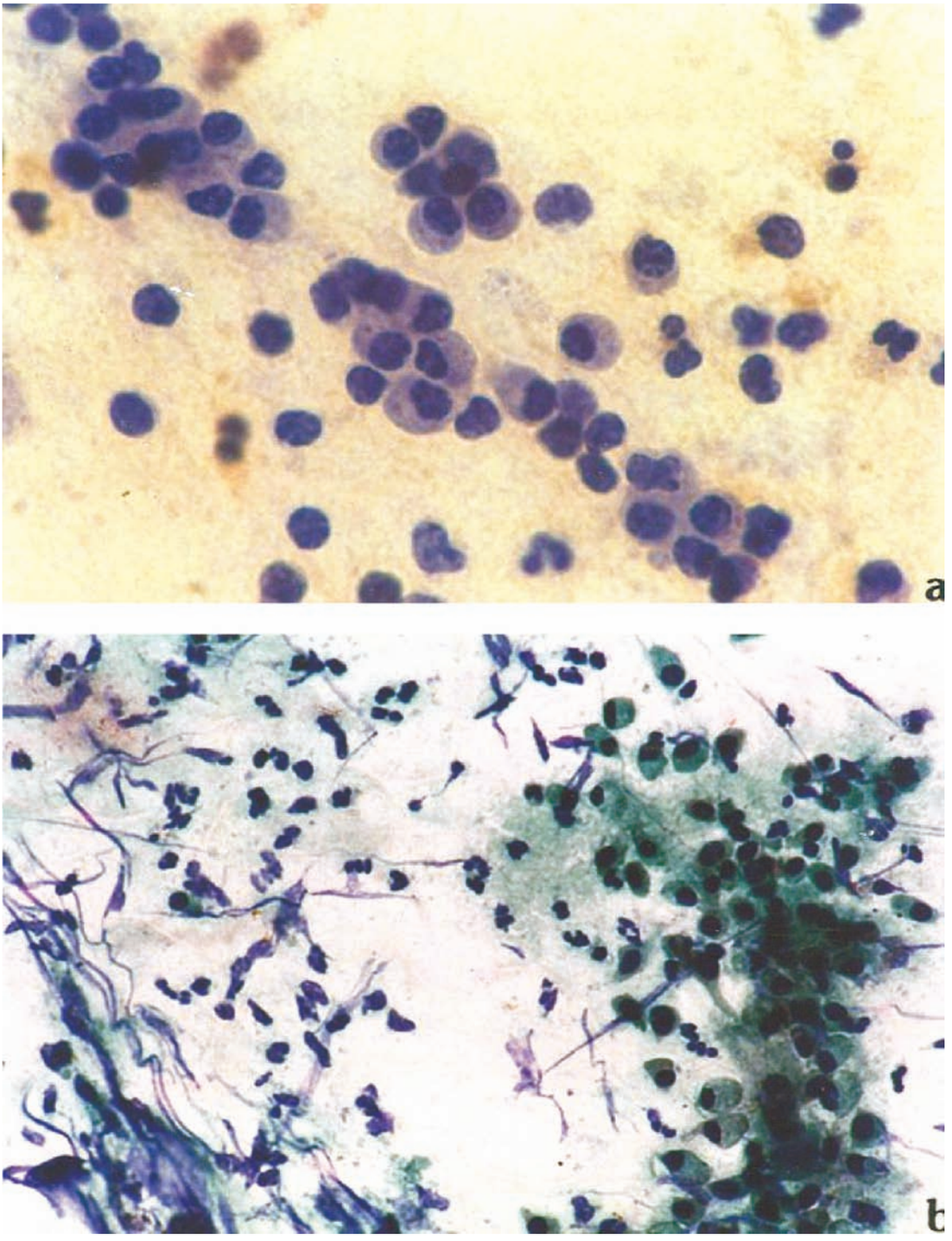


Figure 5.17: Sputum sample of residents of Delhi showing increased number of lymphocytes in sputum (a) along with thick mucus strands and clusters of goblet cells (b). Papanicolaou-stained, x 400

(d) Cytological changes in airway epithelial cells

Two important cytopathological findings in sputum of urban subjects were the presence of metaplasia and dysplasia of bronchial epithelial cells (Figure 5.18). Metaplasia was present in 14 out of 440 (3.2%) control samples. In contrast, 156 out of 980 (15.9%) sputum samples of the residents of Delhi had metaplasia of airway cells ($p<0.001$; Figure 5.19). Dysplasia of epithelial cells was found in 3% (29/980) samples of Delhi, whereas only 3 samples from control subjects (0.7%) had dysplasia ($p<0.05$; Table 5.5, Figure 5.19). Both metaplasia and dysplasia were more prevalent among residents of northern, western and central Delhi compared with residents of southern and eastern parts of the city. Therefore, metaplasia and dysplasia of airway epithelial cells, recognized as early changes towards the pathway of carcinogenesis, were 4 to 5- times more prevalent among the residents of Delhi who were chronically exposed to high level of air pollution including potential human carcinogens like benzene and benzo(a)pyrene.

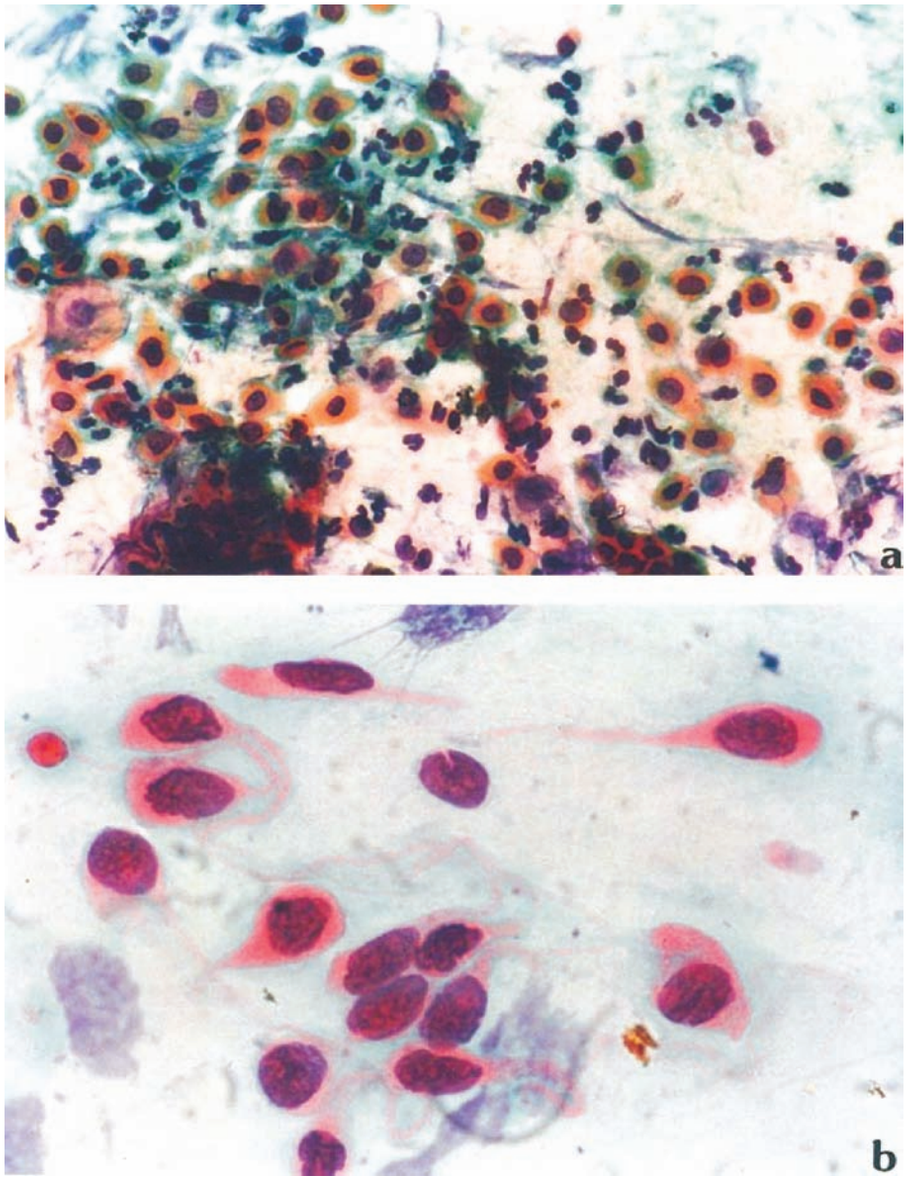


Figure 5.18: Metaplasia of airway cell in sputum of two non-smoking residents of Delhi: (a) trader and (b) an office employee. Keratinization and nuclear features of cells suggest airway injury and consequent faulty repair. Papanicolaou-stained, x 400 (a), x 1000 (b)

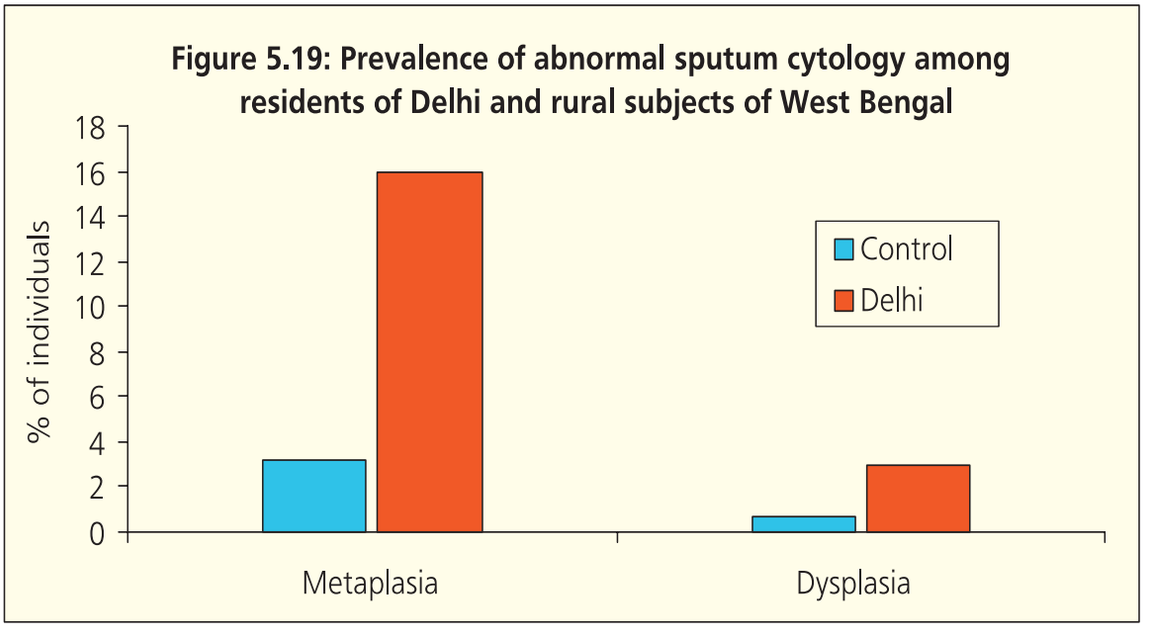


Table 5.5: Prevalence of metaplasia and dysplasia of epithelial cells in expectorated sputum of the residents of different areas of Delhi in comparison with that of control

Locations	Number of sputum samples	Metaplasia of epithelial cells, number (%)	Dysplasia of epithelial cells, number (%)
North Delhi	199	40 (20.1%)*	9 (4.5%)*
South Delhi	182	20(11.0%)*	2 (1.1%)*
East Delhi	208	26(12.5%)*	4 (1.9%)*
West Delhi	202	37 (18.3%)*	8 (4.0%)*
Central Delhi	189	33 (17.4%)*	6 (3.2%)*
Delhi, overall	980	156 (15.9%)*	29 (3.0%)*
Control areas	440	14 (3.2%)	3 (0.7%)

**, p<0.05 compared with control in Chi-square test*

(e) Correlation between particulate air pollution and cellular changes in sputum

The changes in sputum cytology were positively correlated with ambient PM_{10} level. Although the correlation was highly significant in Spearman's rank correlation test for all the cell types ($p<0.001$), the association was stronger for total cell count (rho value 0.795), absolute number of sputum neutrophils (rho =0.761) and eosinophils (rho = 0.644, Table 5.6). It seems therefore that air pollution of Delhi elicited inflammatory changes in the lung and the airways, and the changes are possibly mediated by PM_{10}.

Table 5.6: Spearman's rank correlation between PM10 level and sputum cell count

			Rho (rs) value	p value
PM10	with	Cells/hpf	0.795	<0.001
PM10	with	Neutrophil/hpf	0.761	<0.001
PM10	with	Eosinophil/hpf	0.644	<0.001
PM10	with	Lymphocyte/hpf	0.562	<0.001
PM10	with	Epithelial cells/hpf	0.448	<0.001
PM10	with	AM/hpf	0.581	<0.001

(f) Other cytological changes in sputum

Sputum samples of Delhi's inhabitants displayed several other cytological changes. Ciliocytophthoria, characterized by the presence of disintegrated distal tufts of cytoplasm with intact cilia from columnar epithelial cells was present in 32 out of 980 sputum samples (3.0%) from Delhi compared with 3 of 440(0.7%) of control samples (Table 5.7, Figure 5.5a, 5.20). Ciliocytophthoria is usually associated with respiratory virus infections.

Table 5.7: Cytological changes in Pap-stained sputum. Results are expressed as percentage of individuals with changed cytology

Finding	Control (n=440)	Delhi (n=980)
Ciliocytophthoria	0.7	3.0*
Aggregates of columnar epithelial cells	2.5	6.9*
Goblet cell hyperplasia	2.0	6.4*
Mucus strands	14.3	39.7*
Curschmann spiral	0.7	1.6
Koilocyte	1.1	2.7*
Charcot –Leyden crystal	0	0.6*
Alternaria	12.3	23.1*

**, p<0.05 compared with control*

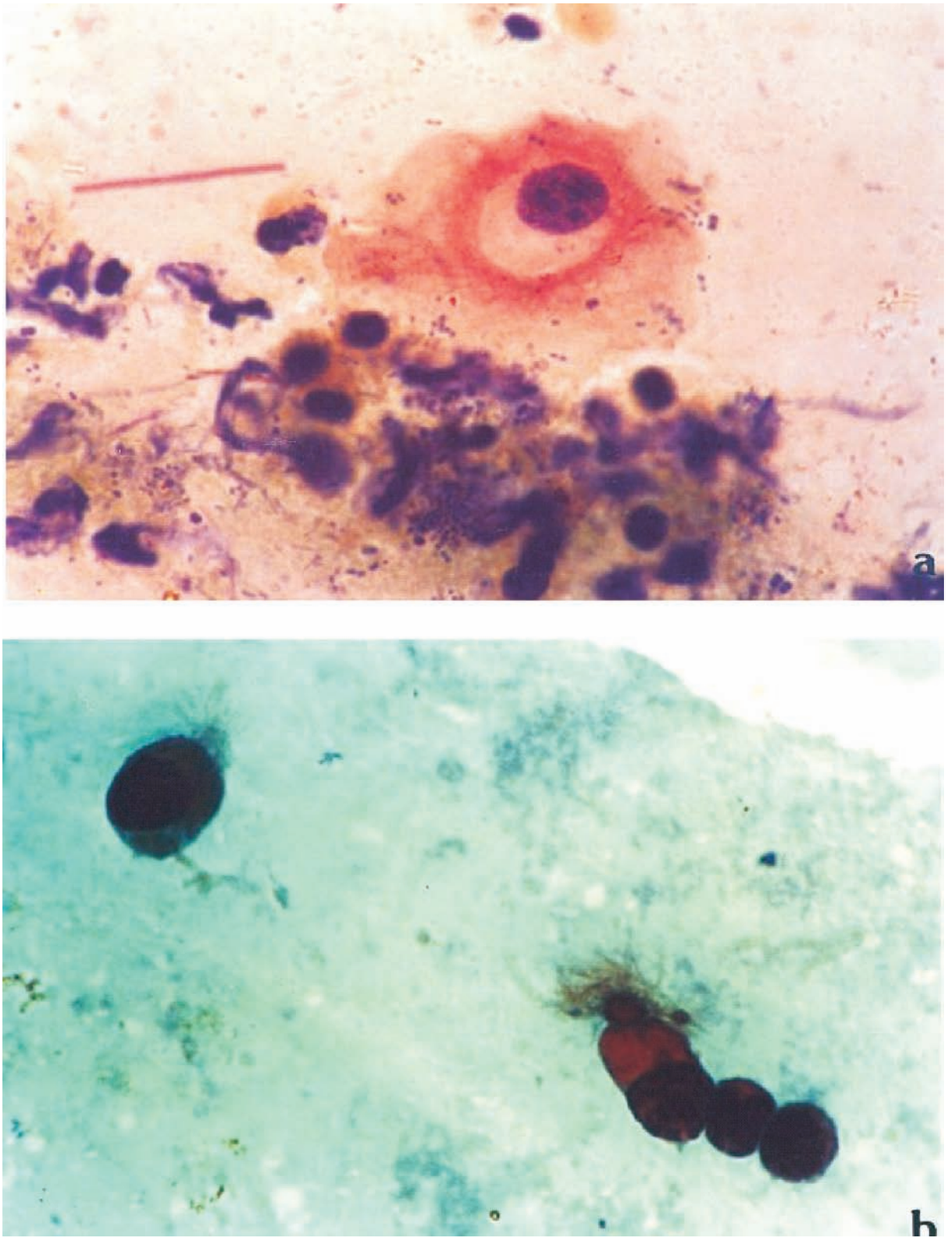
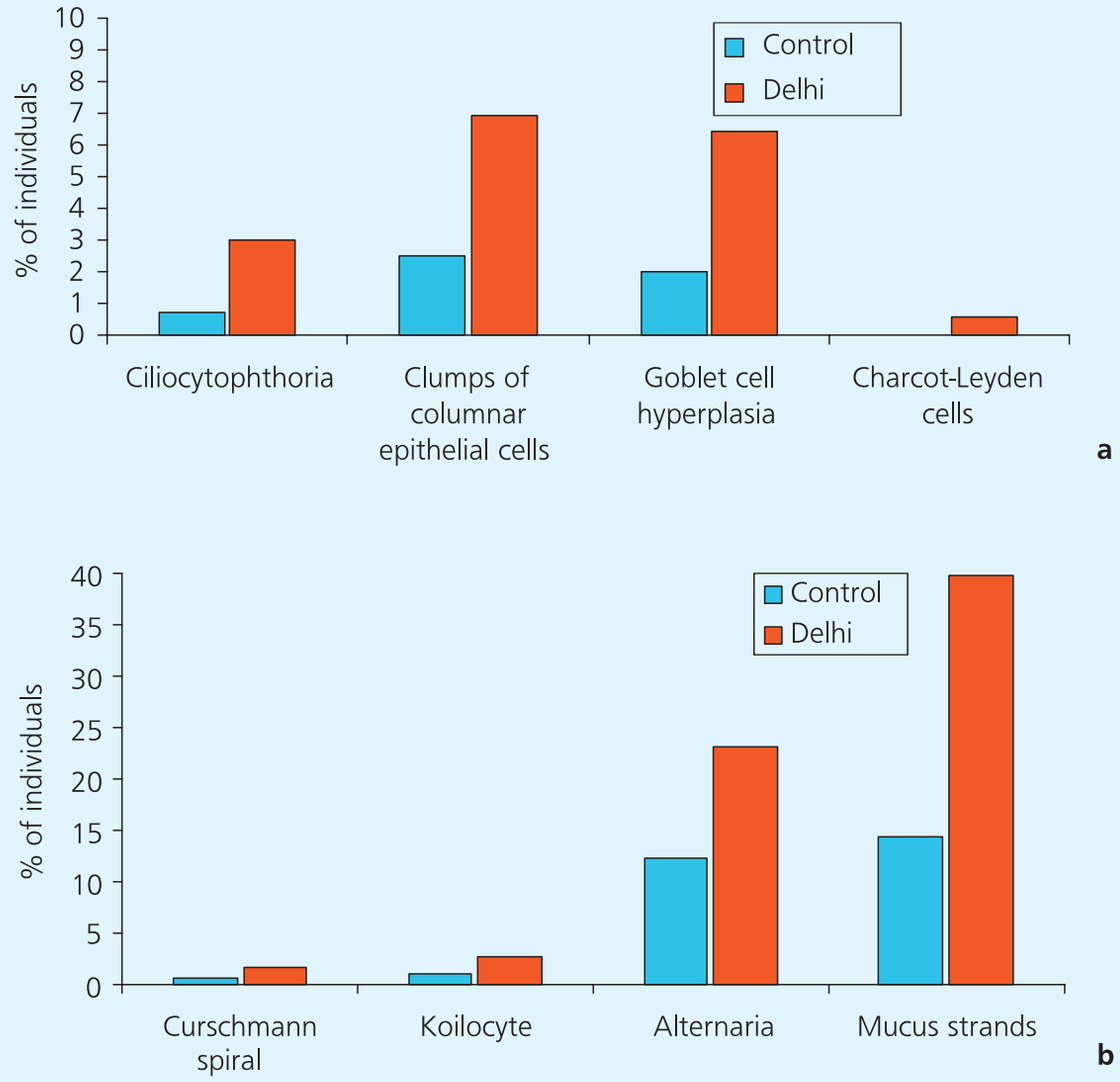


Figure 5.20: Sputum samples showing a loilocyte (a) indicating the possibility of Human Papilloma Virus (HPV) infection and ciliocytopthoria (b) which suggest cellular disintegration associated with viral pneumonia, Papanicolaou-stained, x 1000

Aggregates or clumps of ciliated and non-ciliated columnar epithelial cells were present in 68 (6.9%) sputum samples in Delhi compared with 11 (2.5%) of control samples (Table 5.7, Figure 5.21a). The changes are usually associated with injury to the airway wall.

Figure 5.21: Cytological alterations in the residents of Delhi and controls from rural West Bengal



Goblet cell hyperplasia is usually followed by mucus hypersecretion and accumulation of these cells in the sputum (Figure 5.22). Such condition was present in 63 (6.4%) samples from Delhi and 9 (2.0%) of control (Table 5.7, Figure 5.21a). Goblet cell hyperplasia signifies mucus overproduction and hypersecretion presumably to contain higher load of inhaled pollutants. Indeed, mucus strands were a common feature of the sputum samples of Delhi's inhabitants. In 39.7% of Delhi's samples mucus strands were present compared with 14.3% of control samples (Figure 5.21b, 5.17). Curschmann spiral, representing inspissated mucus plug, was present in sputum of 1.6% of Delhi's residents and 0.7% of controls (Table 5.7, Figure 5.21b, 5.22).

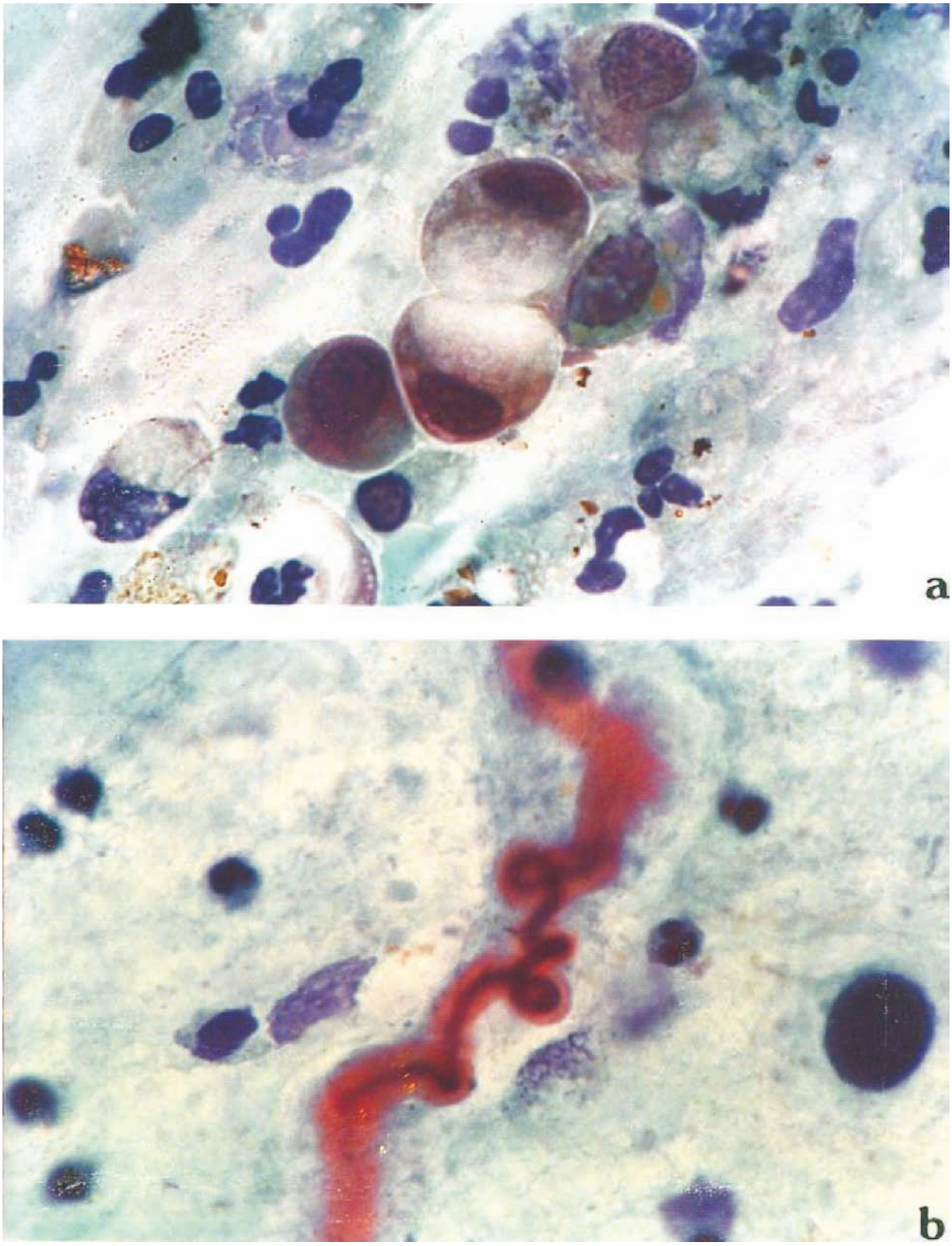


Figure 5.22: Sputum cytology showing hyperplasia of goblet cells (a) and Curschmann's spiral (b) in a non-smoking subject with chronic obstructive pulmonary disease. Papanicolaou-stained, x 1000

Koilocytes are distinguished from other cell types for the presence of perinuclear halo. These cells were present in 26 urban (2.7%) and 5 (1.1%) control samples (Table 5.7, Figure 5.21b, 5.20). Koilocytes are usually associated with human papilloma virus (HPV) infection. Therefore, greater prevalence of HPV infection in airway epithelial cells among the citizens of Delhi is envisaged.

The cell membrane of eosinophils contains several enzymes including lysolecithinase. Together with phospholipase A and D, lsolecithinase spontaneously forms rhomboid-shaped structures named as Charcot -Leyden crystals. These crystals were present in 6 (0.6%) sputum samples of Delhi but none in control samples (Table 5.7). The presence of these crystals indicates allergic condition (Figure 5.21a, 5.23).

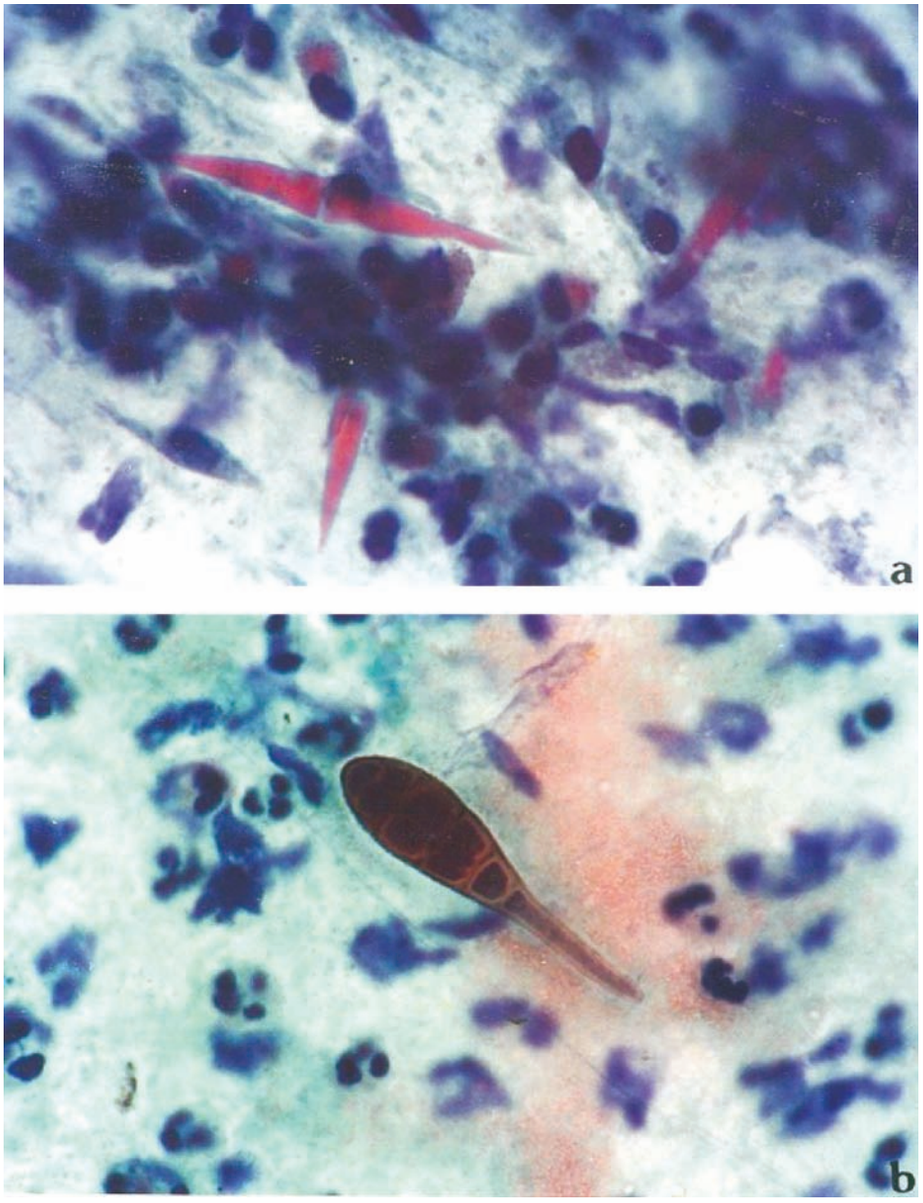


Figure 5.23: Photographs of sputum sample showing presence of Charcot-Laden crystals (a) and conidium of the fungus Alternaria (b) indicative of allergic lung disease and exposure to funal bio-aerosols respectively. Papanicolaou-stained, x 1000

Spores and conidium of the fungus *Alternaria* were detected in 226 (23.1%) sputum samples of Delhi compared with 54 (12.3%) control samples, suggesting greater chances of fungal aeroallergen exposure in Delhi (Table 5.7, Figure 5.21b, 5.23).

5.3.2 Functional alteration of sputum cells

The results described so far documented marked eosinophilia, neutrophilia and elevated AM number in sputum of persons chronically exposed to Delhi's air pollution. Investigations were also carried out to examine whether numerical changes in airway and lung defense cells were associated with functional alterations as well. Functional state of sputum AM and neutrophil was evaluated by cytochemical analysis of the generation and release of elastase.

(a) High elastase activity in AM and neutrophils of citizens of Delhi

Elastase is a tissue-degrading enzyme found abundantly in neutrophils and AM. Elastase helps in airway defense for its bacteria killing activity. But excess production and release of this enzyme may destroy the elastin protein of the alveolar wall, leading to the breakdown of alveolar wall causing emphysema. Localization of elastase was done in this study by enzyme cytochemistry in 93 non-smokers of Delhi and 55 age-and sex-matched non-smoking controls.

Cytochemical analysis revealed increased production and release of elastase by AM and neutrophils in sputum of Delhi's citizens (Figure 5.24). In control subjects, 42± 12% of AM were stained positively for elastase. In contrast, 73±5% AM of the participants from Delhi had detectable elastase activity. The absolute number of elastase-positive AM in sputum was 2.9± 0.8/hpf in control and 9.4±1.9 /hpf in citizens of Delhi ($p<0.001$; Figure 5.25). Moreover, the enzyme activity was moderate to high (2+ to 3+) in 56.7 ± 8.4 % AM of urban subjects compared with 34.2± 9.3% in controls ($p<0.05$), (Table 5.8, Figure 5.26).

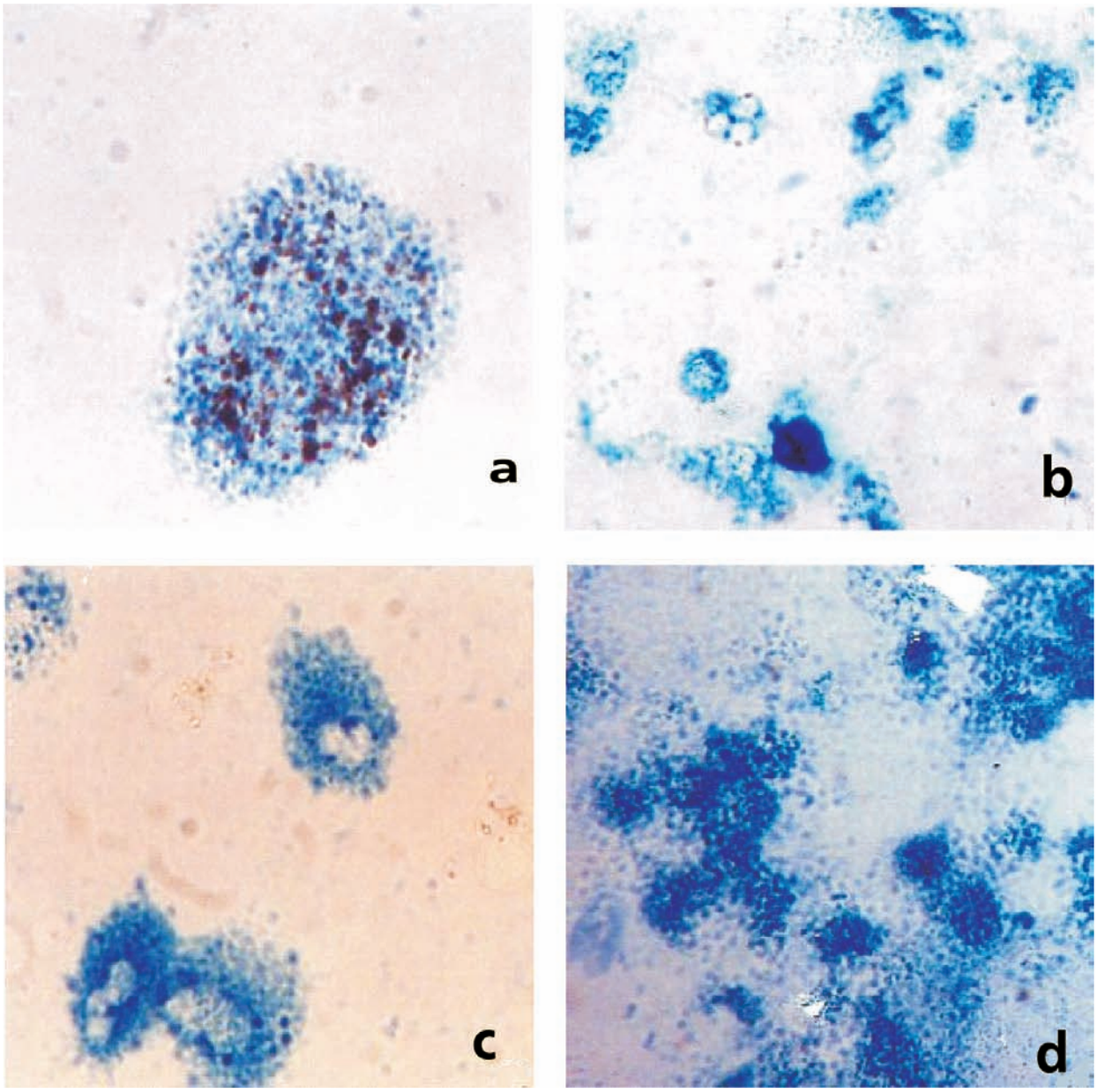


Figure 5.24: Photomicrograph showing cytochemical localization of elastase, a tissue degrading enzyme in neutrophils and alveolar macrophages (AM) in sputum. Elastase activity was moderate in neutroplils of control subjects (b) but high to very high in residents of Delhi (a, c). overproduced elastase is seen released in he surrounding that may cause tissue damage (d). upregulation of elastase production was recorded in AM of the residents of Delhi (a). Elastase-stained (Fast Blue B), x400 (b, c, d), x 1000 (a)

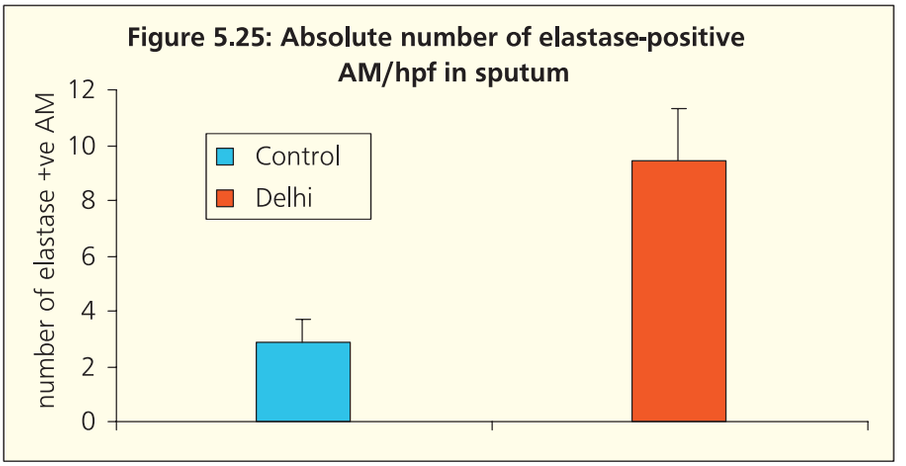
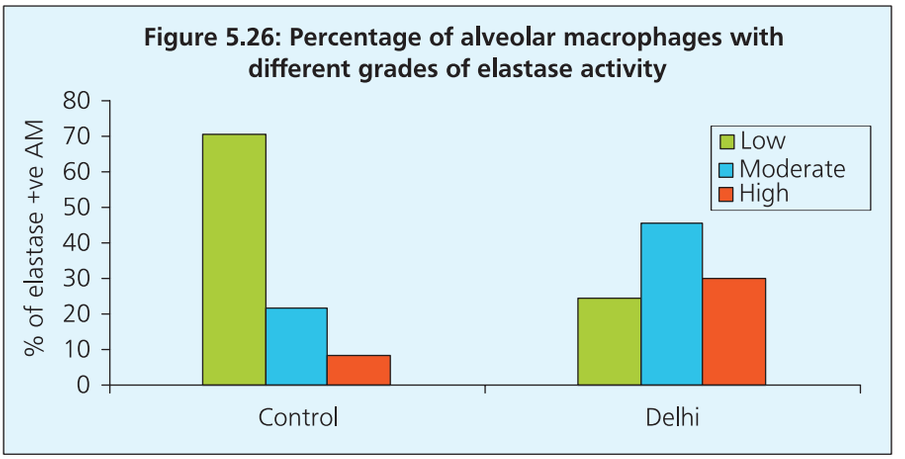


Table 5.8: Percentage of alveolar macrophages with different grades of elastase activity

Group	Low	Moderate	High
Control	70.3 ± 2.2	21.5 ± 1.1	8.1 ± 0.5
Delhi	24.5 ± 2.4*	45.5 ± 5.0*	30.0 ± 7.2*



Neutrophil elastase

All the neutrophils present in sputum of control and Delhi's residents were positive for elastase. But the relative activity of this enzyme was greater in residents of Delhi. Subjective grading of enzyme activity in a scale of 1+ (mild) to 3+ (high) revealed that 64 ± 12% of neutrophils of the residents of Delhi belonged to 3+ category, against 41± 8 % neutrophils of control subjects in similar category ($p<0.05$).

(b) Covert pulmonary hemorrhage among the residents of Delhi

In order to investigate whether chronic exposure to Delhi's air pollution was associated with microscopic hemorrhage inside the lung, Perl's Prussian blue reaction was carried out in 205 control and 411 Delhi's samples (all were never-smokers) for detection of hemosiderin iron in AM, because pulmonary hemorrhage is generally associated with abundance of iron-laden macrophages (siderophages) in sputum. Results showed that 29% of AM of the citizens of Delhi had iron deposits compared with 8% of iron-laden macrophages in sputum of control subjects. Thus, the urban subjects had 3 to 6-fold rise

in the percentage of siderophages (Figure 5.27). The increase in the total number of siderophages in sputum was even greater. The residents of Delhi had a mean of 3.7 siderophage per hpf compared with 0.6 siderophage/hpf in control, thereby demonstrating 7.4-fold increment over control ($p<0.001$; Table 5.9, Figure 5.28a).

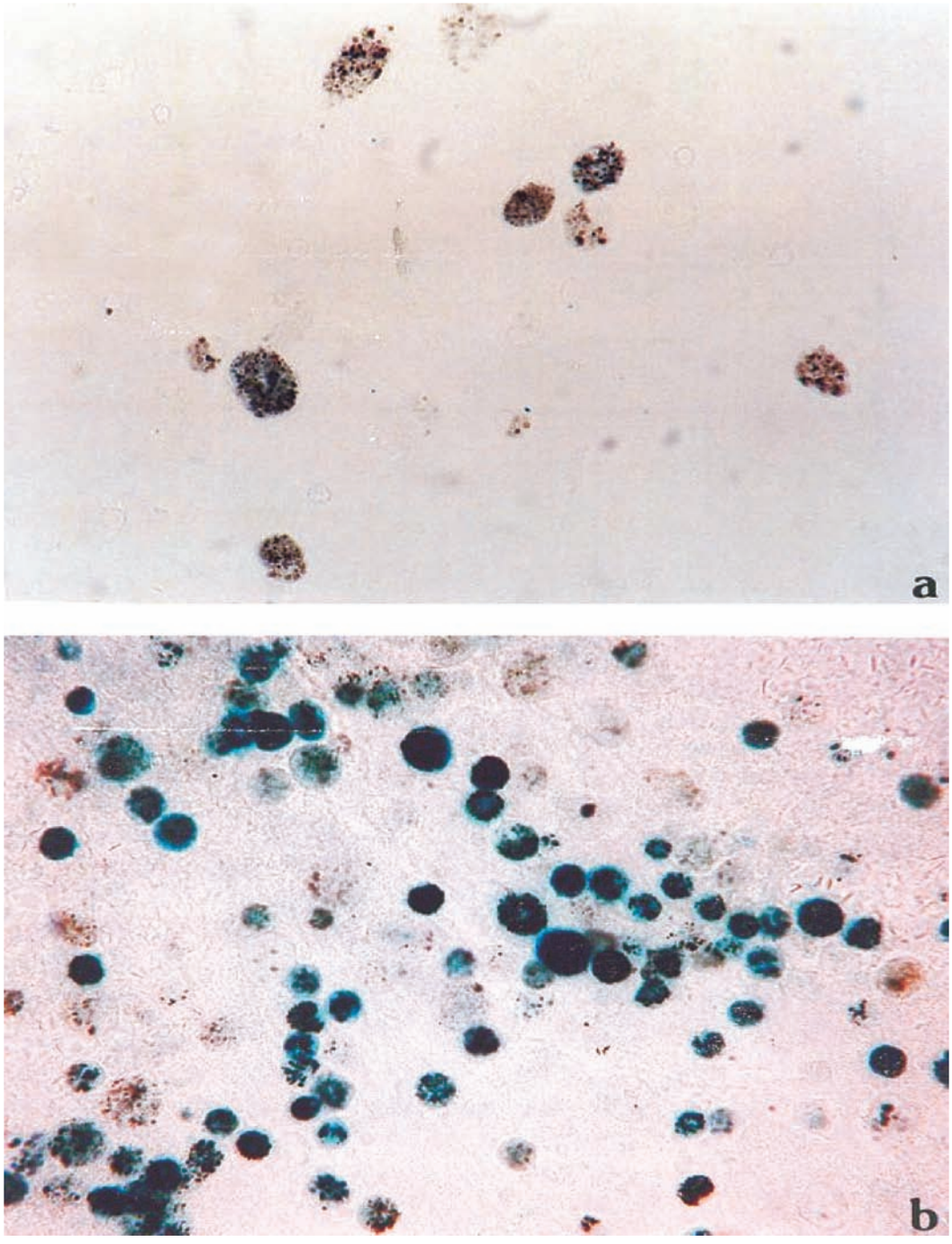


Figure 5.27: Photomicrographs of Perl's Prussian blue reaction in sputum. Note the abundance of blue-stained hemosiderin iron containing alveolar macrophages (siderophages) in a non-smoking office employee of Delhi (b) compared with negligible iron deposition in control subject (a) abundance of siderophages mey suggest covert pulmonary hemorrhage. Perl's Prussian Blue-stained, x 400

Table 5.9: Iron deposition in alveolar macrophages

Siderophage in sputum	Control (n=205)	Delhi (n=411)
Number/hpf	0.6 ± 0.2	3.7 ± 1.1*
Golde score	12± 8	63± 32*
% of subjects with Golde score ≥ 100	1.0	3.9*

*The results are mean ± SD; *, p<0.001 compared with control*

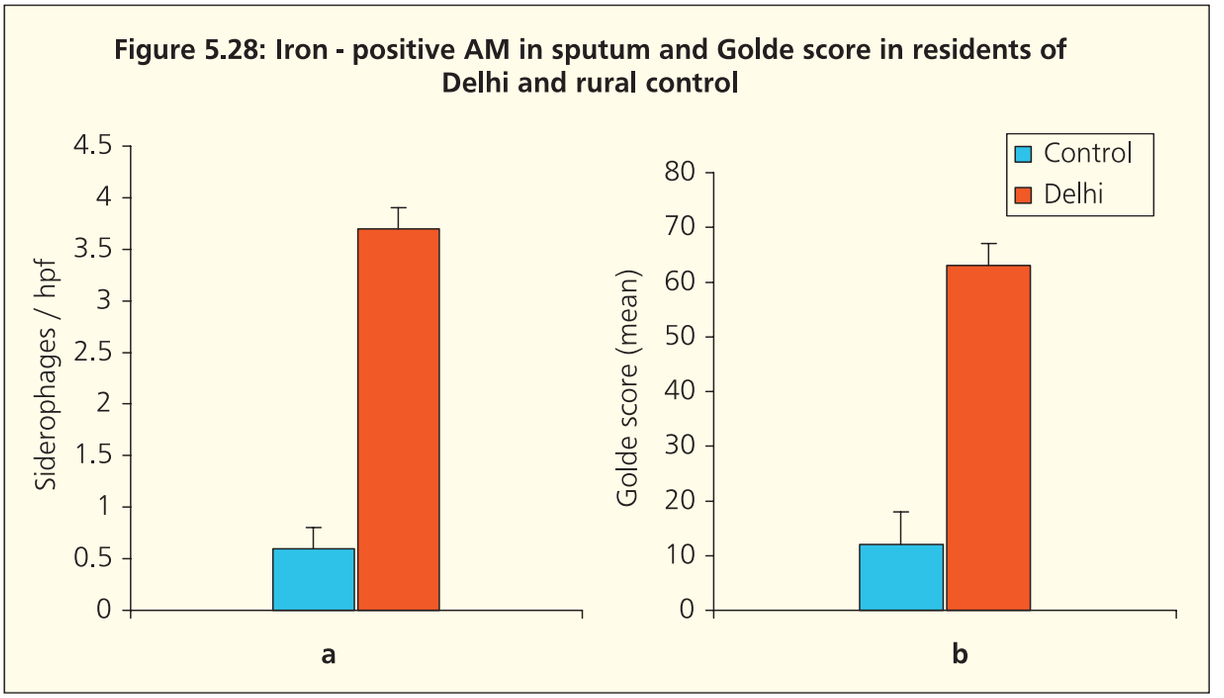


Figure 5.28: Iron-positive AM in sputum and Golde score in residents of Delhi and rural control

The residents of Delhi also exhibited a much higher Golde score compared with controls. They had a mean score of 63± 32 (SD) compared with 12± 8 of controls (p<0.001; Figure 5.28b). In addition, 3.9% of the residents of Delhi had a very high score (≥100) compared with 1% of controls (Table 5.9). Moderate to severe hemorrhage in the lungs is a distinct possibility in these subjects with high Golde score (Figure 5.29).

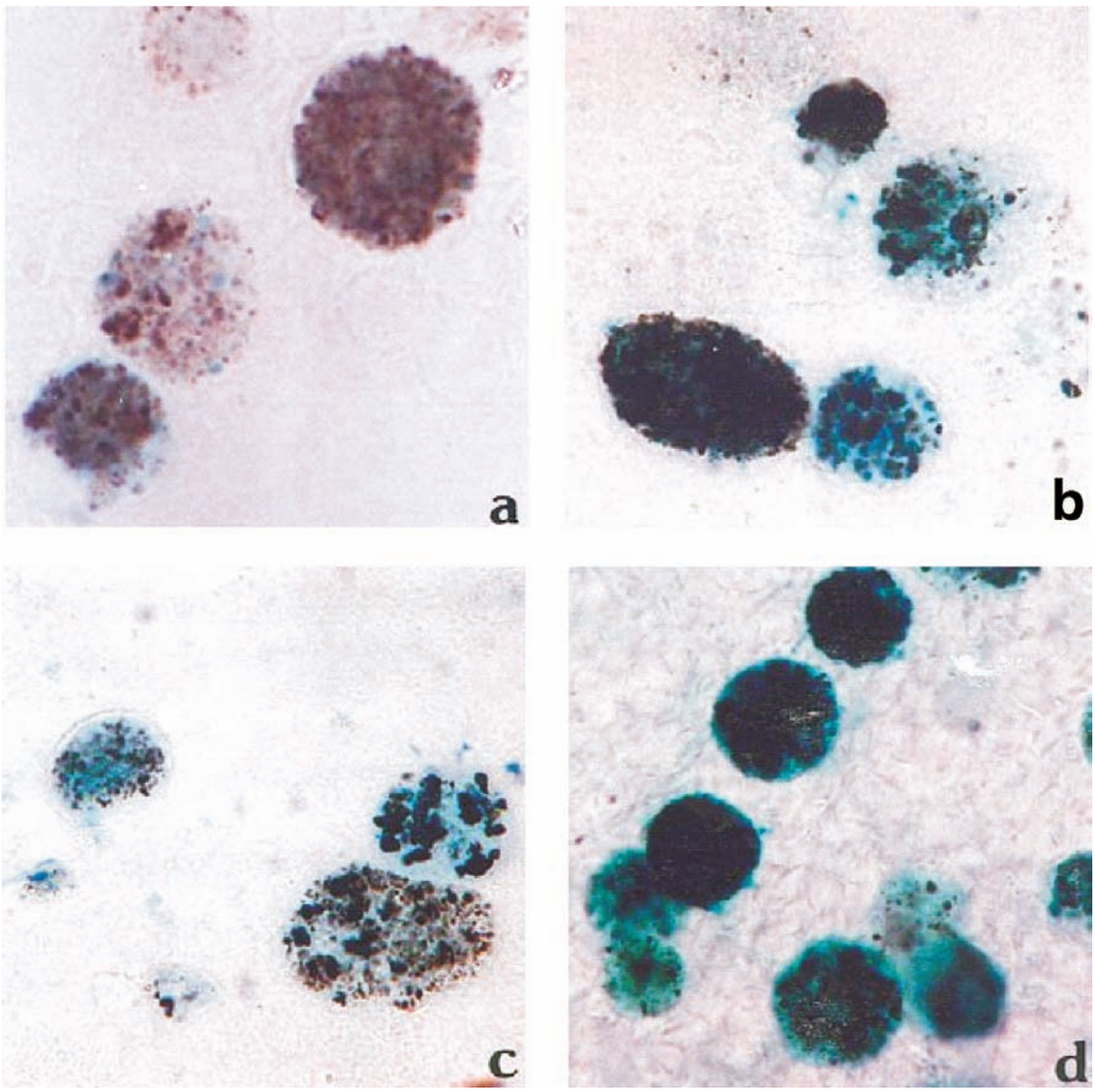


Figure 5.29: Sputum samples showing different grades of iron deposition in alveolar macrophages. Note negligible iron deposition in alveolar macrophages in a control subject (a) compared with moderate to very high deposition in a housewife (b), office employee (c) and a road side hawker (d). Perl's Prussian Blue-stained, x 1000

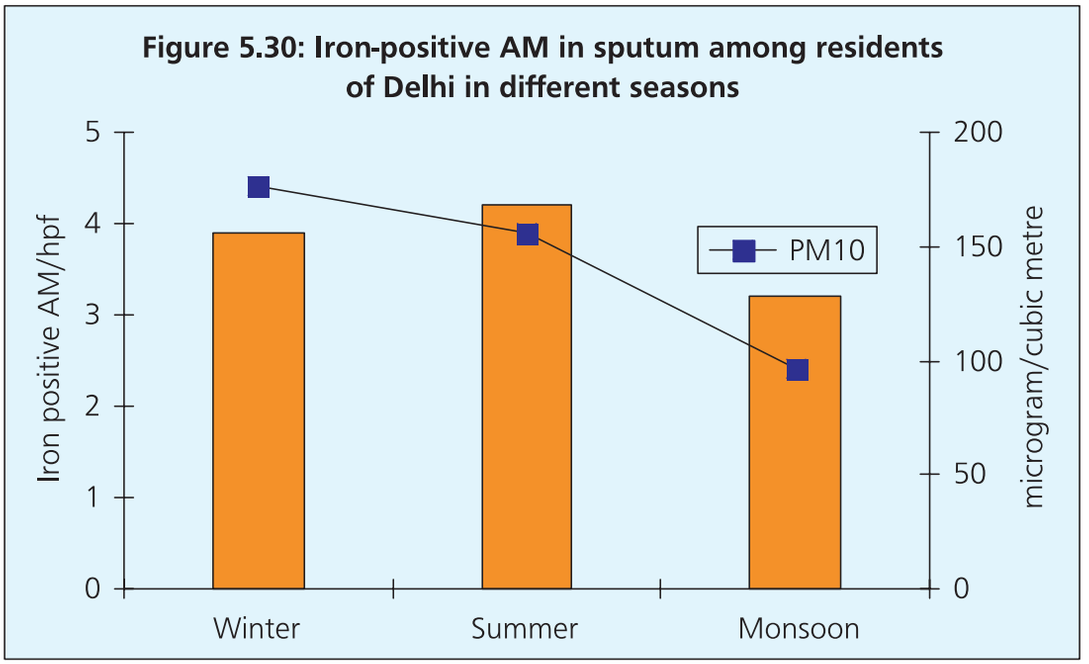
In essence, increased number of hemosiderin-laden macrophages i.e. siderophages in sputum of the never-smokers of Delhi was found compared with their control counterparts, and the degree of iron deposition was greater in urban subjects. Since the presence of siderophages generally indicates covert hemorrhage inside the lungs, microscopic pulmonary hemorrhage is not unlikely in a substantial number of residents of Delhi.

Relationship between siderophage number with season, socio-economic conditions, and vehicular pollution

Siderophage number among the residents of Delhi was higher in summer and winter when air pollution levels were high (4.2 and 3.9/hpf vs. 3.2/hpf in monsoon; Figure 5.30), in persons belonging to low SES (4.5/hpf compared with 3.0 in high SES), and among auto rickshaw and taxi drivers (4.4 and 4.0 /hpf

respectively compared with 2.9/hpf in office employees) of the city who were occupationally exposed to high level of vehicular pollution.

Figure 5.30: Iron-positive AM in sputum among residents of Delhi in different seasons



Relationship between siderophage number and lung function

A strong, negative correlation exists between siderophage number in sputum and spirometric lung function measurements. The correlation was significant for FVC (rs = -0.587, p <0.0005), FEV1 (rs = -0.530, p <0.0005), FEF25-75% (rs = - 0.370, p<<0.0025), and PEFR (rs = - 0.472, p<0.0025). Thus, iron deposition in AM was associated with lung function deficits.

Correlation between cellular lung response and lung function decrement

On the other hand, a negative correlation was found in Spearman's rank correlation test between total cell count and AM number in sputum and spirometric measurements especially the FVC and PEFR (Table 5.10). It implies that the increase in inflammatory cell population in the airways following chronic exposure to urban air pollution of Delhi results predominantly in restrictive type of lung function deficits.

Table 5.10: Spearman's rank correlation test between sputum cell count and lung function

			Rho (rs) value	p value
Total cells in sputum /hpf	with	FVC	-0.228	<0.05
Total cells in sputum /hpf	with	FEV1	-0.169	NS
Total cells in sputum /hpf	with	FEF25-75	-0.115	NS
Total cells in sputum /hpf	with	PEFR	-0.237	<0.05
AM/hpf	with	FVC	-0.264	<0.025
AM/hpf	with	FEV1	-0.203	NS
AM/hpf	with	FEF25-75	-0.209	NS
AM/hpf	with	PEFR	-0.364	<0.0025

NS, not significant in statistical test

5.4 FINDINGS

1. Sputum samples were collected from 550 control (male 357 and female 193) and 1050 residents of Delhi (male 706, female 344). All the subjects were never-smokers.

2. A greater percentage of urban subjects produced sputum representative of lower airways compared with their rural counterparts (93.4% vs.80%, $p<0.05$). This could be attributed to higher level of air pollution in Delhi as particulate pollution increases mucus production and sputum expectoration.

3. Compared with control, sputum samples from the residents of Delhi contained significantly increased number ($p<0.05$) of all the cell types. For example, the absolute number of neutrophils and lymphocytes were increased by 1.6-fold each, eosinophils number by 5.5-fold, alveolar macrophage (AM) number by 1.8-fold and epithelial cells by 1.8-fold. Sputum cytology indicates allergy and inflammatory changes in the lung among the residents of Delhi.

4. Sputum of Delhi's citizens contained 12.9±2.6 AM per hpf in contrast to 6.9±1.6 AM/hpf in controls, and the AMs of former group were heavily loaded with particles resulting in increase of cell size (mean diameter 27.8µm compared with 16.2 in control). Greater load of particles also resulted in impairment of cell division and differentiation because bi-, tri-, and multinucleated AM were present in excess in sputum of Delhi's residents compared with rural controls (20.0% vs. 5.0%; $p<0.05$).

5. Metaplasia and dysplasia of airway epithelial cells were more frequent in Delhi's residents. Metaplasia of airway epithelial cells was present in 15.9% non-smokers of Delhi compared with 3.2% of controls ($p<0.001$). Similarly, dysplasia of airway cells was detected in 3.0% individual of Delhi in contrast to 0.7% of controls ($p<0.001$). Metaplasia and dysplasia are risk factors for cancer in the exposed tissues. Therefore, a greater risk of the disease can be envisioned in Delhi.

6. The citizens of Delhi also had greater prevalence of several cytological changes in sputum compared with rural controls. They had increased presence of ciliocytophthoria (3.0% vs. 0.7% in control), aggregates of columnar epithelial cells (6.9% vs. 2.5%), koilocytes (2.7% vs. 1.1%), Charcot-Leyden crystals (0.6% vs.0%), goblet cell hyperplasia (6.4% vs. 2.0%) and mucus plugs (39.7% vs. 14.3%), spores and conidium of the fungus *Alternaria* (23.1% vs. 12.3%). The findings suggest greater chances of influenza and HPV infection, injury to the airway wall, greater exposure to fungal bioaerosols and hypersecretion of mucus that may obstruct the airways.

7. The changes in sputum cytology were positively correlated with ambient PM_{10} level. Although the correlation was highly significant in Spearman's rank correlation test for all the cell types ($p<0.001$), the association was stronger for total cell count (rho value 0.795), absolute number of sputum neutrophils (rho =0.761) and eosinophils (rho = 0.644). This may indicate association between air pollution in Delhi and inflammatory changes in the lung and the airways.

8. The rise in AM count and percentage of individual with high AM count (≥10 AM/hpf) in Delhi significantly correlated ($p<0.001$) with the city's particulate pollution level. For instance,

AM/hpf value of Central Delhi having very high PM10 level was 16.7 compared to 11.9 of South Delhi, which was less polluted.

9. A negative correlation was found between total cells and AM number in sputum and spirometric measurements especially the FVC and PEFR.

10. The number of iron-laden macrophages (siderophages) was significantly increased in sputum of the citizens of Delhi and the magnitude of iron deposition was much more in the latter group. The average Golde score in Delhi's residents was 63 against 12 in control suggesting covert pulmonary hemorrhage in the lungs. A strong negative correlation was found between siderophage number in sputum and spirometric lung function measurements.

11. A considerable rise in elastase activity in both alveolar macrophages and neutrophil was found among residents of Delhi. The absolute number of elastase positive AM was 2.9± 0.8 /hpf in control and 9.4±1.9 /hpf in residents of Delhi ($p<0.001$). Very high elastase activity was localized in 64% sputum neutrophils of Delhi's residents compared with 41% neutrophils of controls. The finding emphasizes greater risk of damage to the bronchial and alveolar walls that may lead to emphysema.

CHAPTER-6.0

HEMATOLOGICAL, IMMUNOLOGICAL, METABOLIC CHANGES ASSOCIATED WITH AIR POLLUTION

6.1 INTRODUCTION

Chronic exposures to high particulate air pollution, particularly in the ultrafine range, are known to provoke alveolar inflammation that release mediators capable of exacerbating lung disease and increased blood coagulability in eosinophils individuals (Seaton, 1995). The heart can be affected by air pollution exposure, because animal studies by Godleski and coworkers have shown that inflamed lung, as evidenced by neutrophil accumulation in bronchoalveolar lavage fluid, releases mediators that alter the autonomic nervous system control of cardiac rhythm (Godleski et al., 1997). Subsequent studies in human subjects have shown a rise in pulse rate in association with exposures to PM10 (Pope et al., 1999a). Air pollution is associated with lower heart rate variability, implying poor autonomic control (Liao et al., 1999; Pope et al., 1999b). Poor autonomic control of the blood has also been reported in association with air pollution exposure (Gold et al., 2000). PM2.5 has been implicated for these changes (Liao et al., 1999).

Cropper et al., (1997) conducted a pioneering time-series study on the impact of Delhi's particulate air pollution on daily mortality in early 1990's.They found a significant relationship between particulate air pollution and daily deaths from respiratory and cardiovascular problems. In general, the impact of particulate matter on total non-trauma deaths in Delhi was smaller than the US.

Pande and his co-workers (2002) conducted a 2-year (January 1997-December 1998) time-series analysis in Delhi. Emergency room visits for acute asthma, acute exacerbation of chronic obstructive airway disease, and acute coronary events at All India Institute of Medical Sciences (AIIMS) increased by 21.3%, 24.9% and 24.3% respectively on account of higher than acceptable level of air pollutants (CO, NOx, SO2). It was concluded that there is a considerable burden of cardiopulmonary diseases in Delhi due to high level of ambient air pollution (Pande et al., 2002).

Adverse effects of Delhi's air pollution on the respiratory system have been documented in the preceding Chapter. The studies have documented abnormal red cell, neutrophil and platelet levels (Salvi et al., 1999), increase in blood viscosity (Schwartz, 2001), and changes in the number of T-lymphocytes, B-lymphocytes, and NK cells (Salvi et al., 1999) in response to air pollution exposure. In addition, neurotoxicity (Anderson et al., 1995; Kilburn, 2000), change in antioxidant defense of the body (Georgieva et al., 2002), and genotoxicity at the level of chromosomes (Zhang et al., 2005) and DNA (Eastman and Barry, 1992; Moller and Wallin, 1998; Don Porto et al., 2001) have been reported. In the line of these reports it seems important to examine the systemic changes, if any, in people chronically exposed to city's air pollution. Accordingly, hematological and immunological toxicity have been examined in this study.

It has been shown earlier that the residents of Delhi were exposed to higher levels of particulate pollution and benzene than that of rural controls. Recent studies have suggested that these pollutants elicit their toxic effects by oxidative stress through generation of free radicals. Antioxidant enzymes like SOD protect the body from the deleterious effects of free radicals. Considering this, SOD enzyme activity has been measured in this study to explore whether body's antioxidant arsenal has been appropriately activated to combat Delhi's vehicular pollution.

6.2 MATERIALS AND METHODS

6.2.1 Clinical examination and blood pressure measurement

Two physicians clinically examined the participants for general health problems at health check up camps organized in different parts of Delhi and in the village of West Bengal. Arterial blood pressure (BP) was measured by a sphygmomanometer. An inflatable cuff with a meter attached was placed around the subject's arm over the artery, while he/she was seated. Systolic (the force that blood exerts on the artery walls as the heart contracts to pump out the blood) and diastolic (force as the

heart relaxes to allow the blood to flow into the heart) blood pressures (SBP and DBP respectively) were expressed in millimeters of mercury (mm Hg). BP measurements were done in subject with empty bladder, in resting conditions sitting in a chair with back supported and arm supported at heart level. A minimum of two BP measurements was done at an interval of 5 minutes, and the readings were averaged (Figure 6.1, 6.2).

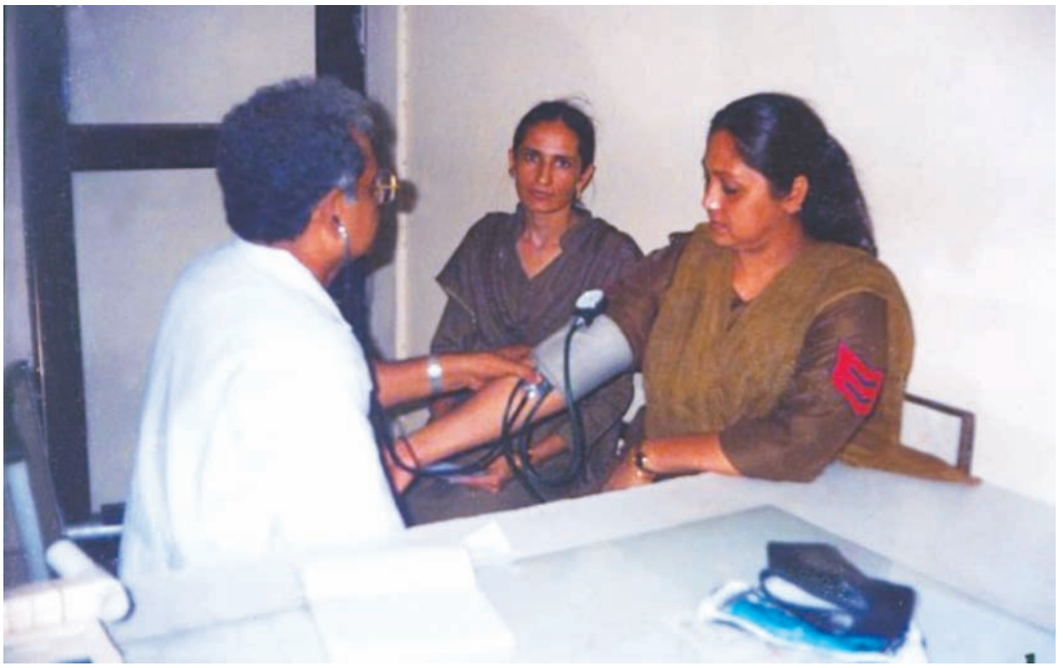


Figure 6.1: Measurement of blood pressure at IP Police Station, Delhi

Figure 6.2: Health check-up camp in progress at Banga Bhawan, Hailey Road, New Delhi (a) and sample collection at Clean Air Station, Paharpur

Figure 6.2: Health check-up camp in progress at Banga Bhawan, Hailey Road, New Delhi (b) New Delhi

(a) Diagnosis of hypertension

Hypertension was diagnosed on the basis of Seventh Report of the Joint Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7, 2003). The condition was confirmed when SBP rosse to 140 mmHg or more, or DBP elevated to 90 mmHg or more, or both of these changes were prevalent in three or more consecutive measurement at 5 min intervals. Hypertension was subdivided into two based on severity of the problem: Stage 1(mild to moderate) when SBP was 140-159 mmHg or DBP was 90-99 mmHg; Stage 2 (severe hypertension) when SBP and DBP cross the 159 and 99 mmHg marks respectively (Table 6.1).

Table 6.1: Classification of blood pressure according to JNC-7

BP Classification	SBP (mmHg)	DBP (mmHg)
Normal	<120	<80
Pre-hypertension	120-139 or	80-89
Stage 1 hypertension	140-159 or	90-99
Stage 2 hypertension	≥160 or	≥100

(b) Diagnosis of pre-hypertension

A diagnosis of pre-hypertension was established when either SBP was between 120 and139 mmHg, or DBP was between 80 and 89 mmHg, or both (JNC-7).

6.2.2 Hematological studies

(a) Collection of blood

Blood samples (5.0 ml) were collected after informed consent by venipuncture using 21-gaugeneedle fitted on 5 ml sterile, disposable plastic syringe (Dispovan, India) from 417 control subjects and 1312 residents of Delhi (Figure 6.3, 6.4). All these subjects were never smokers. A part was used for blood smear preparation on glass slides, and collection of serum for biochemical estimation of liver and kidney function. The rest was anticoagulated with K2EDTA (in vacutainer tubes, Becton Dickinson, USA) for routine hematology (hemoglobin measurement, red blood cell, white blood cells and platelets counts) following the procedures of Dacie and Lewis, 1975 (Figure 6.5, 6.6, 6.7). Differential counts of WBC and examination of blood cell morphology were done from Leishman-stained smears under light microscope (Leitz, Germany) following standard procedure (Dacie and Lewis, 1975). Blood glucose level (random) was estimated at the sampling site by one-touch glucometer (Johnson and Johnson, USA), which was calibrated at regular intervals (Figure 6.8).

Figure 6.3: Health check-up camp in progress at (a) National Physical Laboratory, Pusa Road, New Delhi (b) and Mausam Bhawan, Lodhi Road, New Delhi

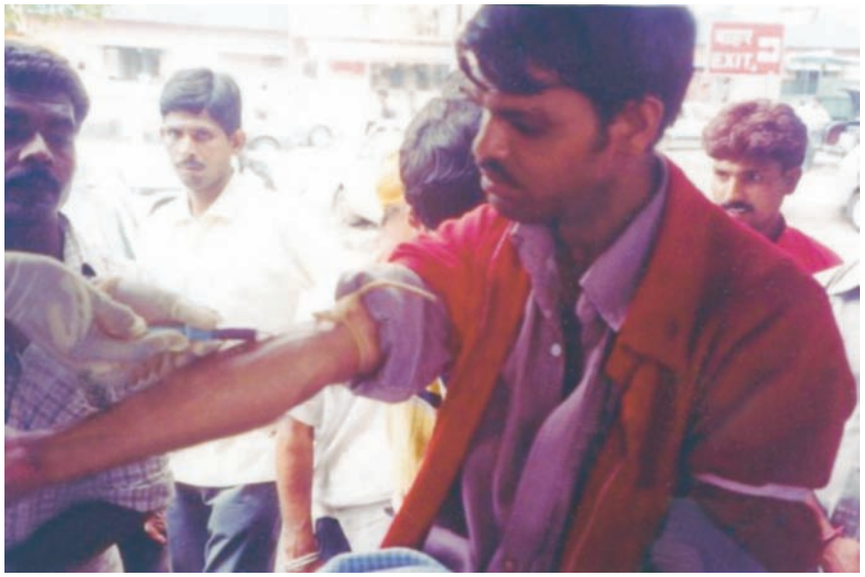


Figure 6.4: Collection of blood samoles of a railway porter at Ajmeri Gate, New Delhi RailwayStation

Red Blood cell (RBC) count

Blood is drawn in a RBC pipette and diluted 1:200 with RBC diluting fluid

↓

After thorough mixing, few drops of the resultant mixture are discarded and then the resultant mixture is discharged under the cover glass of Neubauer's hemocytometer

↓

Corpuscles are allowed to settle for 3 minutes

↓

The number of erythrocytes in 80 small squares is counted under light microscope.

White blood cell (WBC) count

Blood is drawn in a WBC pipette and diluted 1:20 with WBC diluting fluid

↓

After thorough mixing, few drops of the resultant mixture are discarded and then the resultant mixture is discharged under the cover glass of Neubauer's hemocytometer

↓

Corpuscles are allowed to settle for 3 minutes

↓

Total number of leukocytes is counted in 4 corner blocks of Neubauer hemocytometer under microscope

Platelet count

Blood is drawn in a RBC pipette and diluted 1:200 with Rees-Ecker diluting fluid

↓

After thorough mixing, few drops of the resultant mixture are discarded and then the resultant mixture is discharged under the cover glass of Neubauer's hemocytometer

↓

Mixture is allowed to settle for 10 minutes

↓

The number of platelets in 80 small squares is counted under light microscope

Figure 6.5: Total counts of RBCs, WBCs and platelets

Thin blood films are prepared on clean glass slides and air-dried
↓
Blood smears are fixed in methanol
↓
Smears are stained for 5 minutes with undiluted Leishman's stain (Sigma Chem, USA) prepared in absolute methanol
↓
The stain is then diluted by adding distilled water drop-wise on the slides and kept for 10 minutes
↓
The slides are washed in running tap water, air-dried and observed under oil immersion lens of a light microscope (Leitz , Germany) for differential distribution (%) of WBC

Figure 6.6: Differential count of leukocytes

20 µl of whole blood is diluted with 200µl of N/10 HCl
↓
After 10 min, the mixture is vortexed and transferred to the tube of the Hemoglobinometer
↓
The brown solution was diluted drop by drop with distilled water until the color matches that of the standard
↓
The final mark that the mixture reaches is the amount of Hb (g/dl) in peripheral blood

Figure 6.7: Measurement of hemoglobin

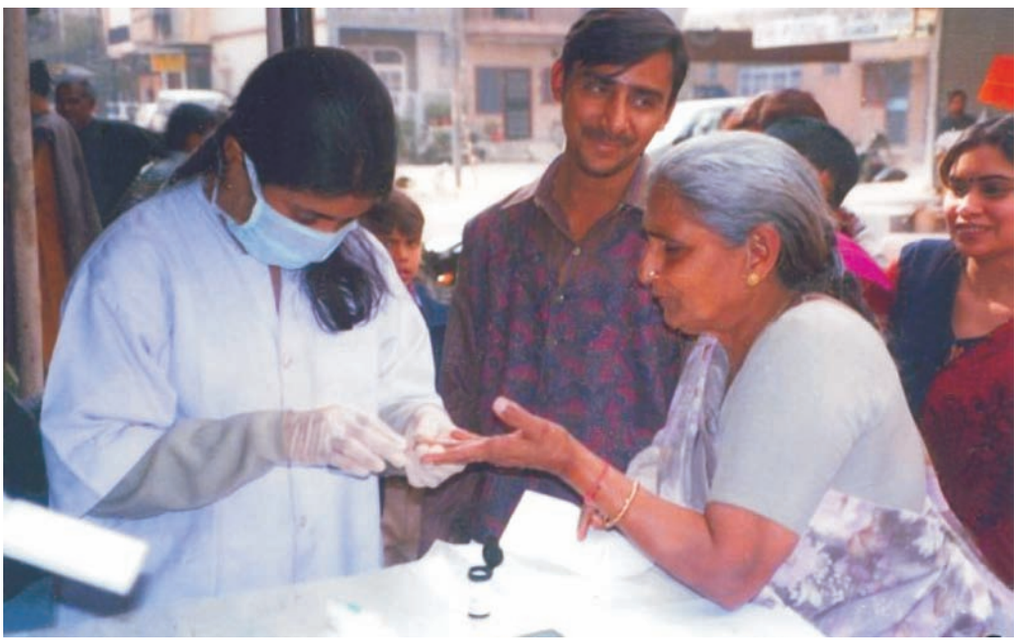


Figure 6.8: Checking of blood glucose (random) level at campsite of Delhi

(b) Total and differential counts

Total counts of red blood cells, white blood cells (WBCs) and platelets were estimated by standard techniques (Dacie and Lewis, 1975; Figure 6.5). Blood smears were prepared immediately following blood drawing. Three slides were made for each person and stained with Leishman's and blindly evaluated by a hematologist. Differential counts of WBC were done from Leishman's-stained slides (Figure 6.6). Morphology of RBC, WBC and platelets was examined.

(c) Detection of blood cell morphological changes

Blood smears on clean glass slides were prepared immediately following blood drawing. Three slides were made for each person and allowed to air-dry. Smears were then stained with Leishman's stain and examined under light microscope (Figure 6.9). Differential count of WBC was done after examining at least 200 cells per slide. The mean of three slides was accepted as individual value. Morphology of RBC, WBC and platelets was examined. Specific observations included percentage of schistocytes, 'target' cells, and toxic granulations in neutrophils. Schistocytes were evaluated as the number of fragmented red cells per 1,000 RBC expressed as a percentage, and was graded mild (<1%), moderate (1-1.9%) and severe (>2%) [Lesesve et al, 2001]. Reading was restricted to the thinner part of the smear.

Figure 6.9: The research team at work at the Biolab of Central Pollution control Board, Delhi

(d) Platelet P-selectin expression

P-selectin expression in non-stimulated, circulating platelets in whole blood was measured by flow cytometry following the procedure of Michelson et al., (2000) in 45 control and 82 participants of Delhi (Figure 6.10). It was based on immediate sample preparation and minimal sample manipulation as RBC lysis and subsequent centrifugation may trigger platelet activation. Within 10 min of blood sampling, 10 µl of whole blood was transferred to polystyrene test tubes containing a saturating concentration of phycoerythrin (PE) –conjugated anti-human monoclonal antibody against CD62P and fluoroscein isothiocyanate (FITC) –conjugated anti-human CD41a monoclonal antibody (Becton Dickinson, USA) and incubated undisturbed for 20 min at 200 C in the dark. Subsequently the samples were fixed for 1 hr with 1 ml cold 1% paraformaldehyde in Ca2+- Mg2+- free phosphate buffered saline (PBS) containing 0.1% sodium azide. The samples were analyzed for forward scatter (FSC), side scatter (SSC), FL-1 (FITC- CD41a) and FL-2 (PE-CD 62P) values of 10,000 events in a flow cytometer (FACS Calibur with sorter, Becton Dickinson, USA) using Cell Quest software (Becton Dickinson, USA). Electronic compensation was used to remove spectral overlap and aligned daily with CaliBrite beads (Becton Dickinson, USA). Platelets were identified in whole blood by the characteristic FSC and SSC and FITC-conjugated CD41a positivity. P-selectin expression was recorded as percentage of PE-CD62P-positive platelets. Mean fluorescence intensity (MFI) was calculated after subtracting the MFI of isotype control IgG from.

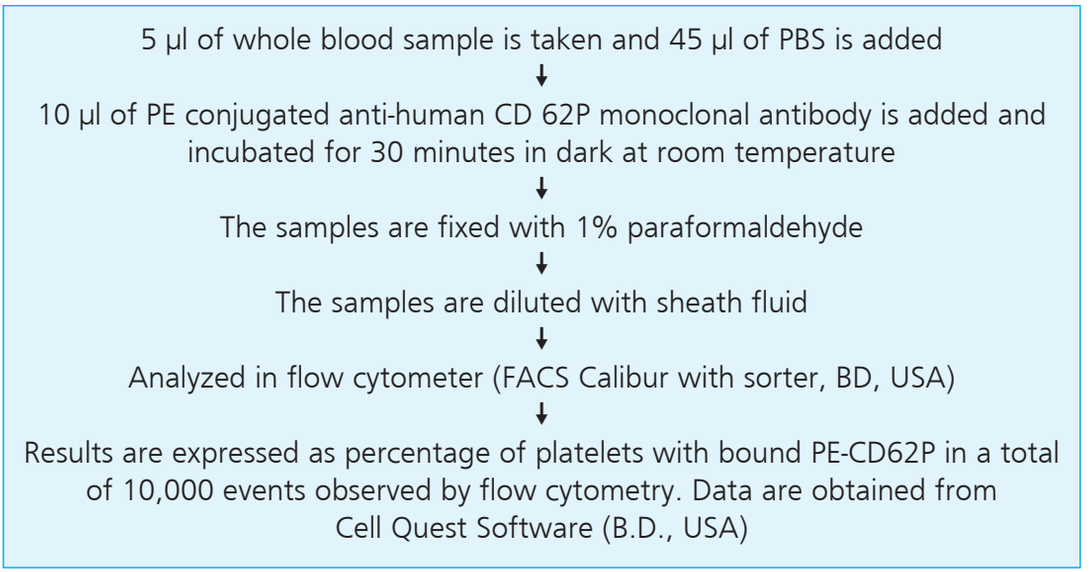


Figure 6.10: Measurement of Platelet activation marker P-selectin

6.2.3 Immunological studies

(a) Lymphocyte subtyping by flow cytometry

The subpopulation of peripheral blood lymphocytes was determined in a flow cytometer (FACS Calibur with sorter, BD, USA) by using a panel of anti-human monoclonal antibodies for lymphocyte surface markers like CD4, CD8, CD20 and CD56 (BD-Pharmingen, USA). An average of 10,000 events was acquisitioned and the results were analyzed using Cell Quest software (BD, USA). Identification of lymphocyte subsets was done by the procedure of Fujimoto et al., (2000; Figure 6.11).

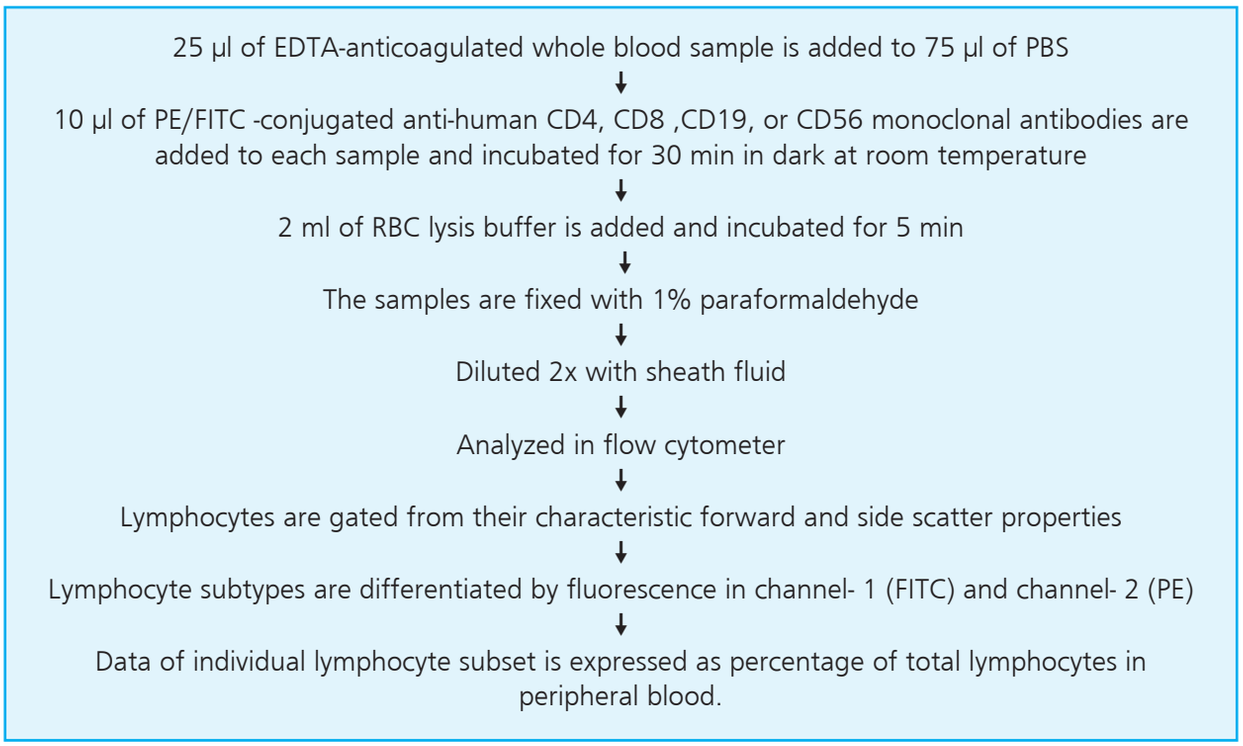


Figure 6.11: Lymphocyte subtyping by flow cytometry

Procedure

Whole blood samples anticoagulated with K3EDTA were analyzed for lymphocyte subsets by flow cytometry within 8 hours of blood drawing. A 25µl aliquot of whole blood was diluted with 75 µl of phosphate buffered saline (PBS, pH 7.3) and the diluted blood samples were incubated with 10 µl each of fluorescence isothiocyanate (FITC) – and phycoerythrin (PE)-conjugated monoclonal antibodies (BD Pharmingen, USA) raised against human lymphocyte surface markers CD4 (T-helper), CD8 (T- cytotoxic/ suppressive), CD19 (B cell), CD 16 and CD56 (natural killer cell) and isotype-matched negative controls for 30 min in the dark at room temperature. Then the erythrocytes were lysed by incubating the samples with 2 ml of RBC lysing solution (BD, USA) for 5 min at room temperature. Thereafter the cells were fixed with 0.5% paraformaldehyde (E. Merck, India), and 15,000 events were acquired and analyzed in a flow cytometer (FACS Caliber with sorter, BD, San Jose, CA, USA). Lymphocytes were identified from their characteristic forward and side scatter profile on dot plots and gated. Data acquisition and analysis of FL-1 (FITC)) and FL- 2 (PE) were done using Cell Quest software (BD, USA). The relative proportion of each lymphocyte subset (such as CD4+ or CD8+) was calculated from statistical package of the Cell Quest software from quadrant gate setting for CD4, CD8, CD19, CD16 and CD56 and isotype controls. Data of individual lymphocyte subset was expressed as percentage of total lymphocytes in peripheral blood.

6.2.4 Liver and kidney function

The concentrations of serum urea, creatinine, bilirubin, total protein, albumin, SGPT and SGOT were measured biochemically by established procedures.

6.2.5 Blood glucose measurement

Blood glucose was measured at the health camps using a portable glucometer (Johnson and Johnson, USA). Diabetes was diagnosed if the random blood glucose level was 200 mg/dl (11.11mmol/L) or higher (Mayo Clinic Protocol-1998).

6.2.6 Assessment of antioxidant status

(a) Measurement of antioxidant enzyme superoxide dismutase (SOD)

SOD is a member of the family of metallo-enzymes and is the most important enzyme in the front line of defense against oxidative stress. It accelerates the dismutation of superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) and molecular oxygen.

$$2\ O2^- + 2H \xrightarrow{SOD} H2O2 + O2.$$

The enzyme was assayed in human RBC spectrophotometrically following the procedure of Paoletti et al., (1986; Figure 6.12).

1 ml of EDTA-anticoagulated whole blood centrifuged at 5000 rpm for 10 min

↓

Red cell pellet washed thrice in saline

↓

100µl of RBC pellet is diluted 9x with cold distilled water

↓

Tea-Dea buffer, EDTA/MnCl2, NADH and mercaptoethanol added to the RBC lysate

↓

Decrease in absorbance is measured at 340nm in UV-spectrophotometer at 1 min interval

Figure 6.12: Measurement of antioxidant enzyme superoxide dismutase (SOD)

One eosinophil of EDTA-anticoagulated whole blood was centrifuged at 5000 rpm for 10 min and the supernatant was discarded. RBC pellet was washed thrice in 0.9% saline. To 100µl of RBC pellet 900 µl of cold distilled water (1: 9 dilution) was added for lysis of the red cells. Following centrifugation at 500g for 5 min, the supernatant was collected for SOD assay.

The absorption of the samples (RBC lysate) was measured at 340 nm in a UV-spectrophotometer (Shimadzu, Japan). SOD in standard and sample will cause proportionate inhibition of the rate of NADH oxidation. This was calculated after measuring the absorbance at 340 nm at 1 min intervals up to 5 min. The absorbance values were graphically plotted against time after mercaptoethanol addition (0, 1,2,3,4, and 5 min), and SOD activity (U/ml) in RBC was calculated from the standard curve following established procedure (Paoletti et al., 1986).

(b) Measurement of total antioxidant status (TAS)

Total antioxidant refers to all the intracellular antioxidant enzymes involved in combating oxidative stress. This mainly includes superoxide dismutase, catalase and glutathione peroxidase.

TAS was determined by commercially available kit (Randox, UK). Whole blood sample was centrifuged at 5000 rpm for 10 min at 4 oC and the supernatant plasma was taken. 20µl of plasma was then added to the chromogen containing Metmyoglobin (6.1µmol/l) and ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) 610 µmol/l and was incubated at 37ºC. Initial absorbance (A1) was measured at 600nm in a UV-vis Spectrophotometer (Shimadzu, Japan). The substrate was then added and the absorbance was recorded after 3 mins (A2)

A2 – A1 = Δ A of sample/standard/blank

Factor = Concentration of standard

(Δ A blank - Δ A standard)

Total Antioxidant Status (mmol/l) = Factor x (Δ A Blank - Δ A Sample)

6.2.7 Statistical evaluation

All data are expressed as mean± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between hematological, immunological and metabolic changes and possible confounders such as RSPM levels. Spearman's rank test for continuous variables and Chi-square test for categorical variables were done. P<0.05 was considered as significant.

6.3 RESULTS

6.3.1 Prevalence of hypertension

Arterial blood pressure values of a total number of 2,218 citizens of Delhi (1543 men and 675 women) and 642 control subjects (425 men and 217 women), all never-smokers, are presented in Tables 6.2, 6.3.

Table 6.2: Prevalence (%) of hypertension in never-smokers

Hypertension	Control (n=642)	Delhi (n=2218)
Only systolic	4.4	2.7
Only diastolic	2.5	16.7*
Systolic+Diastolic	2.6	16.7*
Overall	9.5	36.1*

**, p<0.05 compared with control*

Table 6.3: Magnitude of hypertension in never-smokers

Hypertension	Control (n=642)	Delhi (n=2218)
Systolic hypertension		
Stage 1	6.1	15.4*
Stage 2	0.9	4.0*
Diastolic hypertension		
Stage 1	4.4	23.4*
Stage 2	0.8	10.0*

*Results are expressed as percentage of individuals; *, p<0.05 compared with control*

(a) Systolic hypertension

Systolic hypertension was present in 430 of 2218 (19.4%) residents of Delhi compared with 45 (7.0%) of controls. Systolic pre-hypertension was present in 536 (24.2%) citizens of Delhi and 52 (8.1%) control subjects. Therefore, normal systolic blood pressure was present in 56.4% citizens of Delhi compared with 84.9% of controls.

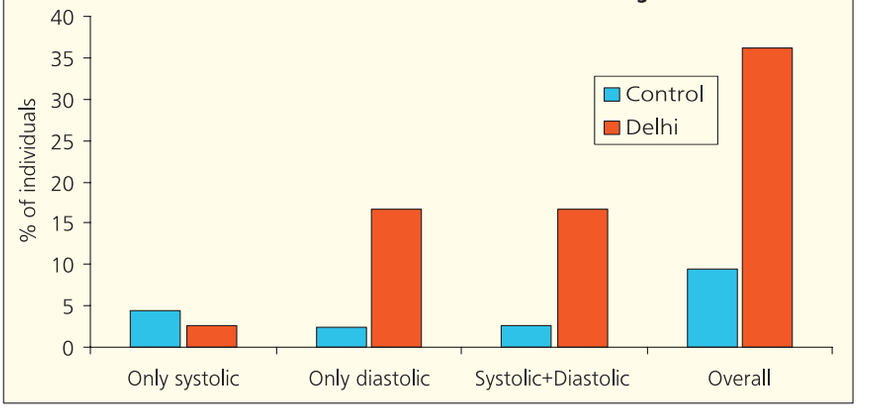
(b) Diastolic hypertension

Diastolic hypertension and pre-hypertension were present in 740 (33.4%) and 238 (10.7%) participants of Delhi, and 33 (5.1%) and 42 (6.5%) control subjects. Thus, normal diastolic pressure was present in 55.9% subjects in Delhi and 88.3% in control.

(c) Persons with systolic plus diastolic hypertension

A total of 370 participants of Delhi (16.7%) and 17 of control (2.6%) had both systolic and diastolic hypertension. Therefore, 430-370=60 individuals of Delhi (2.7% of total) had only systolic hypertension, 370 (16.7%) had only diastolic hypertension and another 16.7% had both systolic and diastolic hypertension. Similarly in control group, 4.4% had only systolic hypertension, 2.5% had only diastolic hypertension and another 2.6% had both systolic and diastolic hypertension (Table 6.2, Figure 6.13).

Figure 6.13: Prevalence of hypertension in never- smokers of Delhi and controls of rural areas of West Bengal



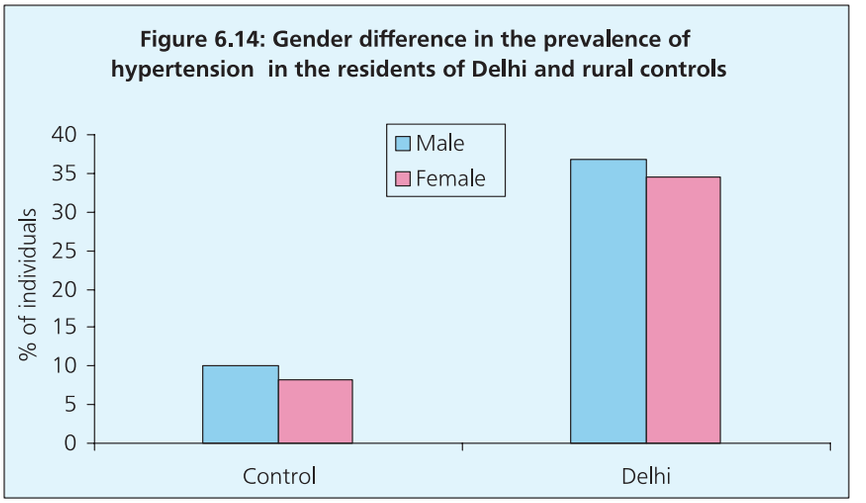
(d) Overall hypertension prevalence: 36.15 in Delhi against 9.5% in controls

Overall, 800 (36.1%) residents of Delhi had hypertension compared with 9.5% (61 out of 642) of control subjects (Table 6.2, Figure 6.13).

(e) Gender difference in the prevalence of hypertension

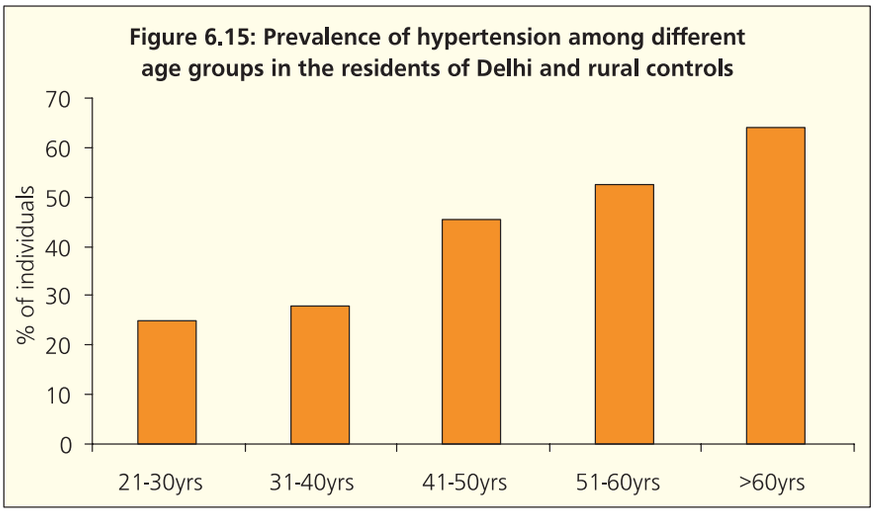
In Delhi, 34.5% of women (233/675) had hypertension compared with 36.7% of men (567/1543). Likewise, 8.3% of control women had hypertension against 10.1% of control men (Figure 6.14). Therefore, men marginally suffered more from hypertension than women both in urban and rural settings.

Figure 6.14: Gender difference in the prevalence of hypertension in the residents of Delhi and rural controls



(f) Hypertension in relation to age

The prevalence of hypertension increased progressively with age. The prevalence in Delhi was 24.8% in 21-30 yr age group, 28.1% in 31-40 yr age group, 45.3% in 41-50yr age group, 52.5% in 51-60yr and 63.9% in 60+ yr age group (Figure 6.15).



(g) Magnitude of hypertension: more severe in Delhi

Out of the 430 citizens of Delhi who had systolic blood pressure ≥ 140 mmHg, 342 (15.4%) had relatively less severe Stage 1 hypertension (SBP 140-159 mmHg) and 88 (4.0%) had more severe Stage 2 hypertension (SBP ≥160 mmHg). In contrast, 39 (6.1%) and 6 (0.9%) of control subjects had Stage 1 and Stage 2 systolic hypertension respectively. Similarly, Stage 1 (DBP 90-99 mmHg) and Stage 2 diastolic hypertension (DBP ≥100 mmHg) were present in 23.4% and 10.0% of the citizens of Delhi compared with 4.4% and 0.8% of control subjects respectively (Table 6.3).

(h) Risk factors of hypertension

(i) Poor air quality

RSPM (PM_{10}) level in ambient air was found to be positively correlated with both systolic and diastolic blood pressure in Spearman's rank correlation test (Table 6.4). The correlation was stronger for diastolic blood pressure (rho = 0.350, $p<0.005$).

Table 6.4: Spearman's rank correlation between RSPM level and blood pressure

Variables	Correlation (rho value)	P value
RSPM and systolic blood pressure	0.320	<0.005
RSPM and diastolic blood pressure	0.350	<0.005

(ii) High socio-economic status and obesity

Conditional logistic regression analysis revealed that the risk factors for hypertension were high socio-economic status, elevated RSPM level, and overweight/obesity (Table 6.5).

Spearman's correlation illustrated a significant positive correlation between BMI with systolic hypertension (rho= 0.297, $p<0.01$), diastolic hypertension (rho= 0.327, $p<0.005$; Table 6.5).

Table 6.5: Conditional logistic regression analysis of hypertension

Variables	OR	95% CI
Gender		
Female	1	
Male	1.16	0.92-1.30
SES		
Low	1	
Medium	1.08	0.89-1.44
High	1.42	1.14-1.78
Air pollution (PM_{10}, µg/m^3)		
50-75	1	
76-100	2.19	1.08-3.57
101-150	2.16	1.06-3.32
151-175	2.71	1.35-5.46
>175	2.99	1.47-6.04
Body weight (based on BMI)		
Normal	1	
Below normal	0.99	0.78-1.42
Overweight	1.32	1.08-1.67
Obese	1.91	1.26-2.91

(iii) Correlation between blood pressure and lung function

Systolic blood pressure was negatively correlated with FVC (rho = -0.574, p<0.001), FEV_1 (rho = -0.586, p<0.001), $FEF_{25-75\%}$ (rho = -0.561, p<0.001), and PEFR (rho = -0.411, p<0.002) values. Similarly, diastolic blood pressure was negatively correlated with FVC (rho = -0.583, p<0.001), FEV_1 (rho = -0.536, p<0.001), $FEF_{25-75\%}$ (rho = -0.631, p<0.001), and PEFR (rho = -0.433, p<0.001).

6.3.2 Hematological studies

(a) Hemoglobin and total count of RBC, WBC, and platelets

Hematological studies were conducted in 417 control subjects and 1312 individuals from Delhi. Only never-smokers were included in this study to eliminate the influence of smoking on hematological parameters. The results showed appreciable increase in hemoglobin, erythrocyte, total leukocyte and platelet levels among the citizens of Delhi (Table 6.6).

Table 6.6: Hematological values of the participants

Parameter	**Control (Male 312, female 105, total 417)**	**Delhi (Male 736, female 316, total 1052)**
Hemoglobin (g/l),		
Male	14.4±0.8	14.8±0.8*
Female	12.2±0.7	12.9±0.7*
Total	**13.8± 0.7**	**14.2 ± 0.7***
Hematocrit (%)		

Male	44.5±1.8	45.2±1.3
Female	39.6 ±1.4	42.3 ±1.2*
Total	**43.2± 1.5**	**44.3 ± 1.2**
RBC (x106/ µl)		
Male	4.7±0.5	5.0 ± 0.5*
Female	4.2±0.5	4.6 ± 0.5*
Total	**4.6 ± 0.5**	**4.9 ± 0.5***
WBC (x103/ µl)		
Male	6752± 687	6976± 422
Female	6876±739	7037± 524
Total	**6783± 623**	**6994 ± 432**
Platelet x106/ µl		
Male	2.3± 0.7	3.1± 0.4*
Female	2.4± 0.6	2.9±0.3*
Total	**2.3± 0.5**	**3.0 ± 0.3**

**, p<0.05 compared with respective control value in Student's 't' test*

Differential count of WBC showed increased number of monocytes and basophils in peripheral blood of the residents of Delhi when compared with that of controls ($p<0.05$). However, we did not find any significant change ($p>0.05$) in the number of neutrophils, eosinophils and lymphocytes (Table 6.7). Spearman's rank correlation test revealed a positive but non-significant correlation ($p>0.05$) between PM_{10} level and blood neutrophil, lymphocyte and eosinophils counts.

Table 6.7: Absolute numbers of leukocytes in peripheral blood

Cell type	**Control (n=417)**	**Delhi (n=1312)**
Neutrophil / µl		
Male	4206 ± 423	4133 ± 542
Female	4174 ± 576	4129 ± 456
Lymphocyte / µl		
Male	2144 ± 375	2332±287*
Female	2330± 269	2304± 226
Monocyte / µl		
Male	170 ± 32	223 ± 52*
Female	187 ± 27	262 ± 56*
Eosinophil / µl		
Male	203 ± 45	236 ± 45*
Female	221 ± 33	258± 57*
Basophil /µl		
Male	14 ± 8	55 ± 22*
Female	24 ±18	48 ± 27*

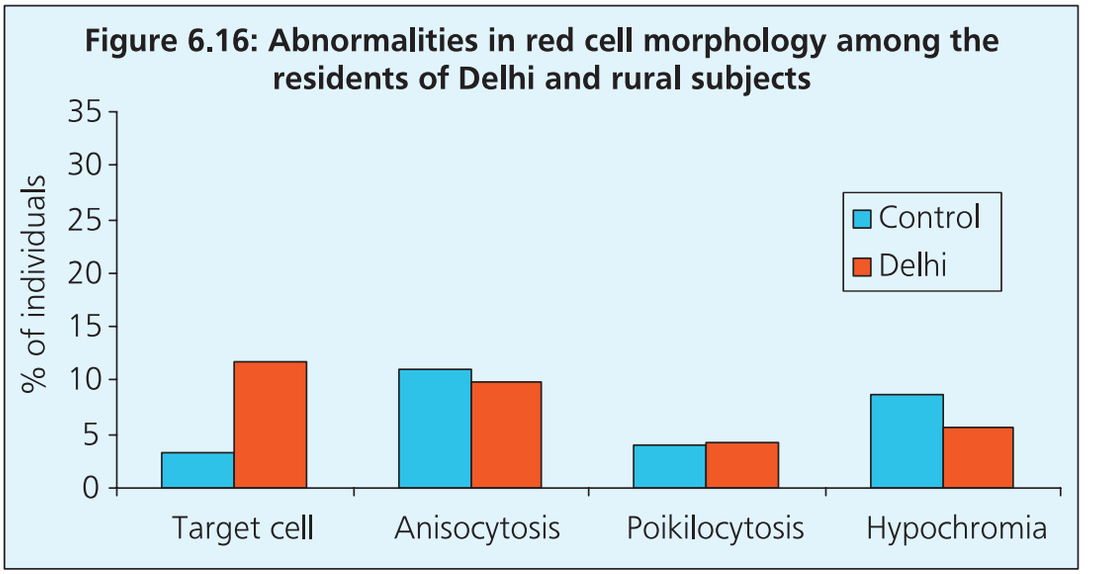
**, p<0.05 compared with respective control value in Student's 't' test*

The residents of Delhi had greater prevalence of several morphological abnormalities of blood cells when compared with that of controls. For example, 11.7% participants from Delhi had abundance of 'target' cells in their peripheral blood compared with 3.3% of controls ($p<0.001$, Table 6.8, Figure 6.16). Target cells are erythrocytes with higher surface-to-volume ratio. Their presence in circulation in excess signifies liver problem related to cholesterol metabolism. It implies that a substantial number of the citizens of Delhi might have liver problem (Figure 6.17).

Table 6.8: Prevalence (%) of abnormal cell types in peripheral blood

Cell type	Control (n= 417)	Delhi (n=1052)
Changes in RBC		
'Target' cell	3.3	11.7*
Anisocytosis	11.0	9.8
Poikilocytosis	4.1	4.3
Hypochromic RBC	8.6	5.7*
Changes in WBC		
Toxic granulation in neutrophil	15.8	34.5*
Metamyelocyte/band cell >20%	4.5	8.1*
Change in Platelet		
Giant platelets	2.6	4.8*

**, p<0.05 compared with respective control value in Chi-square test*



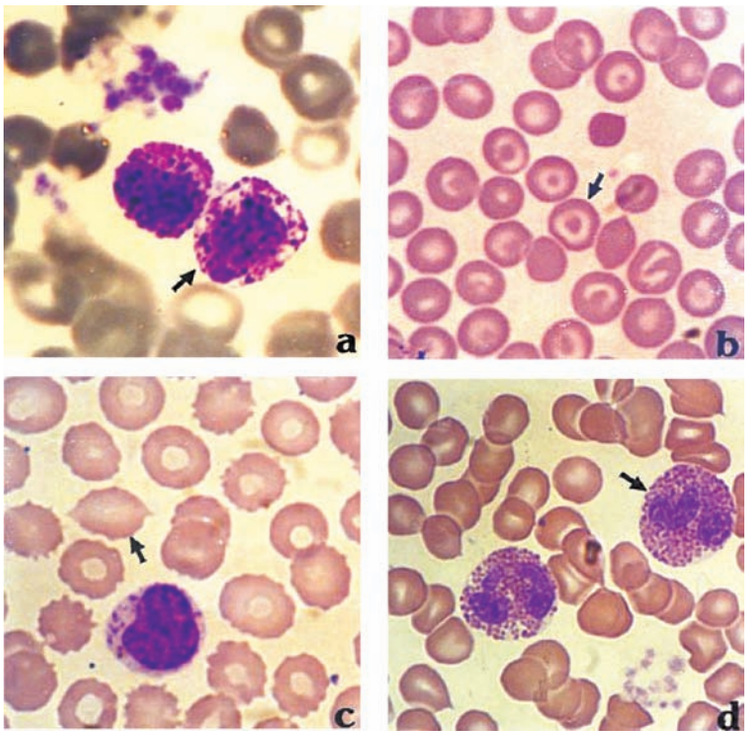
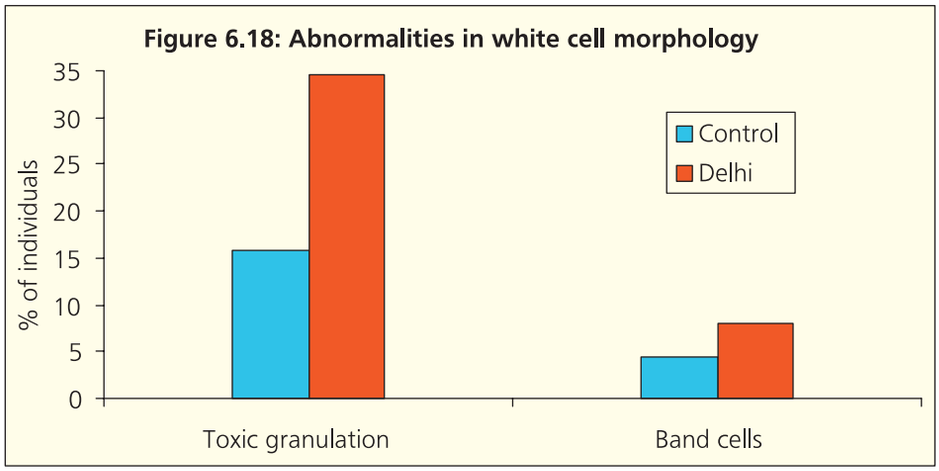
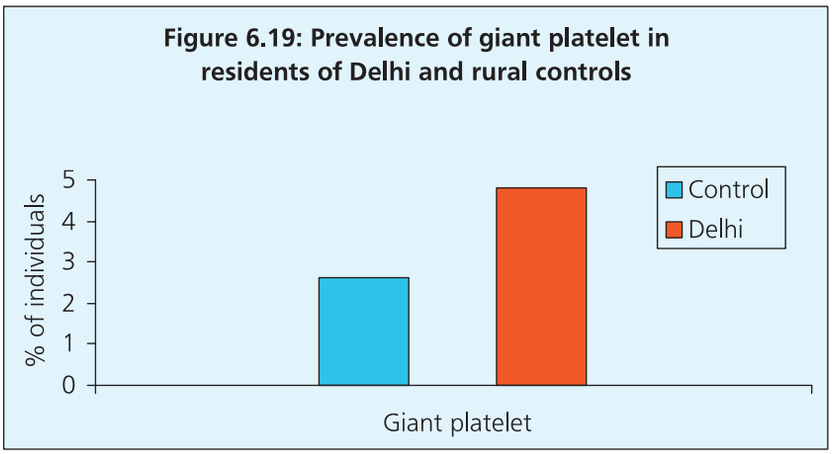


Figure 6.17: Blood film of a resident of Delhi showing abundance of basophils (a), 'target' cells (b), poikilocytosis of red cells in association with small granular lymphocyte with natural killing activity (c) and toxic granulation in neutrophils (d). Leishman's-stained, x 1000

Participants of Delhi and the controls subjects had similar frequencies of aniso-poikilocytosis (changes in red cell shape and size), but the urban subjects had lesser prevalence of hypochromic (RBC with lowered hemoglobin content) cells (Table 6.8). Only a few immature neutrophils like metamyelocytes and band cells are found in peripheral blood under normal circumstances, but their numbers rise following greater demand such as in case of infection and inflammation. Likewise, toxic granulation appears in neutrophil cytoplasm during bacterial infection (Figure 6.17). Higher prevalence of circulating immature neutrophil (8.1 vs. 4.5%, $p<0.05$) and toxic granulation in neutrophil (34.5% vs. 15.8%, $p<0.05$) was found among the residents of Delhi (Figure 6.18), suggesting greater risk of infection and inflammation. The urban subjects also had greater prevalence of giant platelets (platelets with greatly increased size) in circulation (4.8 vs. 2.6%, $p<0.05$), indicating platelet activation (Table 6.2, Figure 6.19, 6.20).

Figure 6.18: Abnormalities in white cell morphology





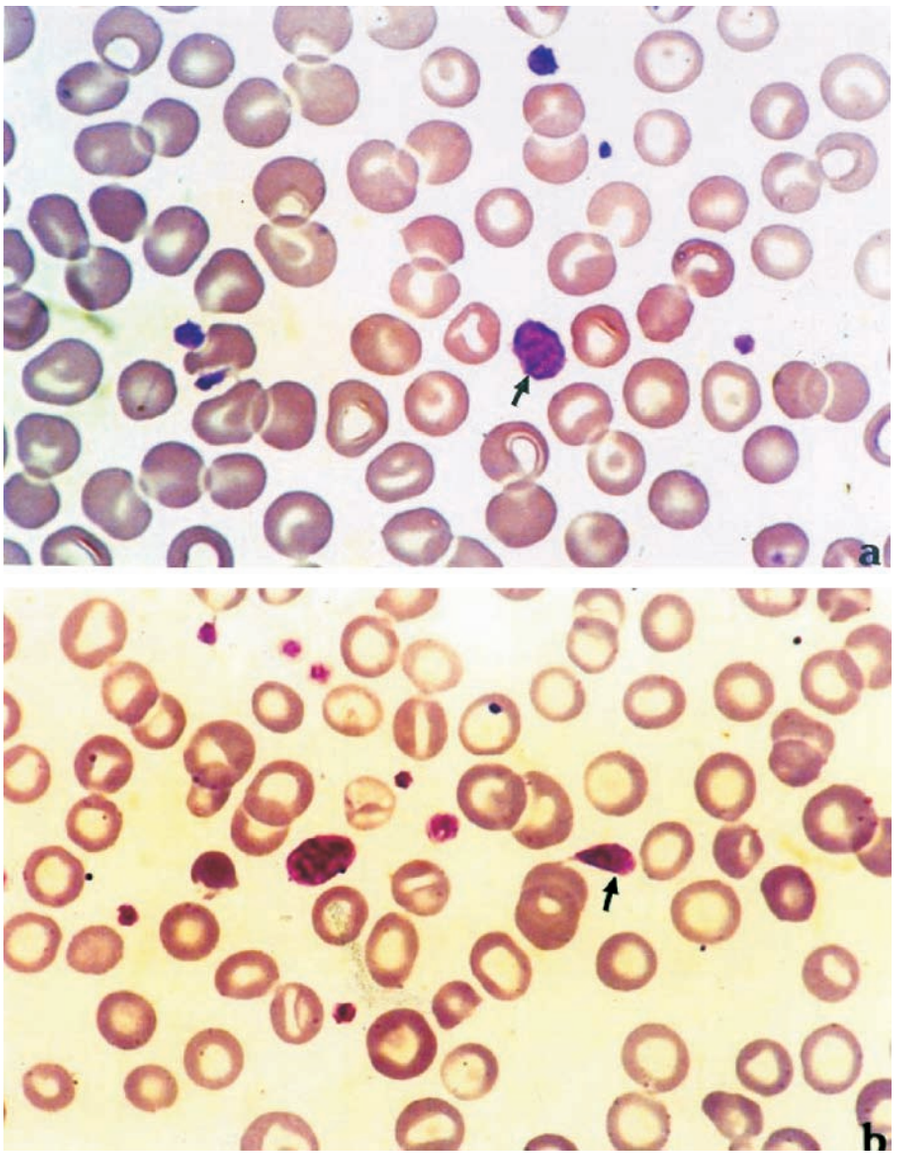


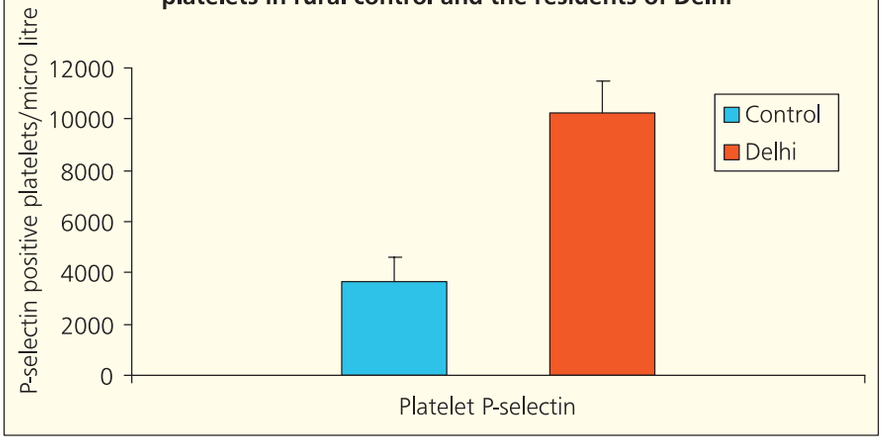
Figure 6.20: Peripheral blood smears from an auto rickshaw drivwr (a) and a roadside hawker (b) showing abundance of giant platelets. Leishman's-stained, x 1000

(b) Upregulation of platelet number and activity

Compared with control subjects, citizens of Delhi had elevated platelet count, especially in men ($p<0.05$, Table 6.6). Platelet activity was evaluated by flow cytometric assessment of CD 62P (P-selectin) expression, a measure of platelet activation, in 45 control subjects (male 30, female 15) and 82 residents of Delhi (male 62, female 20). Compared with 1.6 ± 0.4% CD 62P-positive platelets in circulation of control individuals, participants in Delhi had 3.4 ± 0.6% P-selectin-expressing platelets in their peripheral blood ($p<0.001$).

Estimation of total number of P-selectin expressing platelets in circulation revealed 3,682 ± 960 (mean ± SE) activated platelets/µl of blood in control subjects and 10,268 ± 1232 activated platelets/µl in residents of Delhi, indicating 2.8-times more activated platelets in circulation of the latter group (Figure 6.21). Thus, platelet activity was remarkably upregulated in residents of Delhi.

Figure 6.21: Number of P-selectin -expressing circulating platelets in rural control and the residents of Delhi



6.3.3 Immunological changes

(a) Depletion of CD4+ and increase in CD8+ cells in Delhi

Flow cytometric study of lymphocyte subpopulation was carried out in 75 never-smokers, non-chewers of Delhi and 45 never-smokers, non-chewers of control group. Delhiites had a reduced percentage as well as absolute number of CD4+ T-helper (T_H) cells in their peripheral blood. For example, they had a mean of 33.2% CD4+ cells in their circulation against 42.6% in controls (Table 6.9, Figure 6.22), and the change was statistically significant ($p<0.05$; Figure 6.22). When the changes in CD4+ cells were expressed in absolute numbers, 15.3% decline was recorded among the citizens of Delhi (795 vs. 939/µl in control, $p<0.05$; Table 6.10).

Table 6.9: Percentage of different lymphocyte subtypes in peripheral blood

Cell type and group		Male (Control 30, Delhi 55)	Female (Control 15, Delhi 20)	Total
CD4+cell,	Control	43.6 ± 3.2	40.7± 3.9	42.6±3.0
	Delhi	33.4 ± 3.8*	36.5 ± 3.4	33.2±3.1*
CD8+ cell,	Control	24.4± 2.7	27.4±2.2	25.4± 2.1
	Delhi	32.4 ± 3.8*	26.8 ± 2.5	30.9± 2.2*

Cell type and group		Male (Control 30, Delhi 55)	Female (Control 15, Delhi 20)	Total
CD19 cell,	Control	21.2±2.6	19.8± 2.2	20.7±2.0
	Delhi	13.2 ± 2.5*	16.7 ± 2.0	14.1±2.0*
CD16+CD56+ cell,	Control	10.6± 2.1	11.8±2.2	11.0±2.1
	Delhi	20.8 ± 3.2*	19.4 ± 3.4*	20.4±2.8*

**, p<0.05 compared with control*

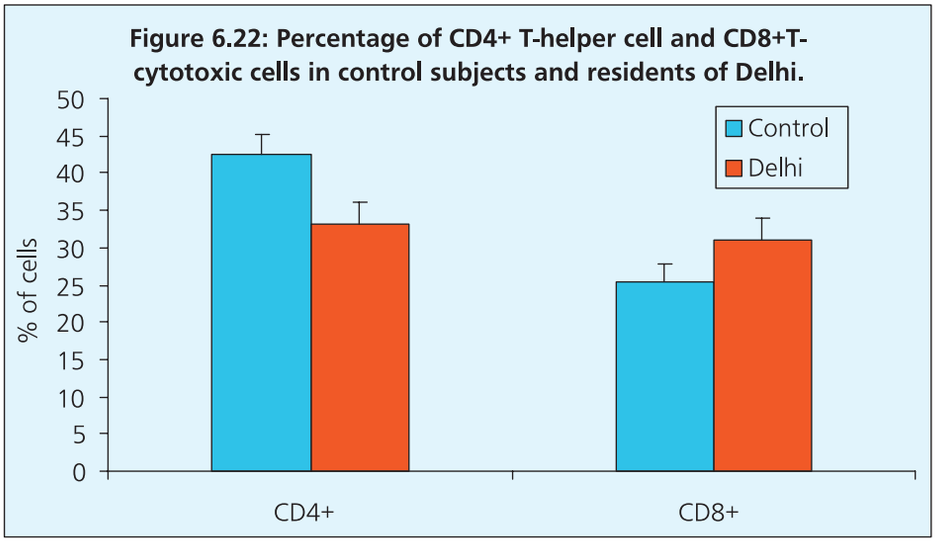


Table 6.10: Absolute number of different lymphocyte subtypes in circulation of the residents of Delhi

Cell type and group		Male (Delhi 55, control 30)	Female (Delhi 20, control 15)	Total (Delhi 75, control 45)
CD4+ / µl,	Control	935± 72	948± 87	939± 65
	Delhi	779 ± 87*	841 ± 72*	795 ± 63*
CD8+ cell / µl,	Control	524± 69	638± 44	562±51
	Delhi	756± 85*	617 ± 54	719 ± 68*
CD19 + cell / µl,	Control	453± 45	462± 51	456± 45
	Delhi	308 ± 48*	385 ± 48*	329 ± 60*
CD16+56+ cell / µl,	Control	228± 42	275±49	243± 44
	Delhi	485 ± 57*	447 ± 88*	475± 65*

**, p<0.05 compared with control*

In contrast to CD4+ cells, the percentage and absolute number of CD 8+ T- cytotoxic/suppressor (TC) cells was increased among the residents of Delhi (Figure 6.23). The mean percentage of CD8+ cells was increased from 25.4% in controls to 30.9% in Delhi (Figure 6.22), and the increment in absolute number of these cells was 28% (719 vs. 562/µl in control, $p<0.05$, Table 6.10). As a result, the CD4:CD8 cell ratio was reduced from 1.37 in control subjects to 1.07 in citizens of Delhi.

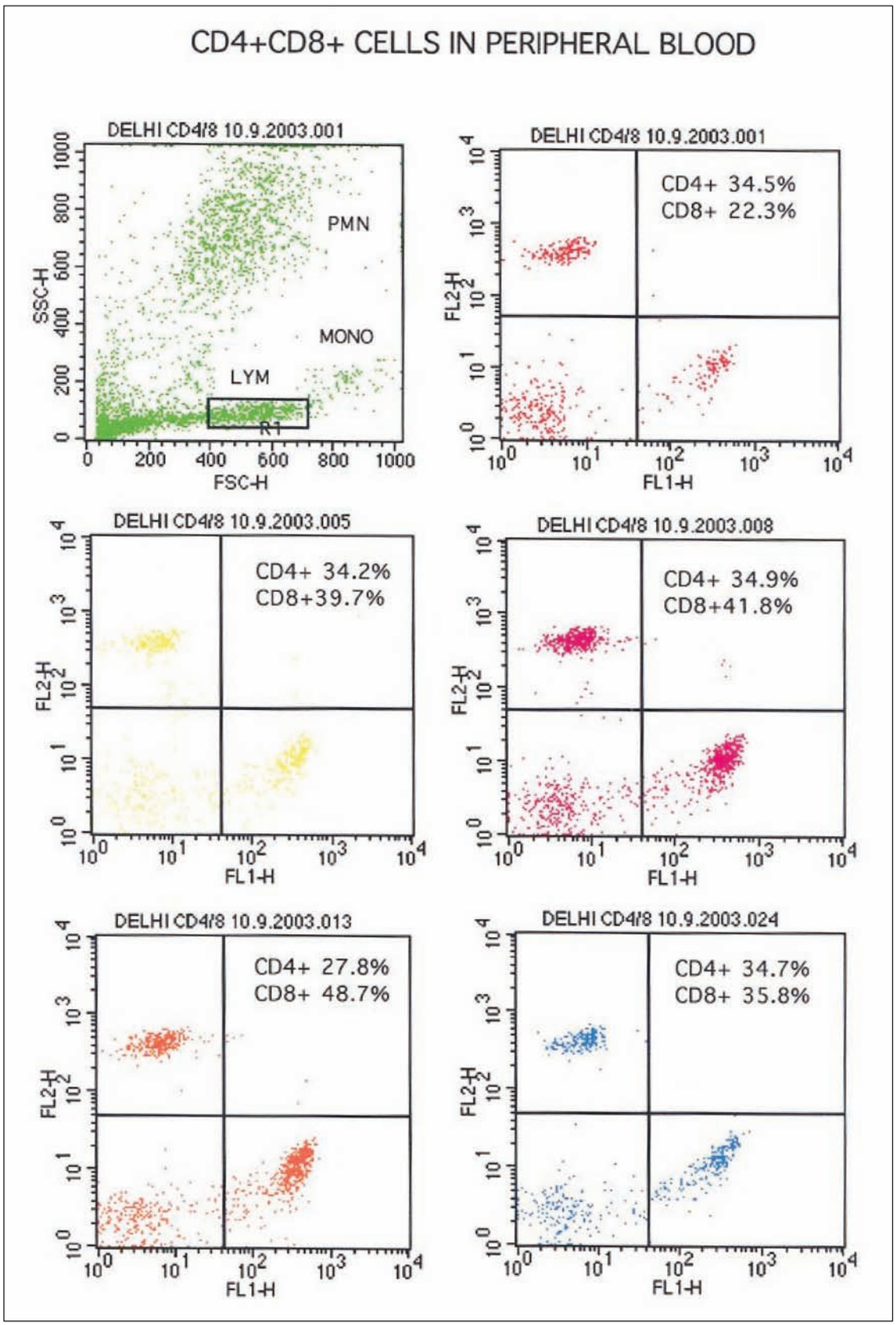


Figure 6.23: CD4+/CD8+ cells in peripheral blood of residents of Delhi

(b) Fall in CD19+ B-lymphocytes

Like CD4+ cells, significant ($p<0.05$) decline in the percentage as well as total number CD19+ B lymphocytes was recorded among the residents of Delhi (Table 6.9, 6.10; Figure 6.24). The reduction in the percentage of B cells was 32% while 28% decline was recorded in the absolute number of B cells in peripheral blood of the residents of Delhi (329 vs. 456/µl in control, $p< 0.05$; Figure 6.25).

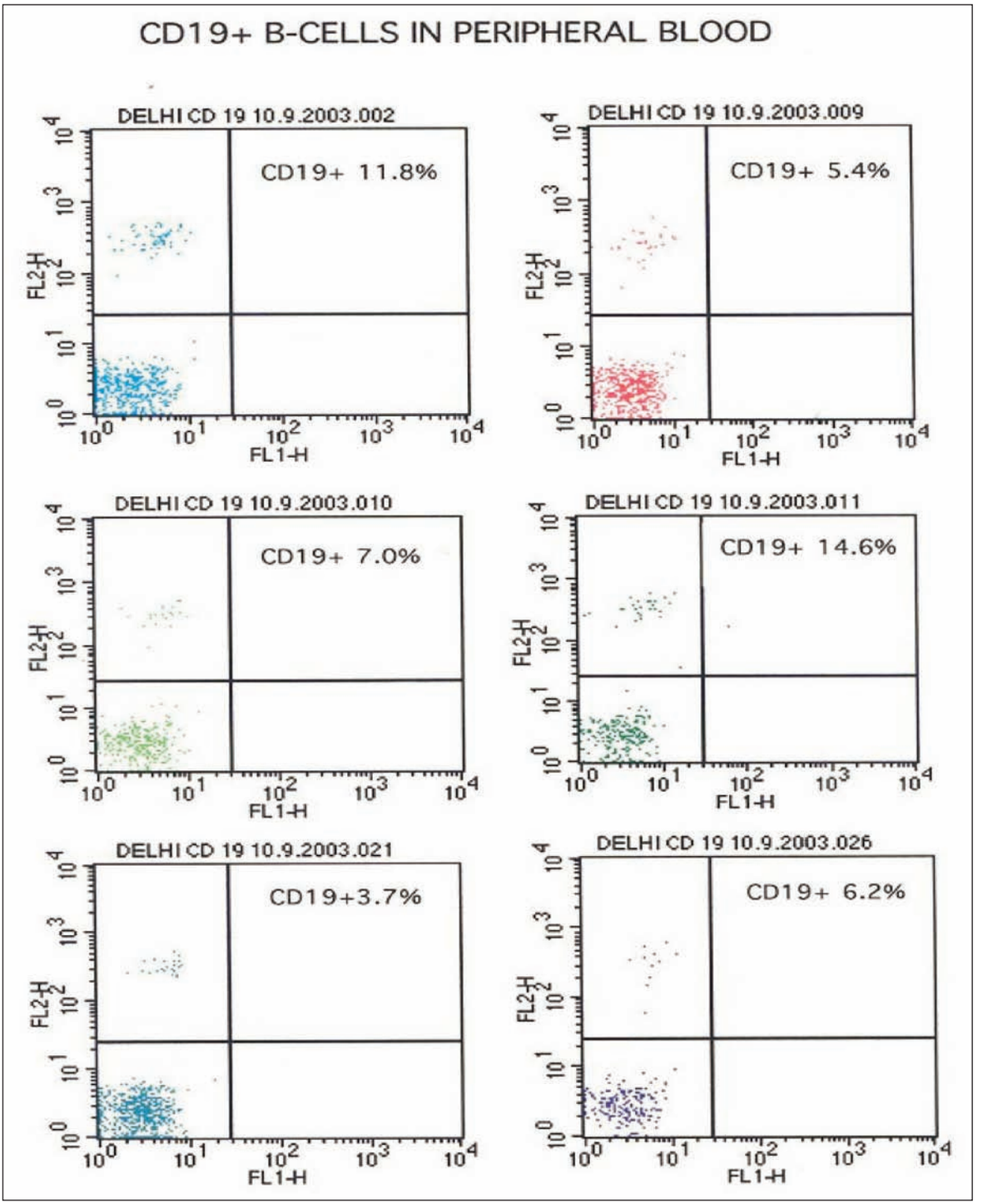
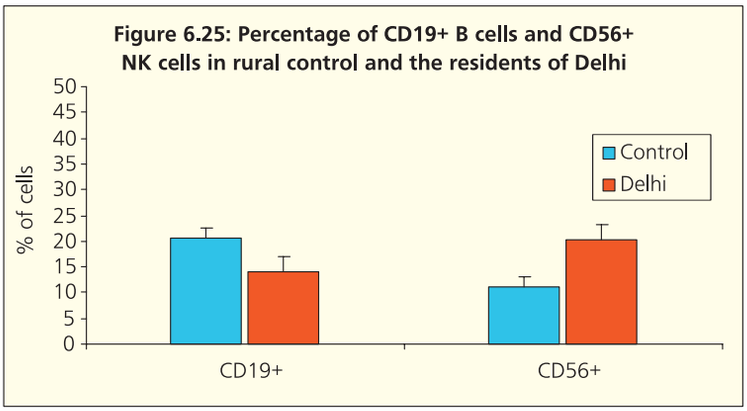


Figure 6.24: CD19+ B cells in peripheral blood of residents of Delhi



(c) Rise in natural killer (NK) cells

The percentage and absolute number of CD 16+CD56+ natural killer (NK) cells was nearly doubled in Delhi. For instance, the percentage of NK cells was increased from 11% in controls to 20.4% in Delhi (Figure 6.25, Figure 6.26). Similarly, the total number of NK cells in circulation was increased from 243/ µl in controls to 475/µl in residents of Delhi (+ 95%), suggesting remarkable increase ($p<0.001$) in the number of natural killer cells in peripheral blood of subjects chronically exposed to Delhi's air pollution (Table 6.9, 6.10).

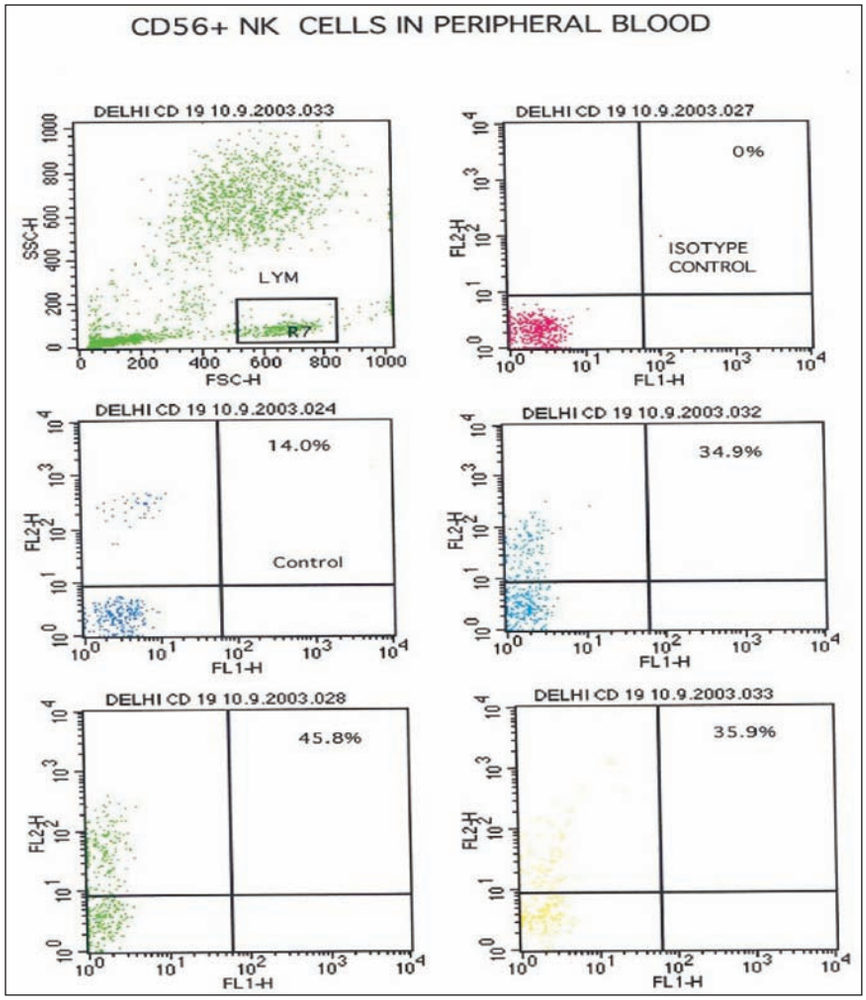


Figure 6.26: CD 56+ cells in peripheral blood of residents of Delhi

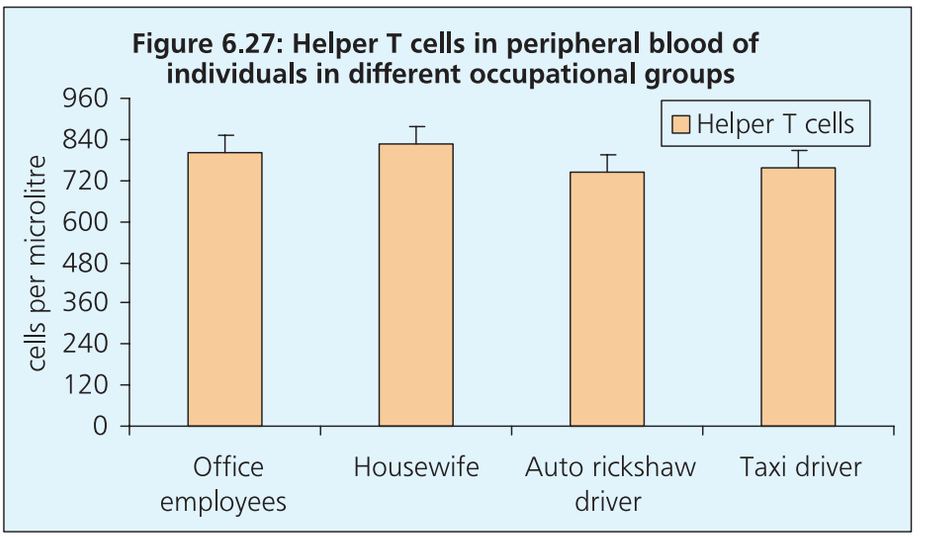
(d) Vehicular pollution and lymphocyte changes

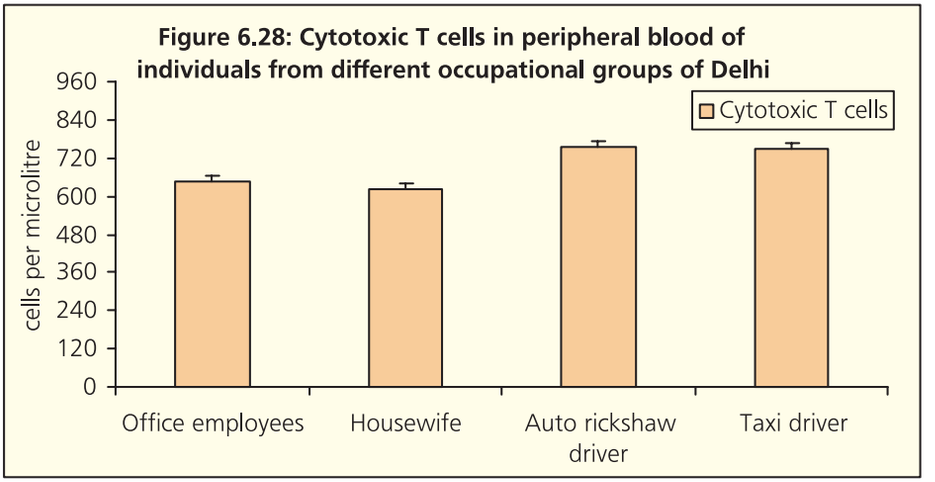
In order to investigate whether occupational exposure to vehicular pollution had any impact on lymphocyte subsets, lymphocyte subtypes in persons of different occupation was compared. Peripheral blood of occupationally exposed persons to vehicular pollution such as auto rickshaw drivers and taxi drivers of Delhi exhibited more severe reduction of CD4+ and CD19+cells and rise of CD8+ and CD56+ cells than city's housewives office employees (Table 6.11; Figure 6.27, 6.28, 6.29, 6.30).

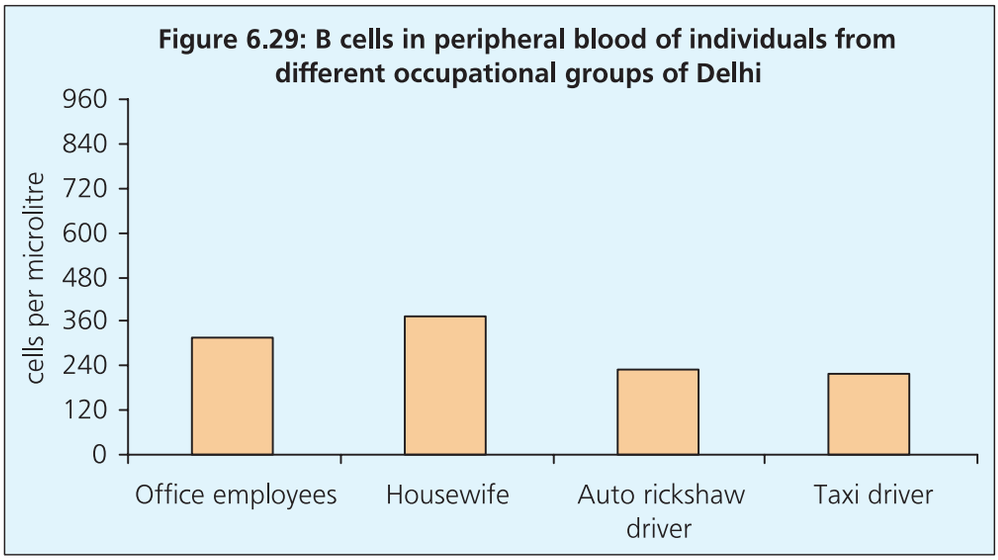
Table 6.11: Absolute numbers of different lymphocyte subtypes in peripheral blood of the residents of Delhi with in relation to occupation

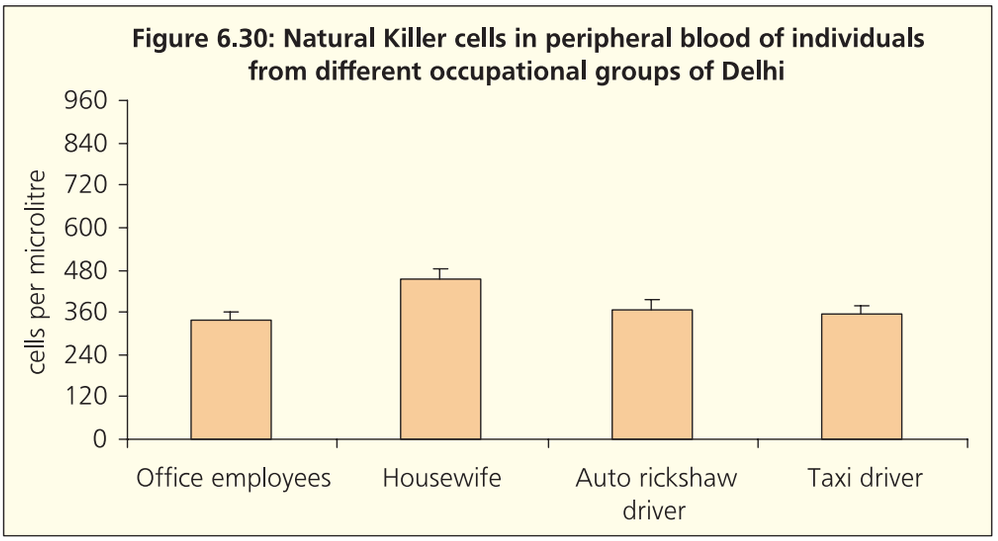
Group	CD4+ cell	CD8+ cell	CD19+ cell	CD16+56+ cell
Office employees	801 ± 224*	646 ± 212*	317 ± 156*	336 ± 99*
Housewife	826 ± 244*	621± 174*	372 ± 108*	456 ± 169*
Auto rickshaw driver	746 ± 244*	753 ± 134*	232 ± 83*	367 ± 102*
Taxi driver	759 ± 148*	748 ± 123*	221 ± 72*	353 ± 96*
Rural control	**939± 65**	**562±51**	**456± 45**	**243± 44**

**, p<0.05 compared with rural control*









(e) Association between immune alterations and air pollution

The changes in the relative proportion and absolute number of different types of lymphocytes in peripheral blood of the citizens of Delhi were correlated with PM_{10} concentration in ambient air in the city. In Spearman's test a negative correlation was found between PM_{10} level and number of CD4+ and CD19+ cells (rho values –0.429 and –0.325 respectively, $p<0.001$), while the correlation was positive for CD8+ and NK cells (rho values 0.531 and 0.785 respectively, $p<0.001$). Thus, alterations in the relative proportion and absolute number of lymphocyte subtypes in peripheral blood were correlated with particulate pollution of the city.

(f) Lymphocyte subtype and benzene exposure

Controlling tobacco/betel quid chewing, passive smoking and alcohol consumption as possible confounders, rise in urinary t,t-MA level was found to be associated with the fall in CD4+ T-cells (OR = 1.38, 95% CI, 1.09-1.71), and CD19+ B cells (OR=1.47, 95% CI, 1.15-2.26).

6.3.4 Assessment of liver and kidney function

(a) Liver function

Liver function was assessed through biochemical measurements of four serum parameters- ALT, AST, albumin an total protein in 302 and 81 subjects of Delhi and control group respectively who were never-smoker and non-user of alcoholic drinks. Moreover, they did not have history of jaundice in past one year. Results showed that the concentrations of all four parameters were higher than the upper limit of the respective normal range in 14 out of 302 (4.6%) of subjects in Delhi. In control, the number of such persons was 1 out of 81 (1.2%). Thus, it seems that liver function was altered in 5.6% residents of Delhi compared with 2.4% of controls (Table 6.12).

Table 6.12: Biochemical assessment of liver function

Serum enzymes and proteins	Normal range	% individuals with higher value in Control (n=81)	% individuals with higher value in Delhi (n=302)
ALT	5-40 IU/l	1.2	4.6*
AST	5-40 IU/l	1.2	4.6*
Albumin	3-4.5 g/dl	1.2	4.6*
Total protein	6-8 g/dl	1.2	4.6*

**, p<0.05 compared with control in Chi-square test*

(b) Kidney function

Kidney function was simultaneously assessed through biochemical measurements of serum urea and creatinine subjects whose liver enzymes were measured. It is evident that the concentrations of serum urea and creatinine were higher in 8 subjects of Delhi (2.6%) and 1 subject in control (1.2%; Table 6.13).

Table 6.13: Biochemical assessment of kidney function

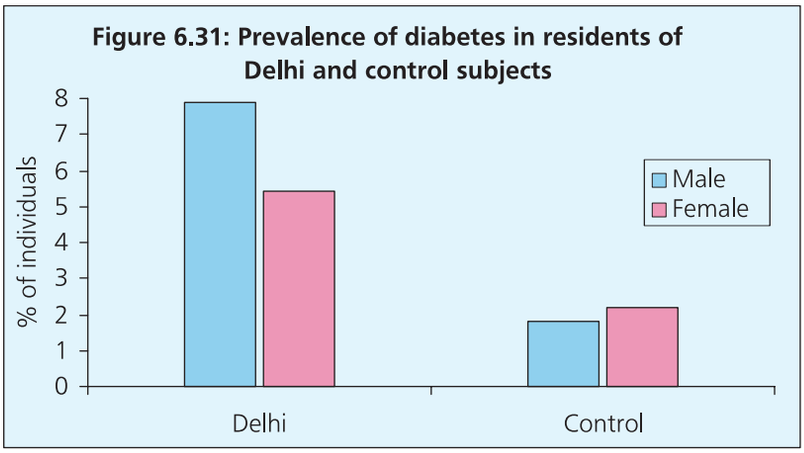
Parameters	Normal range	% with higher value in Control (n=81)	% with higher value in Delhi (n=302)
Serum urea	12-40 mg/dl	1.2	2.6*
Serum creatinine	0.7-1.5 mg/dl	1.2	2.6*

**, p<0.05 compared with control in Chi-square test*

6.3.5 Prevalence of diabetes

Blood glucose level was measured in 1718 (men 1419, women 299) eosinophils of Delhi and 834 in control group (men 654, women 180). The age of the subjects was 40-62 years. Diabetes, diagnosed when random blood glucose level ≥ 200 mg/dl, was present in 128 individuals of Delhi (7.4%) and 16 in control group (1.9%). The difference in the prevalence of diabetes in rural and urban subjects was significant ($p<0.001$).

In Delhi, diabetes was present in 112 men (7.9%) and 16 women (5.4%). In control group, 1.8% men (12/654) and 2.2% women (4/180) had diabetes (Figure 6.31). Thus, diabetes was nearly 4-times more prevalent in Delhi than in rural controls.



6.3.6 Assessment of antioxidant status

Air pollution exposure elicits oxidative stress that could be harmful for the cells. To circumvent such an eventuality, body has a strong efficient antioxidant mechanism. Several enzymes and non-enzymes constitute this antioxidant armory, most important of which is the enzyme superoxide dismutase (SOD).

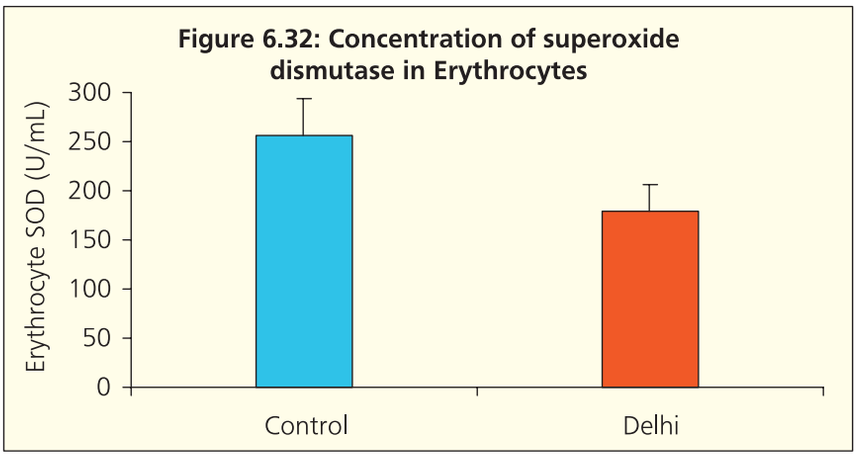
(a) Concentration of SOD

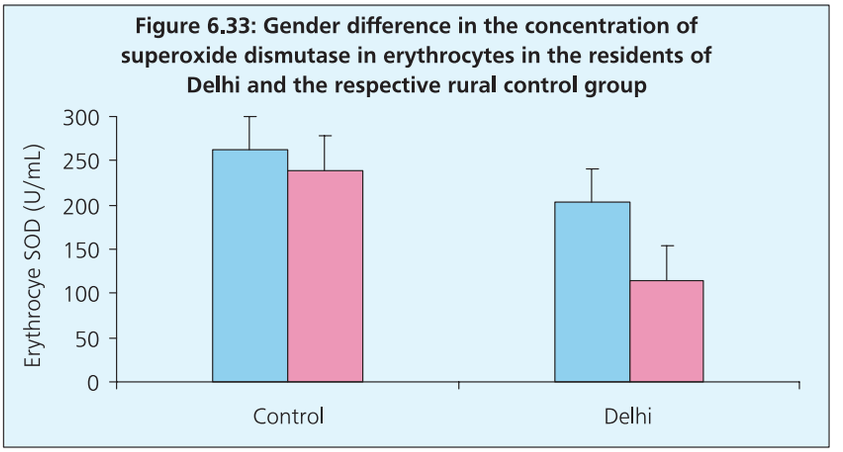
Compared with the control group, a significant depletion ($p<0.05$) of erythrocyte SOD was found among the residents of Delhi (Table 6.14, Figure 6.32). The reduction of SOD was 23% for male and 52% for females (Figure 6.33). Overall, 30% decline in the concentration of SOD in blood was found among the non-smokers of Delhi, implying a significant deficit in antioxidant activity especially in city's women.

Table 6.14: Concentration of superoxide dismutase in erythrocytes

Erythrocyte SOD (U/mL)	Control (n= 40)	Delhi (n=55)
Male (control 28, Delhi 40)	262.5 ± 52.3	204.1 ± 41.7*
Female (control12, Delhi 15)	238.4 ± 41.6	114.3 ± 38.4*
Total	255.3 ±38.4	179.4 ± 27.8*

*Results are mean ±SD; *, p<0.05 compared with respective control group*





(b) Total antioxidant status

Since SOD does not always reflect the antioxidant state of the body *par se*, total antioxidant concentration in plasma was measured by using commercially available kits. The normal reference value of total antioxidant level in blood is 1.3-1.77 mmol/liter of plasma. The control male subjects of this study had higher than the normal limit, but the females had a lower value. Overall the control group had normal total antioxidant level. In contrast, the residents of Delhi had drastically reduced level of total antioxidant. The urban mean was one fourth of the control mean, and 65% lower than the lower limit of normal range (Table 6.15, Figure 6.34, 6.35).

Particulate air pollution could be an important contributing factor to depleted SOD and total antioxidant level, because a negative correlation (rho values - 0.257, and –0.470 respectively, $p<0.05$) was found between PM_{10} level and SOD and total antioxidant status.

Table 6.15: Concentration of total antioxidant in plasma

Total antioxidant in plasma (mmol/L)	Control (n= 40)	Delhi (n=55)
Male (Control 28 , Delhi 40)	2.44±0.83	0.54± 0.28*
Female (Control 12, Delhi 15)	0.84±0.51	0.23 ± 0.19*
Total	1.93±0.62	0.46 ±0.23*

*Results are mean ±SD; *, p<0.05 compared with respective control group*

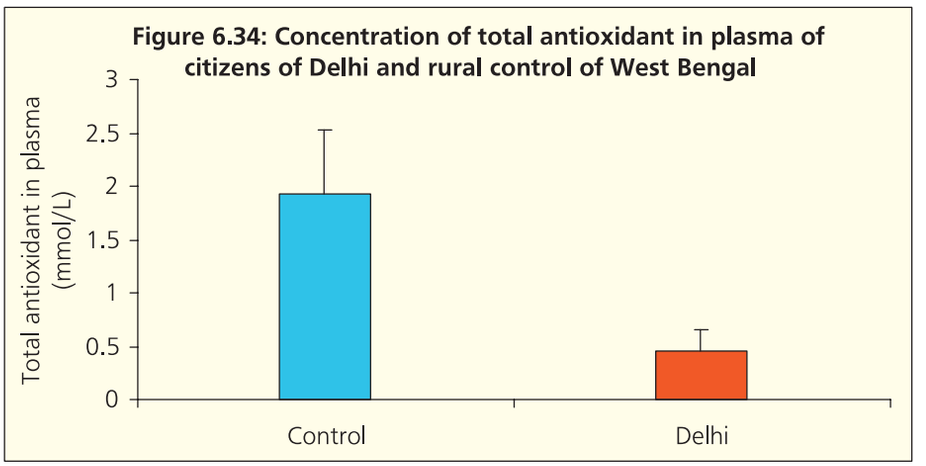
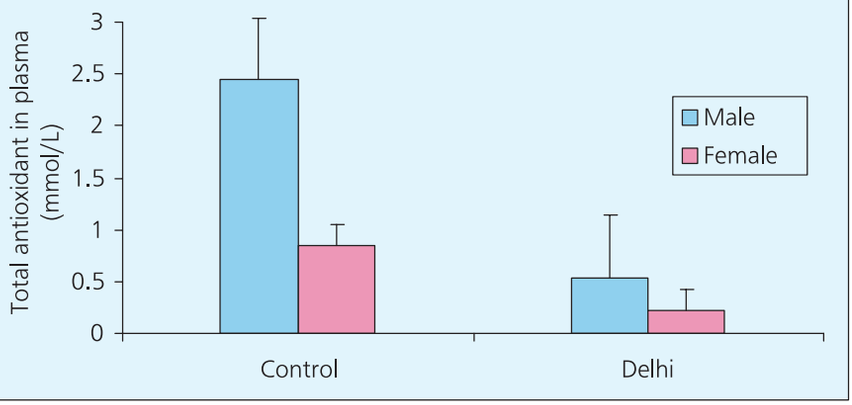


Figure 6.35: Gender difference in the concentration of total antioxidant in plasma of citizens of Delhi and rural control of West Bengal



6.4 FINDINGS

1. Arterial blood pressure of 2218 residents of Delhi and 642 control subjects, all lifetime non-smokers, were measured and analysed for the diagnosis of hypertension according to the criteria of JNC-7 (2003).
2. Overall, 36.1% residents of Delhi 9.5% of control subjects had hypertension. Thus the prevalence of hypertension was nearly 4-times higher in Delhi.
3. Systolic hypertension (systolic blood pressure ≥ 140 mmHg) was present in 19.4% subjects of Delhi compared with 7% of control subjects. The magnitude of systolic hypertension in Delhi was mild to moderate (Stage 1) in 15.4% citizens and severe (Stage 2) in 4% citizens. In control group, 6.1% had Stage 1 and 0.9% had Stage 2 systolic hypertension.
4. Diastolic hypertension (diastolic blood pressure ≥ 90 mmHg) was found in 33.4% citizens of Delhi, compared with 5.1% of controls. Stage 1 and Stage 2 diastolic hypertension were present in 23.4% and 10% residents of Delhi respectively, while in control group 4.4% had Stage 1 and 0.8% had Stage 2 diastolic hypertension.
5. Both systolic and diastolic hypertension was present in 16.7% citizens of Delhi against 2.6% of controls. Thus, in Delhi, 2.7% had only systolic hypertension, 16.7% had only diastolic hypertension and another 16.7% had both systolic and diastolic hypertension. In control subjects, 4.4% had systolic, 2.5% had diastolic and 2.6% had both systolic and diastolic hypertension, making overall hypertension prevalence 36.1% in Delhi and 9.5% in controls.
6. Hypertension prevalence was marginally higher in men both in Delhi (36.5% vs. 34.5%) and in controls (10.1% vs. 8.3%).
7. Like hypertension, the prevalence of pre-hypertension was much more in Delhi as 24.2% of urban subjects had systolic pre-hypertension compared with 8.1% of controls. Similarly, diastolic prehypertension was present in 10.7% citizens of Delhi compared with 6.5% of controls.
8. The prevalence of hypertension increased progressively with age. The prevalence in Delhi was 24.8% in 21-30 yr age group, 28.1% in 31-40 yr age group, 45.3% in 41-50yr age group, 52.5% in 51-60yr and 63.9% in 60+ yr age group.

9. RSPM (PM_{10}) level in ambient air was positively correlated with both systolic and diastolic blood pressure in Spearman's rank correlation test. The correlation was stronger for diastolic blood pressure (rho = 0.350, $p<0.005$). Conditional logistic regression analysis revealed that the risk factors for hypertension were high socio-economic status, elevated RSPM level, and obesity.

10. Systolic and diastolic blood pressure was negatively correlated with FVC, FEV_1, $FEF_{25-75\%}$, and PEFR. Therefore, rise in blood pressure appears to be risk factor for reduced lung function.

11. Hemoglobin concentration showed significant negative correlation (rho values –0.295, $p<0.001$) whereas platelet count correlated positively (rho value 0.354, $p<0.001$) with PM_{10} level. Differential count of WBC showed increased number of monocytes and basophils among non-smoking residents of Delhi when compared with that of control ($p<0.05$).

12. Greater prevalence of several hematological abnormalities like target cells, toxic granulation, anisocytosis, poikilocytosis, hypochromic RBC, immature neutrophils, metamyelocytes and giant platelets were found in the individuals from Delhi in comparison to the control population.

13. Platelet P-selectin was remarkably upregulated in residents of Delhi, suggesting hyper activation of circulating platelets. Estimation of total number of P-selectin expressing platelets in circulation showed 3,682 ± 960 (mean ± SE) activated platelets/µl in controls and 10,268 ± 1232 activated platelets/ µl in residents of Delhi, showing 2.8-times more activated platelets in circulation of the latter group.

14. A significant reduction ($p<0.05$) in the percentage of CD4+ T-helper cells and concomitant increase in the percentage of CD8+ T-cytotoxic cells ($p<0.05$) was found among the residents of Delhi. As a result, the CD4:CD8 cell ratio was reduced. The citizens of Delhi also demonstrated significant fall of CD19+ B cells and rise in CD56+ natural killer (NK) cells in peripheral blood. All these changes were more acute in Delhi's men than in women.

15. Statistical analysis showed a negative correlation of CD4+ and CD19+ cells (rho values –0.429 and –0.325 respectively, $p<0.001$) with PM_{10} values, while the correlation was positive for CD8+ and NK cells (rho values 0.531 and 0.785 respectively, $p<0.001$).

16. Liver function was altered in 5.6% residents of Delhi compared with 2.4% of controls. On the other hand, kidney function was impaired in 2.6% citizens of Delhi compared with 1.2% of controls.

17. Diabetes (random blood glucose >200mg/dl) was recorded in 7.4% residents of Delhi compared with 1.9% of controls. Prevalence of diabetes in Delhi was more in men than in women (7.9% vs. 5.4%).

18. Thirty percent depletion of erythrocyte superoxide dismutase level, and 76% reduction in total antioxidant status were observed among the citizens of Delhi, compared with rural controls. The findings imply down-regulation of body's antioxidant defense and consequent rise in the risk of oxidative stress-mediated cellular injury among the citizens of Delhi. The change could be attributed to city's high level of particulate air pollution, because a significant negative correlation was found between PM_{10} level and superoxide dismutase and total antioxidant status (rho values –0.257, and –0.470 respectively, $p<0.05$).

CHAPTER-7.0

BIOMONITORING OF BENZEME EXPOSURE AND GENOTOXICTY

7.1 INTRODUCTION

Urban air pollution is mixture of thousands of organic and inorganic compounds, some of which may cause damage to the DNA. These compounds are therefore genotoxic, and are considered extremely harmful for human health. The most notable examples are benzene and benzo(a)pyrene, which are genotoxic as well as carcinogenic. In addition, free radicals present in air pollution or generated in respiratory or defence cells such as neutrophils following pollution exposure may cause DNA damage. In view of these, genoxicity in exposed cells of person chronically exposed to Delhi's air pollution was assessed.

As a measure of genetic damage at the level of chromosomes, micronucleus (MN) test was employed. MN is defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus (Schmid, 1975). They originate from aberrant mitoses and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated into the daughter nuclei during mitosis. The MN test is the most frequent technique used to detect chromosome breakage or mitotic interference, events thought to be associated with increased risk for cancer (Stich et al., 1982, Tolbert et al., 1991). MN is useful because it can be studied directly in target cells of the buccal and airway epithelium. Considering these, MN frequency in exfoliated buccal epithelial cells of persons chronically exposed to high level of traffic-related emissions in Delhi was analysed. In addition, single cell gel electrophoresis or alkaline Comet assay in circulating lymphocytes was undertaken for detection of DNA damage.

7.2 MATERIALS AND METHODS

(a) Measurement of t,t-MA in urine

(i) Subjects

The concentration of t,t-MA, a benzene metabolite and a biomarker of benzene exposure, was measured in urine by HPLC-UV. Altogether, 54 samples from non-chewing and never-smoking males were measured: 24 samples from control subjects, 16 from Delhi's office employees, and 14 samples from auto rickshaw and taxi drivers of Delhi who were occupationally exposed to city's vehicular pollution.

(ii) Measurement protocol

Urine (25-50 ml) was collected into 100- ml plastic screw-cap vials for analysis of t,t-MA. Samples were analyzed following the procedure of Ducos et al., (1990). In brief, the samples were protected from light, brought to the laboratory at Kolkata in ice buckets and stored at –20°C until analysis. The samples were thawed and a 1- ml aliquot of each was passed through a Bond elut extraction cartridge filled with 500 mg of SAX sorbent preconditioned with 3 ml of methanol and 3 ml of distilled water. The cartridge was washed with 3 ml of 1% acetic acid solution. Then the t,t-MA was eluted with 3 ml of a 10% aqueous acetic acid solution, and measured in high performance liquid chromatography (HPLC, Waters, USA). Ten microliter fractions of the elute were used per injection in column filled with LiChrosorb C18, 5µM (Waters), and the detector was set at 259 nm. The eluent was a solution of 1% aqueous acetic acid/methanol (90/10). With a flow rate of 1.2 ml /min, the retention time of t,t-MA was 10 min and the duration of an analytical run was 20 min. A stock solution of t,t-MA (100 mg/l, Sigma Chem, USA) was prepared in 10% acetic acid.

(b) Micronucleus (MN) assay

(i) Collection of buccal mucosal and airway epithelial cells

The subject was asked to wash his/her mouth with 0.9% saline (NaCl) water. Then the inner side of the cheeks was scrapped using a sterile spatula to obtain the buccal mucosa, which was then smeared on clean glass slides. Airway epithelial cells were obtained from spontaneously expectorated sputum

samples. For each subject, at least 3 slides each for buccal and airway cells were made: 2 slides were stained with Wright-Giemsa and one with Feulgen for confirmation of MN.

(ii) Wright-Giemsa staining

For Wright-Giemsa staining, the slides were fixed in methanol for 10 min, and stained for 10 min with a mixture of Giemsa stain and distilled water in the ratio 1:1.5, v/v. Thereafter, the slides were washed in running water, air dried and mounted in DPX. The nuclei of the cells stained violet.

(iii) Feulgen staining for DNA

For confirmation of nuclear changes, Feulgen staining for DNA was done following the procedure of Pearse (1991, Figure 7.1). Parallel slides were fixed in Carnoy's fixative for 10 min (ethanol: chloroform: glacial acetic acid in the ratio 6 : 3 : 1, v/v).

Preparation of Schiff's Reagent

Dissolve basic fuschin in boiling distilled water by constant shaking
↓
Cool to 50°C, filter solution and add 1N HCl to the filtrate
↓
Cool to 25°C and add Sodium metabisulphite
↓
Incubate at 4°C for 24 hours
↓
Add activated charcoal with constant shaking, filter solution and store at 4°C in dark
↓
Bring to room temperature prior to use

Procedure

Smeared samples are fixed in Carnoy's fixative for 10 min
↓
Rinsed in distilled water (2 - 3 times)
↓
Incubated in 1N HCl at 37°C for 20 min and at 60°C for 12 min
↓
Stained in Schiff's reagent for 2 hours, rinsed in bisulphate solution (3 times)
↓
Washed in distilled water and dehydrated in graded ethanol, cleared in xylene and mounted in DPX
↓
Observed under light microscope

Figure 7.1: Micronucleus assay in buccal epithelial cell

Reagents used

1N HCl	100 ml
Bisulfite solution	50 ml
Schiff's reagent	50 ml

Preparation of stock solutions

Bisulfite solution

10% H_2SO_4	5 ml
1N HCl	5 ml

Distilled water	190 ml
Schiff's reagent	
Basic Fuchsin	1g
1N HCl	20 ml
Sodium metabisulfite	1 g
Activated charcoal	300 mg
Distilled water	200 ml

Procedure

The fixed slides were rinsed 2-3 times in distilled water, incubated in 1N HCl at 37°C for 20 min and at 60°C for 12 min. Then the slides are stained in Schiff's reagent for 2 hours, rinsed 3 times in bisulfite solution, washed in distilled water, dehydrated in graded ethanol, cleared in xylene and mounted in DPX. DNA stained bright reddish purple or magenta. Using a light microscope, at least 1000 cells were counted at 400x and 1000x magnification and the total number of MN per 1000 cells was recorded.

(c) Single cell gel electrophoresis (Comet assay) in peripheral blood lymphocytes

Analysis of DNA damage in peripheral blood lymphocytes was done following the procedure of Singh et al., (1988) with the modification of Zhu et al., (1999; Figure 7.2).

Procedure

1% Normal melting agarose is coated on pre-frosted slide and immediately solidified at 4°C

↓

0.6 % of low melting agarose is pipetted on the slide, covered with cover slip and allowed to solidify at room temperature

↓

Lymphocyte suspension (10 µl of isolated lymphocyte suspended in PBS mixed with 0.8% of low melting agarose) is transferred on the slide, covered with coverslip and solidified

↓

Coverslips are removed and slides are incubated in Lysis buffer for 1 hr at 4°C

↓

The slides are placed in electrophoretic tank in alkaline electrophoretic buffer for 20 minutes at 25V, 300mA

↓

Slides are washed thoroughly with neutralizing buffer and stained in 20 µl ethidium bromide

↓

Stained slides are immediately observed under green filter in fluorescence microscope

Figure 7.2: Comet assay

Reagents used

Ficoll-Paque (Pharmacia Biotech, USA)	
High melting agarose (SRL, India)	
1% high melting agarose in distilled water	100 µl
Low melting agarose (Sigma Chem, USA)	
0.6% low melting agarose in distilled water	70 µl
0.8% low melting agarose in distilled water	10 µl
Lysis buffer (pH 10.5)	

2.5M Sodium chloride	29.2 g
0.3M NaOH	2.4 g
0.1 M Na2EDTA	7.4 g
10mM TRIS	0.242g
Glass distilled water	200 ml
(10% DMSO (20 ml) and 1% Triton X (0.2ml) in were mixed with the lysis buffer just before use)	
Electrophoretic buffer (pH 12.5)	
0.3 M NaOH	12 g
1.0 mM Na2 EDTA	0.372 g
Distilled water	1000 ml
Neutralization buffer (pH 7.5)	
0.4M TRIS	4.84 g
Distilled water	100ml
Ethidium bromide (25 µg/ml distilled water, Sigma Chem, USA)	20 µl/ slide

Procedure

Lymphocyte separation

EDTA-anticoagulated whole blood was centrifuged for 10 min at 400 g at 20°C with Ficoll-Paque (Sigma Chem, USA) following the instruction of the manufacturer. This procedure separates a buffy coat layer of lymphocyte and platelet by the density gradient centrifugation technique. The buffy coat was pipetted out, collected in Eppendorf tubes and stored at –20°C till further use.

Comet Assay

Frosted microscopic slides were covered with 100 µl of 0.1% high melting agarose, immediately covered with a cover glass and kept at 4oC for 5 min to solidify. Next, the cover glass was removed and 70 µl of 0.6% low melting agarose was added to the slide, covered with a cover glass and kept at 4oC for another 5 min to solidify. Thereafter, 10 µl of the thawed lymphocytes was mixed with 90 µl of 0.8% low melting agarose to form a cell suspension. After gently removing the cover glass, 50 µl of this cell suspension was rapidly added onto the agarose layer, spreaded using a cover slip, and again kept cold for 10 min to solidify. Thereafter the cover glass was removed and 60 µl of 0.8% low melting agarose was added and kept at 4oC for 10 min to solidify. Then, the cover glass was removed and the slides were immersed in freshly prepared lysing solution at 4°C for 1 hr. After lysis, the slides were washed with electrophoretic buffer and then placed on a horizontal electrophoresis tank filled with fresh electrophoresis solution to a level approximately 0.25 cm above the slides for 20 min to allow the unwinding of the DNA and expression of alkali labile damage before electrophoresis. To electrophorese the DNA an electric current of 25 V and 300 mA was applied for 30 min. All of these steps were conducted under dimmed light to prevent additional DNA damage. After electrophoresis, the slides were gently washed to neutralize excess alkali by placing the slides horizontally and flooding them with neutralization buffer. After 55 min the cells were stained with 25 µl of ethidium bromide (Sigma Chem, USA), covered with cover slip, kept in a humidified box and analyzed within 48 hr using a fluorescence microscope (Nikon, Japan).

(d) Statistical analysis

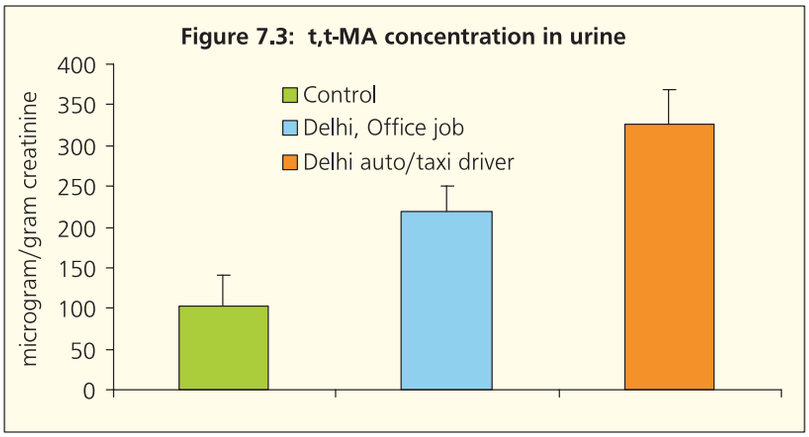
All data are expressed as mean± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between measured outcome and possible confounders such as RSPM levels. Spearman's rank test for continuous variables and Chi-square test for categorical variables were done. $P<0.05$ was considered as significant.

7.3 RESULTS

7.3.1 Concentration of t,t-MA in urine

The concentration of t,t-MA, a benzene metabolite and a biomarker of benzene exposure, was measured in urine by HPLC-UV. The control male subjects (n=24) had a mean of 102± 38 µg/g creatinine of t,t-MA . In contrast, the male residents of Delhi with office jobs (n=16) had 218±102 µg/g creatinine t,t-MA in urine, indicating 2-times more benzene metabolites in their urine ($p<0.001$). Occupationally exposed subjects to vehicular emissions of Delhi such as auto rickshaw and taxi drivers (n=14) had 326± 117 µg/g creatinine of t,t-MA in urine which was 1.5-times more than the office employees of Delhi and 3.2-times more than that of rural controls ($p<0.001$, Figure 7.3). Therefore, the citizens of Delhi in general, and the occupationally exposed subjects to Delhi's vehicular pollution in particular, were several times more exposed to benzene than the age-and sex-matched rural controls.

Figure 7.3: t,t-MA concentration in urine

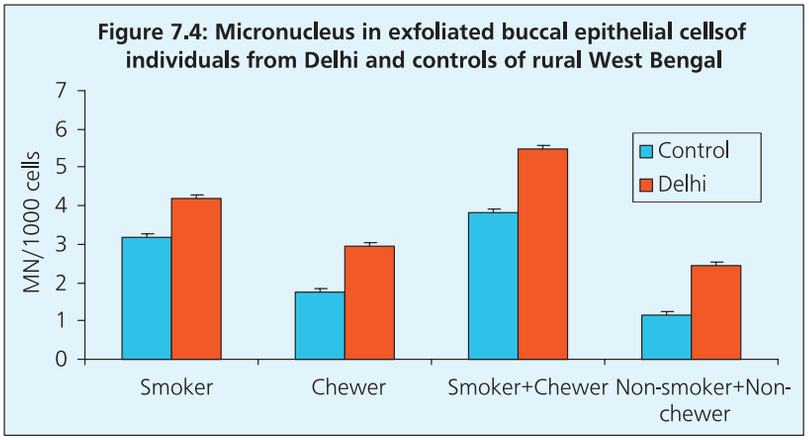


7.3.2 Elevated MN level in buccal epithelium of the residents of Delhi

MN are rare under normal physiological conditions. In control subjects, a mean number of 1.34 ± 0.56 (SD) MN-containing cells per 1000 exfoliated buccal epithelial cells was found in non-smokers. Current smokers among controls had a significantly higher MN count (3.17 ± 1.74 micronucleated cells/1000 cells, $p<0.01$) than never smokers.

In Delhi, even the never-smokers had 3.03 ± 1.46 MN-containing cells per 1000 exfoliated buccal epithelial cells. The smokers had 4.17 ± 1.83 MN per 1000 cells. In general, Delhi's never-smokers had 2.3-times more MN than their control counterparts (3.03 vs.1.34 MN /1000 cells, $p<0.001$, Figure 7.4, 7.5).

Figure 7.4: Micronucleus in exfoliated buccal epithelial cellsof individuals from Delhi and controls of rural West Bengal



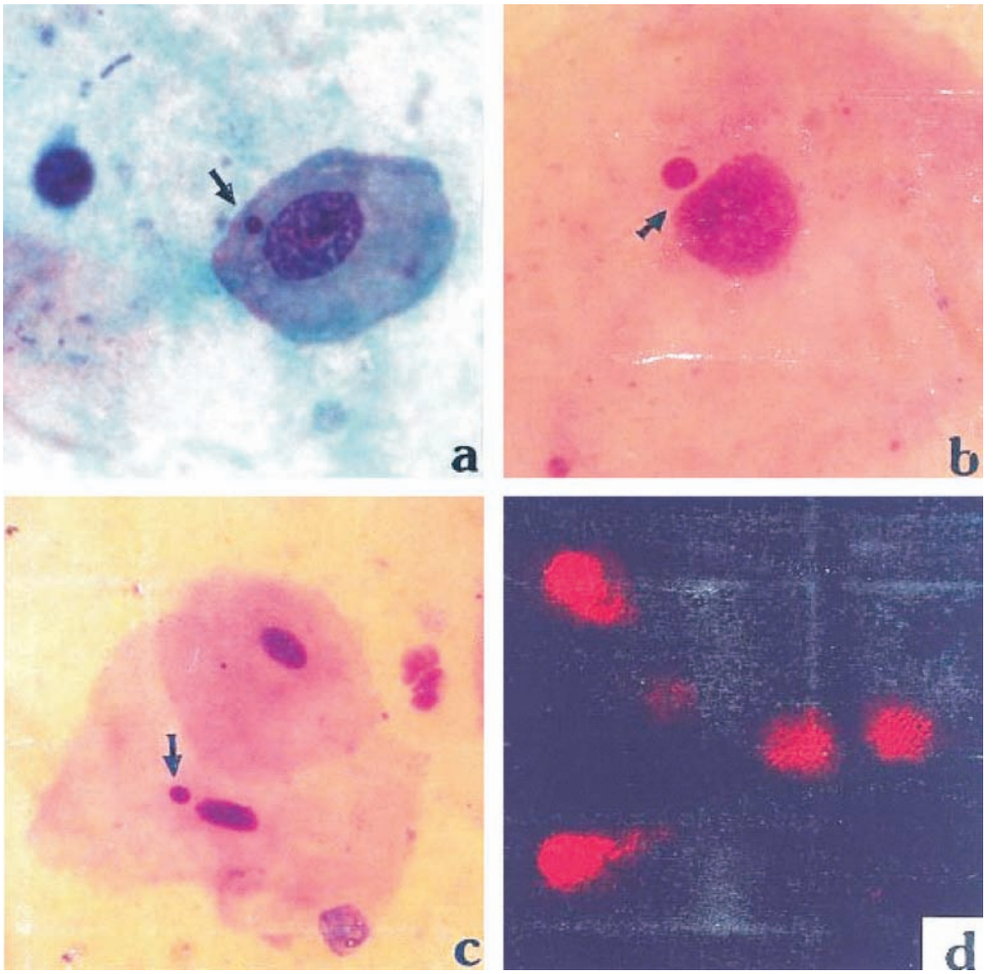


Figure 7.5: Residents of Delhi showing micronucleus in airway (a) and buccal epithelial cells (b, c) and 'comet' in peripheral blood lymphocytes (d). Papanicolaou stained, x 1000 (a), Giemsa-stained x 1000 (b, c) and Single Cell Gel Electrophoresis (Comet assay, x 400 (d)

Like smoking, tobacco chewing could enhance MN number, because genotoxic materials are present in chewing tobacco. To eliminate the effect of that confounder, the MN number of non-smoking, non-chewing individuals from control and urban group was compared. Non-smokers and non-chewers in control group had a mean MN count of 1.15 ± 0.43 per 1000 cells. In contrast, non-smokers and non-chewers residing in Delhi had 2.46 ±0.84 per 1000 cells, indicating more than 2-fold rise in MN number in the latter group. Smokers plus chewers of Delhi had 32% more MN than only smokers (Table 7.1, Figure 7.4) while only chewing increased the MN count by 19% (from 2.46 to 2.93/1000 cells), suggesting synergistic effect of chewing on MN formation in smokers.

Table 7.1: Micronucleated cell per 1000 exfoliated buccal epithelial cells

Group	Control	Delhi	% change over control
Smoker	3.17 ± 1.74	4.17 ± 1.83*	32
Chewer	1.76±0.69	2.93±1.68*	66
Smoker plus chewer	3.82±1.54	5.49±2.32*	44
Non-smoker plus non-chewer	1.15 ± 0.43	2.46 ±0.84*	114

**, p<0.05 compared with respective control value*

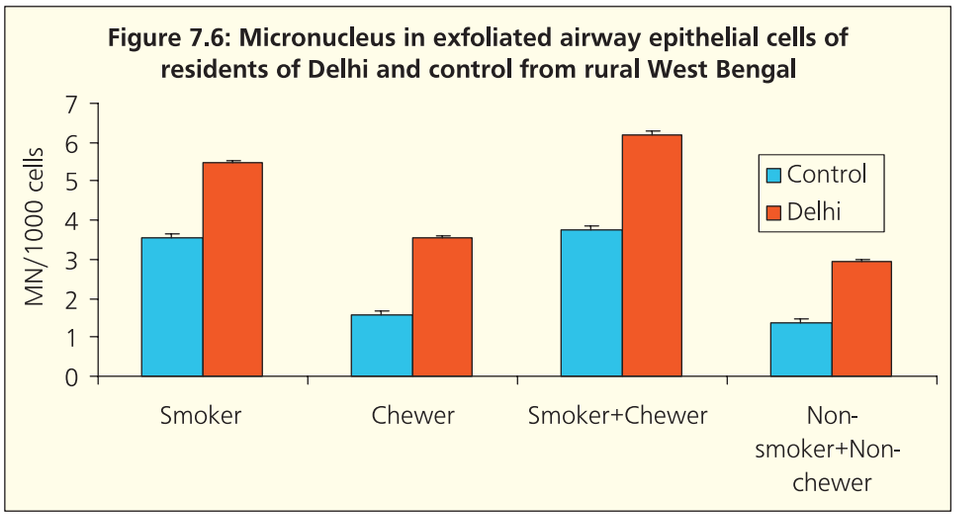
(a) Increased MN count in exfoliated airway epithelial cells in sputum of the residents of Delhi

Presence of MN was examined in airway epithelial cells exfoliated in sputum. In control subjects who were non-smokers and non-chewers, 1.35 ± 0.73 (SD) MN-containing cells per 1000 exfoliated airway epithelial cells in sputum were recorded. Smokers had significant, 2.6-times more MN-containing airway cells, but chewers showed only modest 15% rise in MN count, showing a weak relationship between tobacco chewing and MN formation in airway epithelial cells, although the association was strong for MN development in the oral cavity where the lining cells are in direct contact with the tobacco products. In Delhi, the non-smokers and non-chewers had 2.92 ±1.24 MN-containing cells per 1000 exfoliated airway epithelial cells, which was 2.2-times higher ($p<0.05$) than their control counterparts (Table 7.2, Figure 7.6). Delhi's smokers had 1.9-times more MN in airway cells, while chewers showed 21% rise in MN per number. Smokers plus chewers had 13% more MN than only smokers, suggesting a small additive effect of chewing on MN formation in airway epithelial cells of smokers (Figure 7.5).

Table 7.2: Micronucleated cell per 1000 exfoliated airway epithelial cells

Group	Control	Delhi	% change
Smoker	3.57 ± 1.59	5.47 ± 2.20*	53
Chewer	1.56±0.76	3.53±1.78*	126
Smoker plus chewer	3.77±1.29	6.19±2.32*	64
Non-smoker plus non-chewer	1.35 ± 0.73	2.92 ±1.24*	116

**, p<0.05 compared with respective control value*



(b) MN frequency in never-smoking and non-chewing residents of different areas of Delhi

Exfoliated buccal and airway epithelial cells collected for MN assay from 1396 individuals throughout the city who never smoked or chewed tobacco or tobacco products. It was found that MN frequency was lowest in persons who reside in South Delhi, where the level of air pollution was lowest in the city. Conversely, highest MN frequency was found in residents of North Delhi, where the level of air pollution was highest in Delhi (Table 7.3, Figure 7.7, 7.8).

Table 7.3: Micronucleus (MN) count in buccal and airway epithelial cells of non-chewers and never-smokers of different areas of Delhi

Area	n	MN/1000 buccal epithelial cells	MN/1000 airway cells
East	255	2.23 ±1.32	2.73 ±1.32
Central	326	2.89 ± 1.45	3.29 ± 1.53
North	254	3.12 ±1.83	3.49 ±1.83
West	236	2.48 ±1.42	3.10 ±1.42
South	325	1.65 ±1.17	2.12 ±1.17
Total Delhi	**1396**	**2.46±0.84**	**2.92 ±1.24**

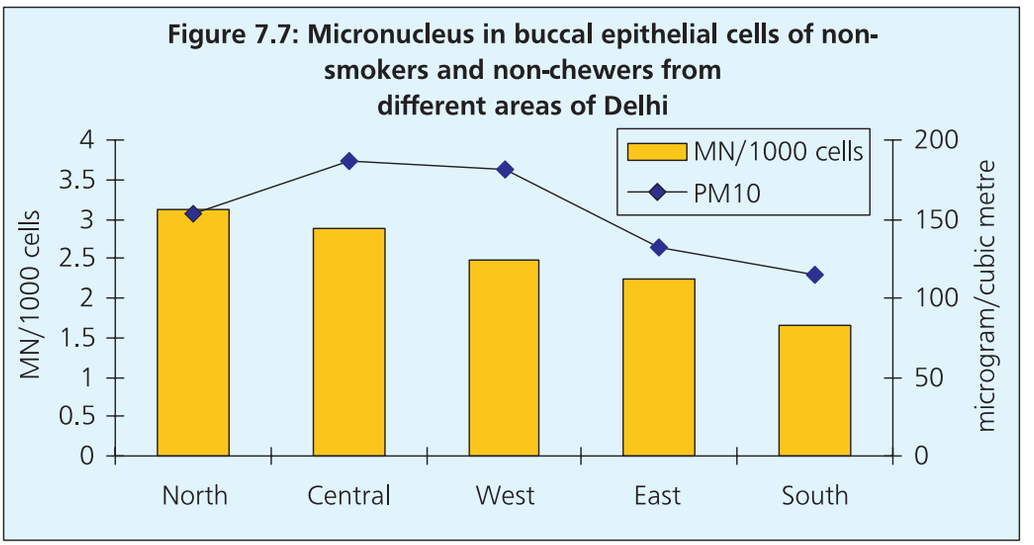


Figure 7.7: Micronucleus in buccal epithelial cells of non-smokers and non-chewers from different areas of Delhi

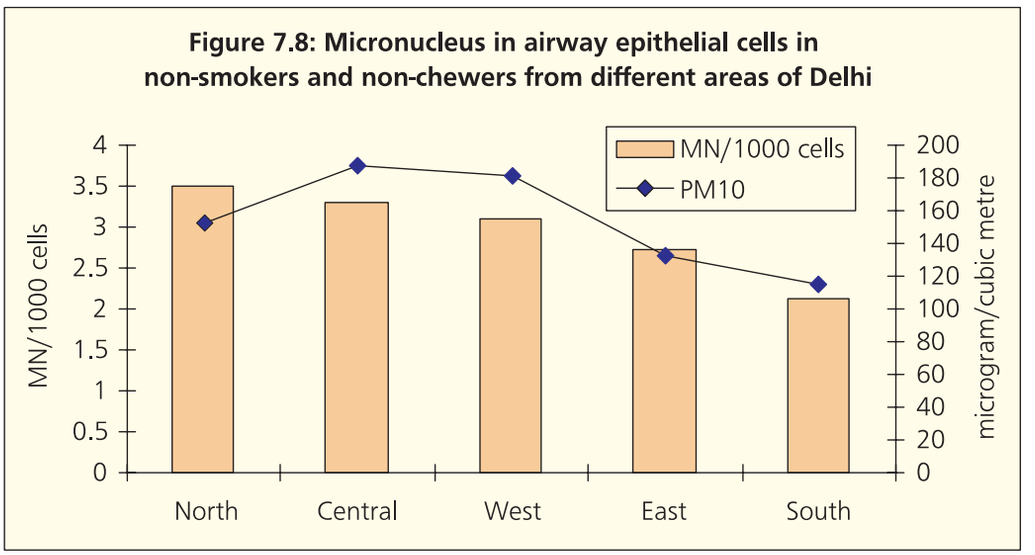


Figure 7.8: Micronucleus in airway epithelial cells in non-smokers and non-chewers from different areas of Delhi

(c) MN count among the residents of different localities of Delhi

(i) East Delhi

East Delhi had a mean of 2.23 and 2.73 MN per 1000 airway and buccal epithelial cells respectively, which were considerably lower than the Delhi's average. It suggests a relatively better air quality in the eastern part of the city. However, Shahdara had a poor air quality, and this was reflected in MN count. Residents of this locality had highest MN count in all of eastern Delhi. In contrast, residents of Vasundhara Enclave displayed lowest MN count, indicating lower concentration of genotoxic pollutant in air of this region (Table 7.4).

Table 7.4: Micronucleus count (number/1000 cells) among the non-smoking and non-chewing residents of East Delhi

Area	n	Buccal epithelial cells	Airway epithelial cells
East Arjun Nagar	96	2.03±0.89	2.23±1.13
Shahdara	62	2.61±0.73	3.15±1.27
Gandhi Nagar	75	2.45±1.06	3.28±1.22
Vasundhara Enclave	22	1.27±0.54	1.87±0.89
Total	**255**	**2.23±1.32**	**2.73±1.12**

Results are mean ± SD

(ii) Central Delhi

The mean MN count of the residents of Central Delhi was higher than the Delhi's average. Highest count was observed among the street hawkers and shopkeepers of the ITO area, which, incidentally had highest PM_{10} and benzene levels in the city. On the other hand, lowest MN count was recorded among the residents of Pusa Road, which had a relatively cleaner air (Table 7.5).

Table 7.5: Micronucleus count among the non-smoking and non-chewing residents of Central Delhi

Area	n	MN/1000 buccal epithelial cells	MN/1000 airway cells
ITO	32	4.25±2.73	4.93±2.54
Ajmeri Gate	45	3.64±2.12	4.33±2.27
Nizamuddin	35	3.68±1.86	4.08±2.38
Hailey Road	37	1.85±1.12	1.98±0.77
Old Rajinder Nagar	45	2.15±1.32	2.42±1.42
New Rajinder Nagar	34	2.15±1.20	2.92±1.25
Pusa Road	33	2.32±1.27	1.82±1.02
Karol Bagh	41	3.34±1.86	3.85±2.24
Hari Nagar	24	2.53±1.24	3.15±1.69
Mean	**326**	**2.89±1.45**	**3.29±1.53**

(iii) North Delhi

Residents of northern part of Delhi had the highest Mn count, suggesting poor air quality with respect to the concentrations of potential mutagens like benzene and benzo(a)pyrene. Highest MN count of the city was found among the residents and traders of Chandni Chowk-Daryaganj area. In contrast, a relatively lower level of MN was found in Civil lines and Rohini, both were quiter places than the rest of the areas examined in this study (Table 7.6).

Table 7.6: Micronucleus count among the non-smoking and non-chewing residents of North Delhi

Area	n	MN/1000 buccal epithelial cells	MN/1000 airway cells
Chandni Chowk	24	4.37±2.48	5.08±2.75
Civil Lines	21	2.83±1.43	2.90±1.65
Darya Ganj	14	3.92±2.39	4.35±2.84
Kamla Nagar	17	3.29±2.43	3.02±2.21
Virendra Nagar	16	2.33±2.12	3.05±2.22
Kalyan Vihar	18	2.78±1.87	2.83±1.87
Ashok Vihar	35	3.65±1.76	3.97±2.12
Rohini	24	2.57±1.25	2.91±2.11
Shalimar Bagh	29	2.89±1.79	3.01±2.24
Sangam Park Extension	22	2.82±1.86	3.90±2.12
Shahzada Bagh	34	2.77±1.82	3.55±2.32
Mean	**254**	**3.12 ±1.83**	**3.49 ±1.83**

(iv) West Delhi

In West Delhi, highest MN count was recorded in Janak Puri, and lowest in Inder Puri.The average MN count of the people of western Delhi was slightly lower than Delhi's average (Table 7.7).

Table 7.7: Micronucleus count among the non-smoking and non-chewing residents of West Delhi

Area	n	MN/1000 buccal epithelial cells	MN/1000 airway cells
Paschim Vihar	53	2.69±1.22	3.18±1.33
Janak Puri	52	2.82±0.87	3.42±1.45
Tilak Nagar	43	2.41±1.12	3.27±1.61
Jaidev Park	32	2.50±1.2	3.25±1.16
Kangan Heri	24	1.44±0.88	2.76±1.24
Inder Puri	32	2.37±1.08	2.31±1.32
Mean	**236**	**2.48 ±0.73**	**3.10 ±1.12**

(v) South Delhi

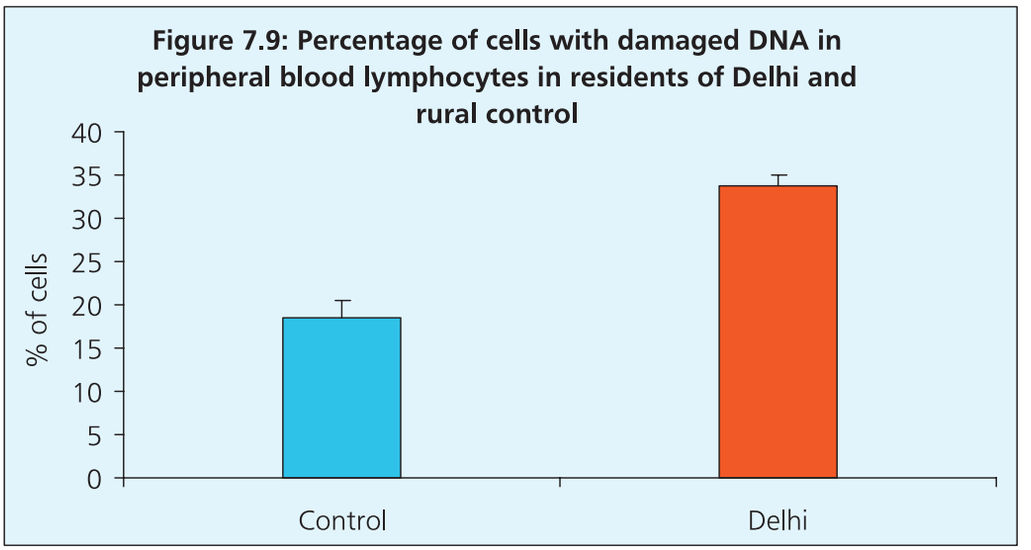
Residents of South Delhi had the lowest MN count in Delhi, suggesting better air quality in this area. Vasant Place had the lowest MN count in entire Delhi, indicating best air quality in this area among all the areas covered under this study (Table 7.8).

Table 7.8: Micronucleus count among the non-smoking and non-chewing residents of South Delhi

Area	n	MN/1000 buccal epithelial cells	MN/1000 airway cells
R.K.Puram	22	1.65±0.63	2.36±1.04
Sarojini Nagar	21	1.10±0.45	2.48±1.12
Vasant Place	22	0.63±0.25	0.82±0.22
Safdarjung Enclave	16	1.12±0.34	2.75±1.14
Lajpat Nagar	34	2.52±1.27	2.73±1.19
Green Park	25	2.00±0.89	2.52±1.03
Yusuf Sarai	25	2.64±1.27	2.80±1.14
Tughlakabad Industrial Area	42	1.23±0.69	1.35±0.66
Srinivas Puri	20	1.95±0.78	2.22±0.82
Nehru Place	53	1.34±0.58	1.87±0.49
Lodhi Road	45	1.81±0.73	2.15±1.22
Mean	**325**	**1.65 ±0.63**	**2.12 ±0.82**

7.3.3 DNA damage in lymphocytes

The frequency and extent of damage in DNA of peripheral blood lymphocytes was examined by Comet assay in 12 control and 15 Delhi's residents who were non-chewer and never smokers. Compared to control subjects, significantly higher ($p<0.05$) frequency of DNA damage was recorded in lymphocytes of the residents of Delhi. In citizens of Delhi 33.8% lymphocytes had damaged DNA against 18.5% of controls ($p<0.05$; Figure 7.9). The tail length of Comet, an indicator of the extent of DNA damage, was 2.8± 0.2 (SD) µm in Delhi compared with 1.7±0.2 µm in control subjects ($p<0.05$); (Table 7.9, Figure 7.10). Therefore, MN and Comet assays showed significantly increased genotoxity among the residents of Delhi, as compared with rural controls (Figure 7.5) although the test was conducted on limited population.



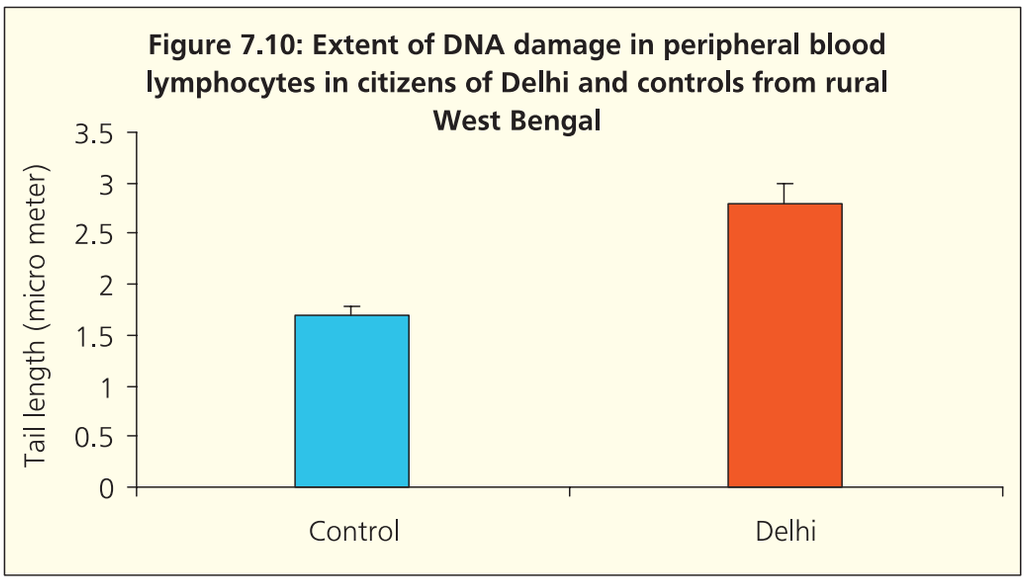


Table 7.9: Assessment of DNA damage by COMET Assay in peripheral blood lymphocytes

	n	Percentage of cells with damaged DNA	Extent of damage (Average tail length in µm)
Delhi	15	33.8±7.3	2.8 ± 0.6
Control	12	18.5±4.3*	1.7± 0.3*

*Results are mean± SD; *, p<0.05 compared with rural*

7.3.4 Association between air pollution exposure and MN formation

(a) Association with RSPM

In order to investigate whether the observed high MN count in buccal and airway epithelial cells among the residents of Delhi were due to higher level of air pollution in city, the data was analyzed by

Spearman's rank correlation study. RSPM level of the city was positively correlated with MN formation in buccal (rho=0.40, p<0.01) as well as airway epithelial cells (rho=0.44, p<0.01), and DNA damage in lymphocytes (rho= 0.37, p<0.05, Table 7.2).

(b) Correlation of MN formation with ambient benzene and benzo(a)pyrene (B(a)P]) levels

A strong correlation (rho=0.77, p<0.001) was found between benzene level in ambient air and MN frequency in buccal and airway epithelial cells, cells on direct line of contact with inhaled pollutants. B(a)P concentration in breathing air of Delhi also showed a significant, positive correlation with MN in buccal and especially airway cells. But the strength of this correlation was weaker than elicited by benzene (rho=0.33 and 0.41 for buccal and airway cells, p<0.05; Table 7.10).

Table 7.10: Spearman's rank correlation between air pollution levels and micronucleus formation

Variable	Correlation (rho value)	P value
RSPM and MN in buccal cells	0.40	<0.01
RSPM and MN in airway cell	0.44	<0.01
Benzene and MN in buccal cell	0.77	<0.001
Benzene and MN in airway cell	0.77	<0.001
B(a)P and MN in buccal cell	0.33	<0.05
B(a)P and MN in airway cell	0.41	<0.05

In essence, it is apparent that the greater prevalence of genotoxicity in buccal and airway epithelial cells, and peripheral blood lymphocytes of the residents of Delhi could be attributed, at least in part, to city's air pollution level with special reference to benzene, benzo(a)pyrene, which usually enters the body being adsorbed on the surface of RSPM.

7.4 FINDINGS

1. High concentration of t,t-MA was found among the subjects of Delhi than the age-and sex-matched rural controls. For example, the control subjects had a mean of 102µg/g creatinine of t,t-MA in urine in contrast to the 218 µg/g creatinine in Delhi's office employees and 326 µg/g creatinine in auto rickshaw and taxi drivers of the city.
2. Study of the micronucleus (MN) frequency among the residents of Delhi revealed increased mean MN containing cells per 1000 exfoliated cells in comparison to the control group. In general, Delhi's non-smokers had 2.3-times more MN than control non-smokers (3.03 vs.1.34 MN /1000 cells, p<0.001), which may indicate chromosomal damage.
3. RSPM (Respirable Suspended Particulate Matter) level of the city positively correlated with MN formation in buccal (rho=0.4, p<0.1) as well as airway epithelial cells (rho=0.44, p<0.05).

4. A strong correlation (rho=0.77, $p<0.001$) was also found between benzene level in ambient air and MN frequency in buccal and airway epithelial cells. B(a)P concentration in breathing air of Delhi also showed a significant, positive correlation but the strength of this correlation was weaker than elicited by benzene (rho=0.33 and 0.41 for buccal and airway cells, $p<0.05$).

CHAPTER-8.0

NEUROBEHAVIORAL SYMPTOMS

8.1 INTROUCTION

Vehicular pollution is a mixture of thousands of chemical agents, some of which are neurotoxic. Therefore it is possible that cumulative exposure may result in neurological problems affecting the behavior. To test this hypothesis, the prevalence of neurobehavioral problems among the residents of Delhi has been ascertained by questionnaire survey. In addition, plasma catecholamines (CA) and acetyl cholinesterase (AChE) enzyme activity have been measured in order to explain the underlying mechanism of neurobehavioral alterations, if any.

8.2 METHODOLOGY

(a) Questionnaire survey for neurobehavioral problems

A total number of 919 subjects aged between 28 and 55 years were enrolled in neurobehavioral symptom survey. They include 272 control individuals (male 182, female 90), and 647 residents of Delhi (male 412 female 235). All the participants were never-smokers and non-chewers. The subjects were interviewed in Hindi, English (Delhi) and Bengali (control) by the research staff. A neurobehavioral symptom questionnaire, adopted from the subjective symptom questionnaire accompanying the World Health Organization Neurobehavioral Core Test Battery (WHO, 1986), Wechsler's memory scale (Wechsler, 1945) and 21-item Beck depression inventory (Beck et al., 1961) was administered to them.

The Beck's depression inventory (BDI) includes 21 parameters- sadness, pessimism, sense of failure, dissatisfaction, guilt, expectation of punishment, dislike of self, self-accusation, suicidal ideation, episodes of crying, irritability, social withdrawal, indecisiveness, change in body image, retardation, insomnia, fatigability, loss of appetite, loss of weight, somatic preoccupation, and low level of energy. Highest score on response to each question related to the above parameters was 3, and a total score of 5-9 was normal; 10-18: mild to moderate depression; 19-29: moderate to severe depression; and 30-63: severe depression (Beck et al., 1961).

In addition, the questionnaire focused on other symptoms like burning sensation in extremities (feeling of burn in distal and terminal portions of the body such as hand and foot), tingling (repetitive moving pin prick-like sensation), numbness (temporary loss of sensation), vertigo (an illusionary sensation that the body or surrounding environment is revolving), and dizziness (sensation of unsteadiness with a feeling of movement within the head, giddiness). A five-point rating scale using simple and clear words like 'never', 'rarely', 'sometimes', 'frequently' and 'very frequently' was used in the questionnaire to elicit a better response for these symptoms. Afterwards, answers like 'never' and 'rarely' were considered as absence of that symptom, while responses like 'sometimes' frequently' and very frequently' were recognized as having such symptoms.

(b) Measurement of plasma catecholamine (CA) by HPLC-ECD

The concentrations of dopamine (DA), epinephrine (adrenalin, E), and norepinephrine (norepinephrine, NE) in blood plasma of 23 control subjects (male 15, female 8) and 45 (male 28 and female 17) residents of Delhi, aged 28-55 years who were all lifetime non-smokers and non-chewers, were measured by high performance

liquid chromatography with electrochemical detection (HPLC-ECD) following the procedure of Davies and Molyneux (1982).

Reagents required

Epinephrine (Sigma Chem, USA)	0.1 mg / ml in 0.1 M perchloric acid
Norepinephrine (Sigma Chem, USA)	0.1 mg / ml in 0.1 M perchloric acid
Dopamine hydrochloride (Sigma Chem, USA)	0.1 mg / ml in 0.1 M perchloric acid
Octane-1-sulfonate (Qualigens, India)	
Aluminium oxide (E Merck, Germany)	
Perchloric acid (Qualigens, India)	
Zinc Sulfate	
Mobile phase	1000 ml
Acetate citrate buffer, pH 5.2	780 ml
Citric acid monohydrate	5.75 g
Sodium acetate	6.8 g
Glacial acetic acid	1.05 ml
Sodium hydroxide	2.4 g
Methanol (HPLC grade, Qualigens, India)	220 ml
Octane sulfonic acid	1.081 g
Washing buffer	
Distilled water	100 ml
1M Sodium metabisulfite	100 µl
Tris HCl buffer, 0.5 M, pH 8.6	1 ml
Eluting buffer	
Sodium metabisulfite	4 mg
$HClO_4$, 0.1M	50 ml
Tris HCl buffer, 0.5 m	
Tris (hydroxymethyl aminomethane, 2 M, (Sigma Chemicals, USA)	24.2 g in 100 ml distilled water
HCl, 10 M	30.93 ml in 100 ml distilled water
(To 250 ml of tris solution 12.2 ml of HCl solution/l was added)	
Internal standard	10 ml
Sodium metabisulfite, 400 µM	0.008 g
$HClO_4$, 0.1 M	600 µl
3,4-dihydroxybenzylamine(DHBA, Sigma Chem, USA)	1 mg/ml

Procedure

Venous blood (5-ml) was collected after informed consent in vacutainer tubes (BD, USA) containing K3EDTA as anticoagulant. The subjects were requested to take rest in supine position in a relaxed environment before blood drawing to measure basal plasma CA level. Plasma was separated by centrifugation at 5000 rpm for 10 minutes at 4 oC of anticoagulated blood. Plasma samples were deproteinized by centrifuging with 10% zinc sulfate solution at 800g at 4°C. Two hundred microliter of 3, 4 dihydroxybenzylamine prepared as 0.1 µM solution in 0.1 M perchloric acid (containing 400 µM sodium metabisulfite) was added as internal standard to 2 ml of plasma samples. Then 400 µl of 0.5 M tris HCl (pH 8.6) and 20 mg of activated alumina were added and the contents of the tube were shaken gently for 15 min. Following centrifugation at 600g for 2 min, the supernatant was removed

and the catecholamines were eluted from alumina into 50 µl of 0.6 M perchloric acid containing 400 µM sodium metabisulfite. The mixture was centrifuged at 800g for 30 min and 20 µl of the supernatant was injected into a reverse phase high performance liquid chromatographic column (Waters Novapak C 18 column, 3.9x150 nm, coupled into an electrochemical detector Waters 464 pulsed ECD; Waters, USA). The mobile phase consisted of 780 ml of acetate-citrate buffer pH 5.2; 220 ml of methanol and

(c) Plasma acetylcholinesterase (AChE) assay

Plasma cholinesterase was determined in 45 control subjects (male 34 female 11) and 66 Delhi's residents (male 43 female 23), aged 28-55 years and all lifetime non-smokers and non-chewers, following the procedure of Ellman et al., (1961; Figure 8.1). In brief, plasma was isolated from venous blood by centrifugation at 5000 rpm for 10 minutes at 4 °C. Isolated plasma were serially diluted with 0.1M Na-phosphate buffer (150x) and 1000µl of the sample was then incubated with 10mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 0.1M Na-phosphate buffer for 10 mins at room temperature (in dark); 50µl of acetylthiocholine iodide (Sigma, USA) was added to the solution and mixed thoroughly. Absorbance was measured in a UV-vis spectrophotometer (Shimadzu, Japan) at 412 nm for 180 secs starting 60secs after mixing of acetylthiocholine iodide. Each sample was tested in triplicates and plasma (butyl) cholinesterase activity was measured as micromole of substrate (acetylthiocholine iodide) hydrolyzed per minute per liter at 30°C.

Procedure

3.0 ml of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) is taken in a 3.0 ml glass spectrophotometric cuvette and 20µl of plasma sample is added to it

↓

100 µl of acetylthiocholine iodide (Sigma Chem, USA) is added to the solution and mixed thoroughly

↓

Absorbance is measured at 406 nm, 60sec after mixing of acetylthiocholine iodide

Figure 8.1: Acetylcholinesterase assay

(d) Statistical analysis

All data are expressed as mean ± standard deviation. The collected data were processed and analyzed in EPI info 6.0 and SPSS (Statistical Package for Social Sciences) software. Logistic regression analysis using generalized estimating equations (GEEs) was used to examine the relationship between measured outcome and possible confounders such as RSPM levels. Spearman's rank test for continuous variables and Chi-square test for categorical variables were done. $P<0.05$ was considered as significant.

8.3 RESULTS

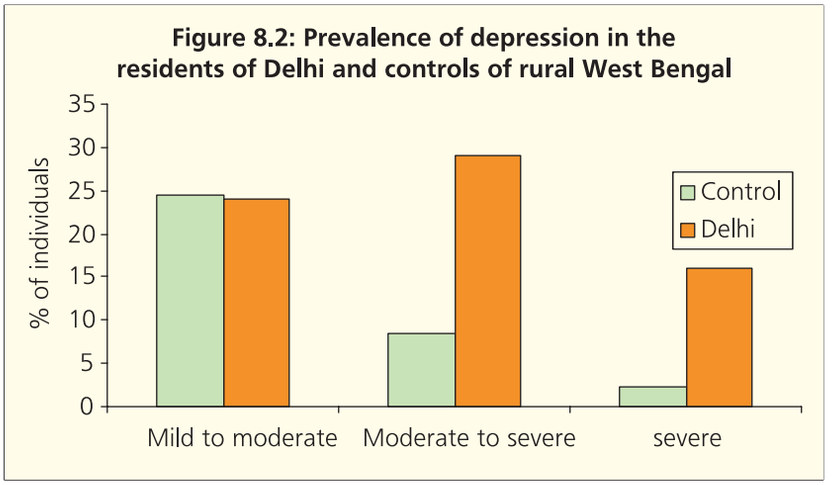
(a) Greater prevalence of depression and other neurobehavioral symptoms among Delhiites

Depression was evaluated by 21- questions Beck Depression Inventory (BDI). A remarkably increased prevalence of depression was found in citizens of Delhi. Compared with 35.4% of control subjects with different grades of depression, 69% of Delhiites had depression (Table 8.1, Figure 8.2). Depression was moderate to severe in 29% and severe in 15.9% citizens of Delhi, which were 3.4-6.6-times more than that of controls.

Table 8.1: Prevalence (%) of depression among rural and urban subjects

Depression	BDI Score	Control (n=272)	Delhi (n=647)
Absent	5 – 9	64.6	31.0*
Mild to moderate	10 –18	24.4	24.1
Moderate to severe	19 –29	8.5	29.0*
Severe	30 – 63	2.4	15.9*

*Results are expressed as percentage of individuals; BDI, 21-question Beck's depression inventory; *p<0.001 compared with control in Chi-square test*



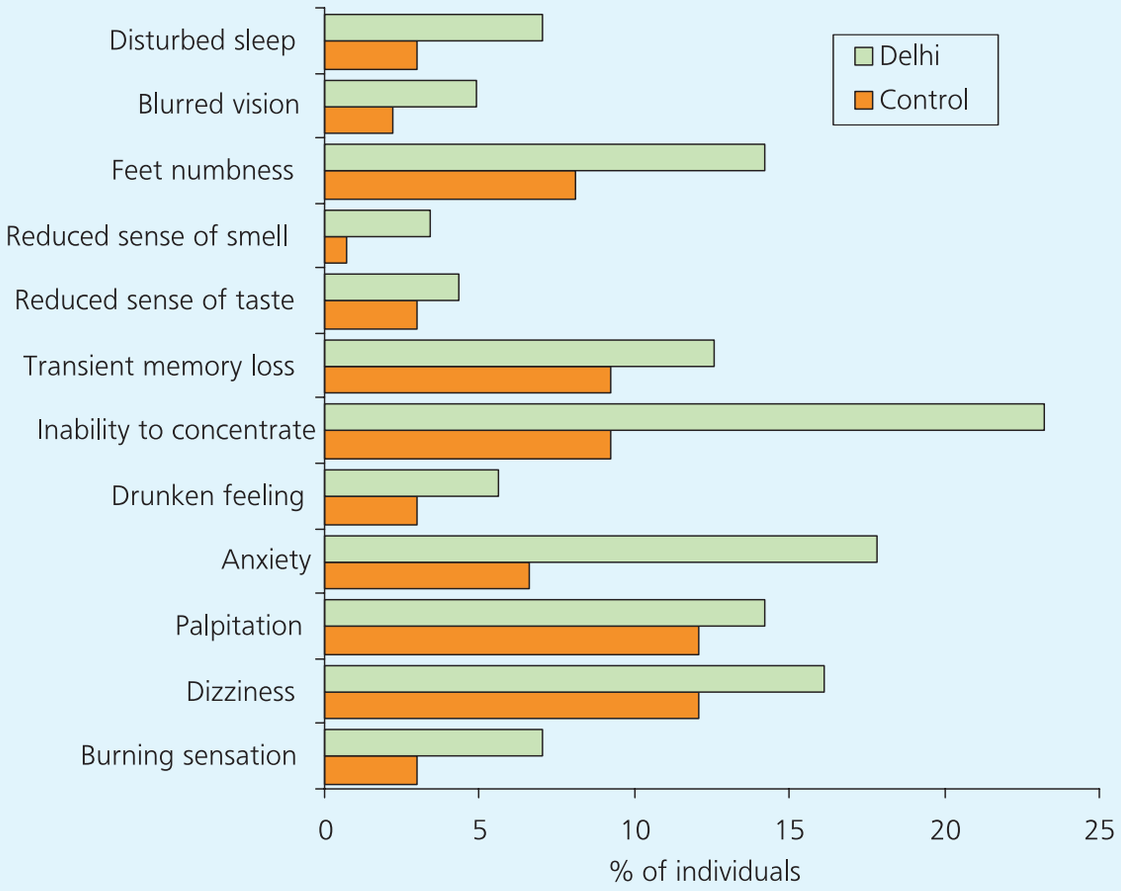
Besides depression, burning sensation in extremities (7 vs. 3%), anxiety (17.8 vs. 6.6%), drunken feeling (5.6 vs. 3%), inability to concentrate (23.2 vs. 9.2%), transient loss of memory (21.6 vs. 9.2%), reduced sense of smell (0.7 vs. 3.4%), blurred vision (4.9 vs. 2.2%), and sleep disturbance (7 vs. 3%) were more prevalent in Delhi's residents (p<0.05, Table 8.2, Figure 8.3).

Table 8.2: Prevalence of neurobehavioral symptoms

	Control (n=272)	**Delhi (n=647)**
Burning sensation in extremities	3.0	7.0*
Vertigo/dizziness	12.1	16.1
Palpitation	12.1	14.2
Anxiety	6.6	17.8*
Drunken feeling	3.0	5.6*
Inability to concentrate	9.2	23.2*
Transient loss of memory	9.2	21.6*
Reduced sense of taste	3.0	4.3
Reduced sense of smell	0.7	3.4*
Feet numbness	8.1	14.2*
Blurred vision	2.2	4.9*
Difficulties in sleeping	3.0	7.0*

*n, number of subjects; *, p<0.05 compared with control in χ^2 test*

Figure 8.3: Prevalence of neurobehavioral symptoms in the citizens of Delhi and controls from rural West Bengal



Controlling for age and passive smoking as possible confounders, logistic regression analysis showed positive association between PM_{10} level and the prevalence of transient loss of memory (OR = 1.43; 95% CI, 1.13-2.12), burning sensation in extremities (OR=1.87; 95% CI, 1.16-3.34), and depression (OR= 1.43; 95% CI, 1.04-2.35).

Similarly, benzene exposure (t,t-MA level) was positively associated with transient loss of memory (OR= 1.55, 95%CI, 1.12-2.35), inability to concentrate (OR=1.44, (95% CI, 1.10-1.95) and anxiety (OR= 2.27, 95% CI, 1.56-3.21. Besides pollution exposure, however, there could be other factors that influence the onset of depression. Stress and strain of urban life and pressure at workplace could have important bearing on depression in urban subjects.

Overall, the results demonstrated significantly higher prevalence of depression and other neurobehavioral problems, such as anxiety, transient memory loss, difficulty in concentrating, reduced sense of smell and sleep disturbance among the residents of Delhi compared with rural controls. High levels of RSPM and benzene in Delhi's air were found to be significantly associated with these symptoms.

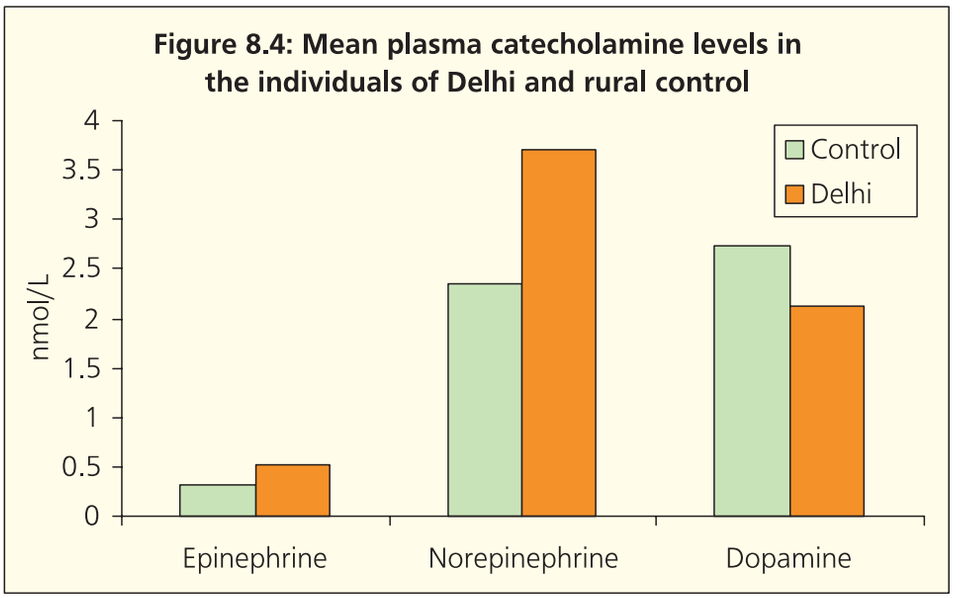
(b) Changes in plasma CA levels

Residents of Delhi had significantly elevated ($p<0.05$) epinephrine (E) and norepinephrine (NE) levels in plasma as compared with controls. The increase was 64.5% above mean control level in case of E, while 58.5% increase was recorded for NE. However, 22% depletion of dopamine level ($p<0.05$) was found in inhabitants of Delhi as compared with control (Table 8.3, Figure 8.4).

Table 8.3: Mean plasma catecholamine levels

	Control (n=23)	Delhi (n=45)
Epinephrine (nmol/l)	0.31 ± 0.12	0.51 ± 0.26*
Norepinephrine (nmol/l)	2.34 ± 0.82	3.71 ± 0.89*
Dopamine (nmol/l)	2.73 ± 0.45	2.13 ±0.36*

*Results are mean± SD; *, p<0.05 compared with rural*



Controlling for age and passive smoking as possible confounders, logistic regression analysis showed positive association between PM_{10} level and the concentration of E (OR = 1.33; 95% CI, 1.08-1.67) and NE (OR=1.47; 95% CI, 1.10-2.12) in plasma. Similarly, a positive association was found between t,t-MA excretion and plasma E (OR = 1.23; 95% CI, 1.05-1.52), and NE (OR=1.34 95% CI, 1.12-1.71).

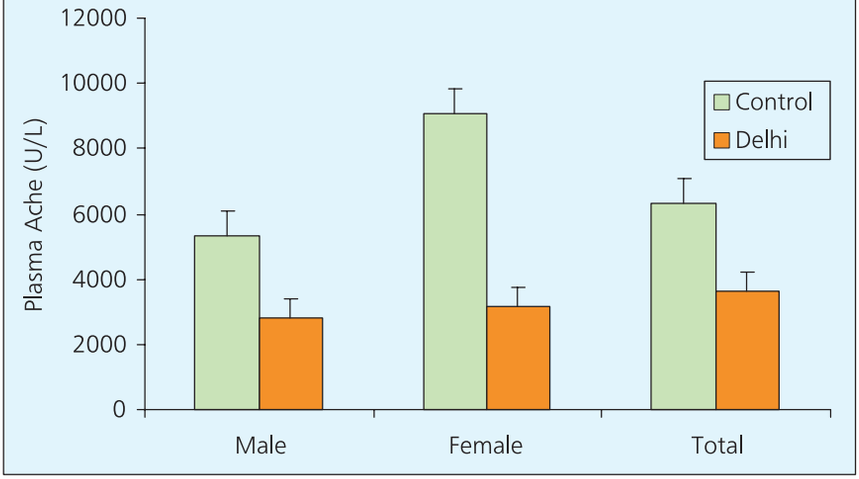
(c) Plasma cholinesterase level

Compared to control subjects, plasma acetyl cholinesterase (AChE) concentration was reduced by 43% in the residents of Delhi ($p<0.001$). The difference between control and Delhi groups was most pronounced in case in females where 65% reduction in AChE level was recorded (Table 8.4, Figure 8.5). Thus, the residents of Delhi had depleted level of AChE in plasma, suggesting perhaps alteration in cholinergic neurotransmission.

Table 8.4: Plasma levels of acetyl cholinesterase

Plasma AChE (U/L)	Control (45)	Delhi (n=66)	P value
Male (Control 34, Delhi 43)	5339.9 ± 716	3784 ± 651	<0.05
Female (Control 11,Delhi 23)	9069.1 ± 879	3151 ± 439	<0.001
Total	6325.1 ± 456	3603 ± 549	<0.001

Figure 8.5: Plasma acetyl cholinesterase level among individuals of Delhi and rural control



In essence, significant elevation of plasma epinephrine and norepinephrine levels was found along with marked decline in plasma cholinesterase level in citizens of Delhi compared with rural controls. The results underscore neurological changes in urban subjects that may, in part, explain their neurobehavioral problems.

8.4 FINDINGS

1. Depression and other neurobehavioral symptoms were screened by standardized questionnaire survey in 272 control subjects and 647 Delhi's residents, aged 28-55 years, who were all lifetime non-smokers and non-chewers of tobacco. The percentage of depressed persons was 2-times more in Delhi (69% vs. 34.5%). Moreover, the magnitude of depression was much more in Delhi. About 16% citizens of Delhi showed severe depression compared with 2.4% control subjects.
2. Besides depression, Delhi's residents had increased prevalence of several other neurobehavioral symptoms like anxiety, burning sensation in extremities, inability to concentrate, transient loss of memory, and palpitation.
3. PM_{10} level was found to be positively associated with increased prevalence of transient loss of memory (OR = 2.23; 95%CI, 1.23-4.12), burning sensation in extremities (OR=3.87; 95% CI, 2.16-5.34), and depression (OR= 1.83; 95% CI, 1.24-3.05) although there may be other additional contributing factors like stress
4. Benzene exposure, measured in terms of t,t-MA level in urine, was positively associated with transient loss of memory (OR= 1.55, 95%CI, 1.12-2.35), inability to concentrate (OR=1.44, (95% CI, 1.10-1.95) and anxiety (OR= 2.27, 95% CI, 1.56-3.21).

5. Subjects from Delhi had elevated epinephrine (E), norepinephrine (NE) levels in blood plasma than their rural counterparts. In contrast, plasma dopamine (DA) level was declined. Elevated t,t-MA excretion was found to be associated with rise in plasma E (OR = 2.15; 95% CI, 0.98-4.52), and NE (OR=2.24 95% CI, 1.22-3.61) levels.
6. Compared to control subjects, plasma acetyl cholinesterase concentration was reduced by 43% in the residents of Delhi ($p<0.001$). The difference between control and Delhi's residents groups was most pronounced in case in females where 65% reduction in AChE level was recorded. Thus, the residents of Delhi had depleted level of AChE in plasma, suggesting alteration in cholinergic neurotransmission.

CHAPTER-9.0

IMPROVEMENT OF RESPIRATORY HEALTH FOLLOWING INTERMITTENT EXPOSURES TO CLEANER AIR

9.1 INTRODUCTION

A frequently asked question is whether the adverse health effects of air pollution is reversible, and whether staying at a cleaner environment for at least some hours of a day has any beneficial effects on respiratory health. To address these questions, health data of persons employed at Paharpur Business Centre (PBC), Nehru Place, New Delhi was compared with that of Delhi's office employees, in general. PBC is a provider of office facilities and air consultancy services to various corporate and multinational companies. The indoor air quality (IAQ) is maintained at PBC since 1996 conforming to the standards for indoor air quality laid down by American Society for Housing, Refrigeration, and Air-conditioning Engineers (ASHRAE).

For maintenance of a good IAQ, an eco-friendly approach is being followed at PBC. First, outside air is washed with water in scrubber before entering inside the building to reduce the water-soluble components like SO_2, NO_2 and SPM. Then the washed air is passed through a green house of sleeted plants that effectively remove formaldehyde, benzene and carbon monoxide from air and enrich the air with fresh oxygen (Fig. 9.1). The processed air is then passed through micro-V filter to remove bacteria and pumped into the building as fresh air. The entire building is a no smoking zone, which is strictly maintained. There are several offices in the building and a number of people work there for 8-10 hours per day. In this study the answer was sought to the question whether staying in a much cleaner environment for a substantial duration had any beneficial effect on their respiratory health.

Figure 9.1: Cleaning of air using plants at Clean Air Station Paharpur, Nehru Place, New Delhi

9.2 MATERIALS AND METHODS

A total number of 94 subjects working at Paharpur Business Center (PBC), male 61 and female 33 who were life time non smokers were enrolled. The participants were aged between 24 and 56 years with a median of 39 years (Fig. 9.2). Prevalence of respiratory symptoms and lung function impairment was evaluated by questionnaire survey and spirometry respectively. The findings were compared with Delhi's other office workers who were never smokers (n=1382, median age 42 year).

Figure 9.2 Camp in progress at Paharpur Clean Air Station, Nehru Place, New Delhi

9.3 RESULTS

It is evident from Table.9.1 that indoor air of PBC had low pollutant levels during 2002-2003.

Table 9.1: Air quality of Paharpur

Pollutant	Daily average concentration at PBC	
	In 2002	In 2003
SPM	92 µg/m^3	91 µg/m^3
RSPM	46 µg/m^3	45 µg/m^3
NO_2	20 µg/m^3	20 µg/m^3
SO_2	23 µg/m^3	21 µg/m^3
Formaldehyde	BDL	BDL
Benzene	BDL	BDL

BDL, below detection level

The prevalence of both URS and LRS in recent past (3 months) was significantly lower ($p<0.05$) among subjects employed at PBC than in rest of the city. Compared with URS, the change was more noticeable for LRS (Figure 9.3). Similarly, the prevalence of headache and eye irritation was significantly less ($p<0.05$) in persons employed at PBC (Figure 9.4). However, there was no change in the prevalence of asthma between these two groups (Table 9.2).

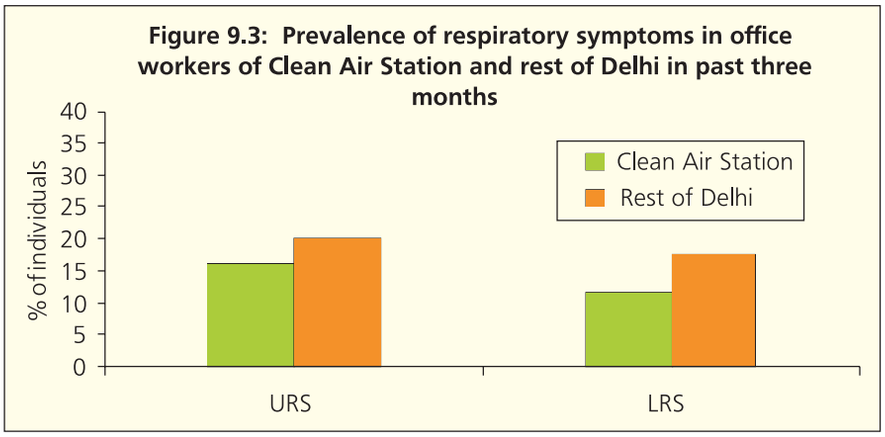


Figure 9.3: Prevalence of respiratory symptoms in office workers of Clean Air Station and rest of Delhi in past three months

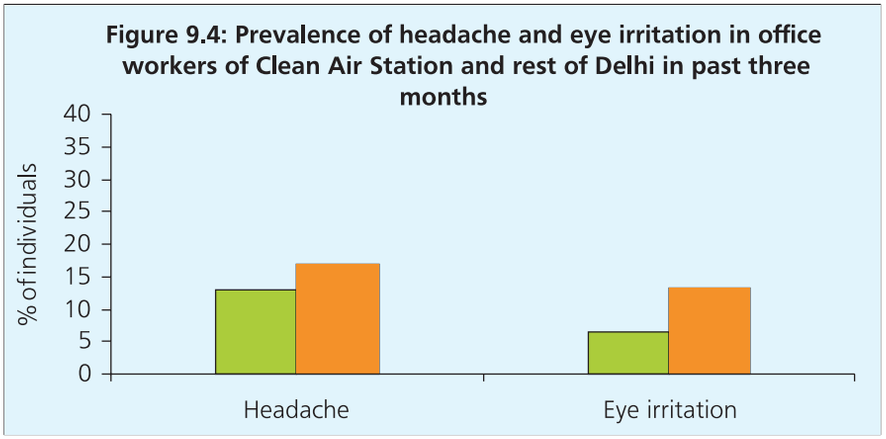


Figure 9.4: Prevalence of headache and eye irritation in office workers of Clean Air Station and rest of Delhi in past three months

Table 9.2: Prevalence (%) of respiratory symptoms in past three months

	Delhi, in general (n=1382)	Paharpur (n=94)	% change
Respiratory symptoms			
URS	20.1	16.0*	-20
LRS	17.7	11.7*	-34
Medically-diagnosed asthma	3.5	3.2	-9
Headache	16.8	12.8*	-24
Eye irritation	13.2	6.4*	-52

**, p<0.05 compared with Delhi, in general*

Lung function was reduced in 23.4% employees of PBC compared with 35.4% of non-smoking office employees of the city (Figure 9.5). Thus, there was 34% decline in the percentage of individuals with lung function deficits in PBC. In general, 27.4% non-smoking residents of South Delhi was found, where PBC was located, had lung function deficits. Prevalence among PBC employees was even lower than that, although a large section of PBC employees travel from other parts of the city. Besides lesser percentage of subjects with deficient lung function, the magnitude of deficit was much less among PBC employees as severe reduction in lung function was present in 3.2% employees against Delhi's average of 5.5% among office employees. Likewise, PBC employees had lower prevalence of COPD than city's average (Table 9.3). Therefore, stay in a cleaner environment for 8-10 hours a day appeared to have beneficial effects on lung function.

Figure 9.5: Lung function pattern in office workers of Clean Air Station and rest of Delhi

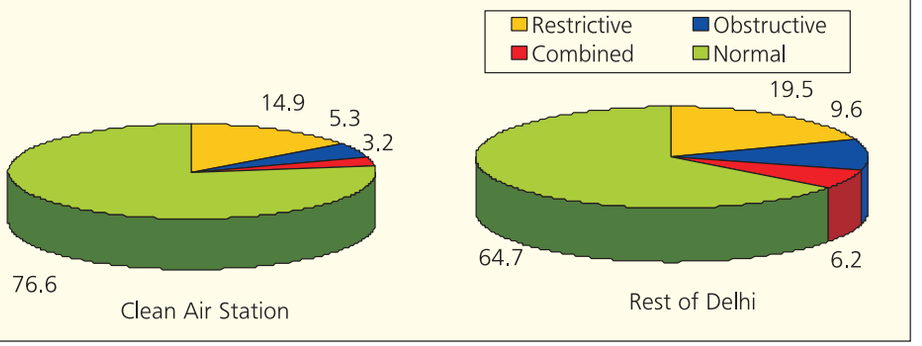


Table 9.3: Prevalence (%) of lung function decrement

Variables	Delhi, in general (n=738)	Paharpur (n=94)	% change
Lung function deficits			
Restrictive type	19.5	14.9*	-24
Obstructive type	9.6	5.3*	-45
Combined type	6.2	3.2*	-48
Overall	35.4	23.4*	-34
Severe deficit in lung function	5.5	3.2*	-42
COPD	3.0	2.1*	-30

**, p<0.05 compared with Delhi, in general*

Sputum cytology of persons employed at PBC showed 19% lesser number of cells, and 30-33% lower number of alveolar macrophages, siderophages (Figure 9.6) and metaplastic squamous epithelial cells (Figure 9.7). The findings imply reduced exposure to particulates and a lower risk of microscopic hemorrhage in lung among PBC employees. Similarly, PBC employees had 36% lower micronucleus count (Figure 9.8) and 20% lower prevalence of hypertension (Figure 9.9), suggesting diminished risk of genotoxicity and cardiovascular diseases (Table. 9.4).

Figure 9.6: Sputum cytology in office workers of Clean Air Station and rest of Delhi

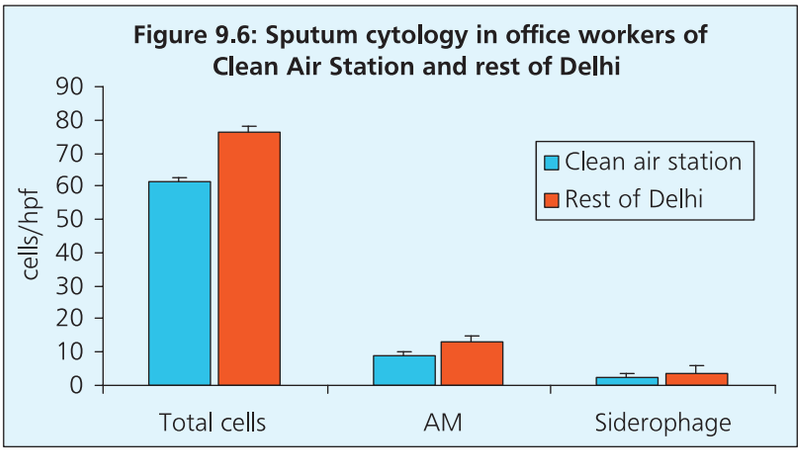


Figure 9.7: Prevalence of squamous metaplasia in office workers of Clean Air Station and rest of Delhi

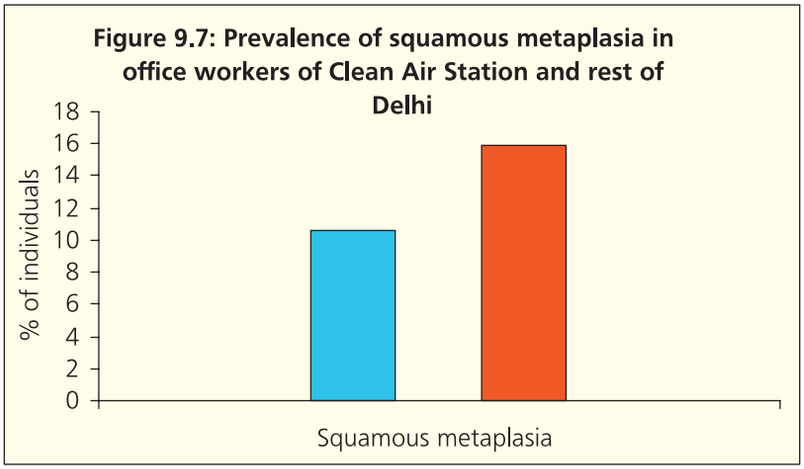
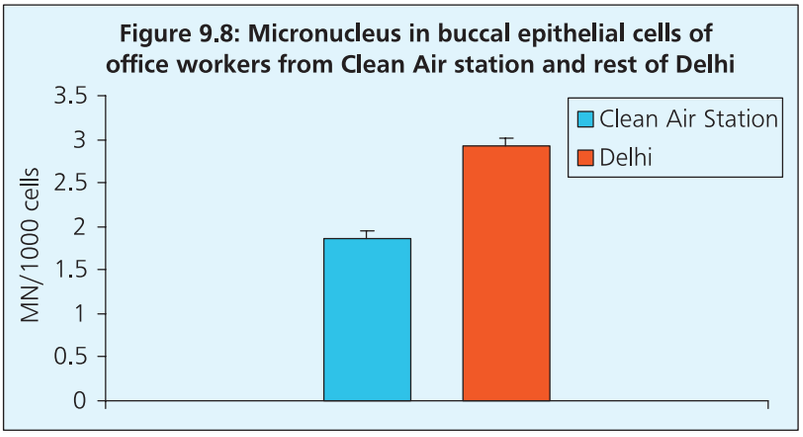


Figure 9.8: Micronucleus in buccal epithelial cells of office workers from Clean Air station and rest of Delhi



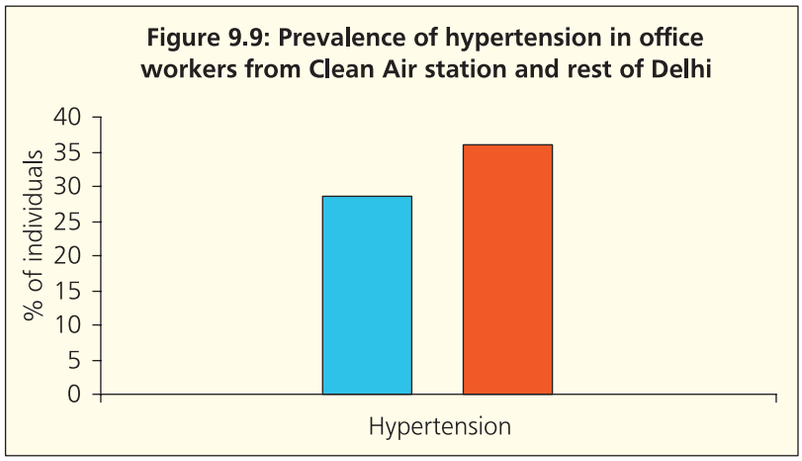


Table 9.4: Comparison of sputum cytology, genotoxity and hypertension data

Parameters	Rest of Delhi	Paharpur	% change
Total cells /hpf in sputum	76.2	61.4*	-19
AM/hpf in sputum	12.9	8.7*	-33
Siderophage/hpf in sputum	3.7	2.6*	-30
Metaplasia (% individuals)	15.9	10.6*	-33
MN/1000 airway cells	2.92	1.86*	-36
Hypertension (% individuals)	36.1	28.7*	-20

**, p<0.05 compared with rest of Delhi*

9.4 INFERENCE

The study has demonstrated that stay in a cleaner indoor environment for 8-10 hours a day reduces the prevalence and magnitude of health impairments associated with chronic exposures to air pollution.

CHAPTER-10.0

DISCUSSION

The study has demonstrated a multitude of health problems among the residents of Delhi who were chronically exposed to high level of air pollution primarily from road traffic. They had higher prevalence of respiratory symptoms, lung function decrement, pulmonary inflammation, platelet hyperactivity, immune alterations, neurobehavioral problems and genotoxic changes relative to rural controls.

Air pollution and respiratory symptoms

Respiratory symptoms, in general, reflect underlying problems in the airways and the alveoli. Therefore, greater prevalence of respiratory symptoms among the residents of Delhi may suggest elevated frequency of airway and alveolar damage. More than 100 published studies have assessed the relationship between exposure to PM and excess mortality and morbidity in humans (Samet, 2002). Morbidity ranges from pulmonary function decrements to respiratory symptoms, and to hospital and emergency department admissions. Several of these epidemiological studies have described a close association between traffic flow and respiratory symptoms (Wjst et al., 1993) and decreased lung function (Brunekreef et al., 1997). Chhabra et al., (2001) investigated the effects of urban air pollution on respiratory health in Delhi. They found that chronic respiratory symptoms excepting wheeze, and reduced lung function were significantly more common in high pollution zones (Chhabra et al., 2001). In Mumbai, Kamat and associates (1992) observed that respiratory symptoms and lung function were associated with NOx and PM, but not with SO_2 (Kamat et al., 1992). Like the present finding, air pollution in Bangkok was associated with higher prevalence of respiratory symptoms especially among the traffic policemen, emphasizing the health impact of vehicular pollution (Karita et al., 2001). Cumulative exposure to benzene through respiration could be an important contributor, because increase in both upper and lower respiratory symptoms has been reported in benzene- exposed subjects (Pappas et al., 2000). In many cases fungi, pollen, grains, insect and other materials of biological origin act as allergen (D'Amato et al., 2001; Mutius, 1998). The most important fungal aeroallergens in India belong to the genus *Alternaria, Candida, Aspergillus, Neurospora and Helminthosporium* (Singh and Kumar, 2002). *Alternaria* is ubiquitous in the environment and is easily isolated from soil, grains, human skin and the atmosphere (Conant et al., 1968). Conidia of *Alternaria* and hyphe of *Aspergillus, Alternaria* and *Candida* were frequently detected in sputum samples of citizens of Delhi of this study. Presence of these fungi is usually accompanied by acute inflammatory reaction in the airways. It seems that abundance of bioaerosols of fungal origin along with particulate pollution and benzene are major contributors to respiratory symptoms among the residents of Delhi.

Greater prevalence of respiratory symptoms was observed in women both in Delhi and in control areas. Most of the women participants of this study were housewives who cooked regularly for 2-5 hours per day. Burning of cooking fuel emits high level of particulate pollutants, which these women were chronically exposed to. Moreover, in many of the Asian countries including India, indoor air pollution from cooking oil vapours is a major contributor to pollution in women folk (Lam 2005).

Compared with rural controls, symptoms of rhinitis like runny or stuffy nose and sneezing were more prevalent in Delhi. Exposures to city's air pollution could be implicated for this, because long-term exposure to air pollution increases prevalence of respiratory symptoms such as allergic rhinitis and atopy (Penard-Morand et al., 2005). Air pollutants may promote airway sensitization by acting as adjuvant. Rhinitis is significantly associated with SO_2, CO and NOx while no relation was found between rhinitis and PM_{10} and ozone (Hwang et al., 2006). However, a recent study in European cities has shown that ambient particles (PM_{10}) from combustion sources act as carrier of allergens such as pollen that could

elicit allergic reactions in the airways following inhalation (Namork et al., 2006). Headache, skin and eye irritation are common problems among the residents of Delhi. Stress is an important factor which may contribute to headache. Saxena et al., (2003) found higher prevalence of redness of the eye, eye irritation and ocular surface disorder without any change in visual activity among the residents of Delhi. The authors suggested that the changes were due to high level of air pollution in Delhi (Saxena et al., 2003). Compared with Delhi, a lower prevalence of headache and eye irritation has been found among the residents of Mumbai (Kamat et al., 1992). The difference can be linked to the relatively lower level of ambient air pollution in Mumbai, where the current ambient $PM_{2.5}$ level is 43 $\mu g/m^3$ (Kumar and Joseph, 2006).

Among the adult population of Delhi 5.6% current asthma and 3.6% physician-diagnosed and treated asthma was found. Our findings are in general agreement with a recent multicentric population study of asthma prevalence in non-smokers of North India in which the overall prevalence of asthma was 2% (Gupta et al., 2006). In the present study, the prevalence of current asthma as well as medically diagnosed asthma was significantly higher than that of rural controls. The reason for this difference is only speculative. Obesity is positively associated with asthma prevalence. Since the problem is more common in Delhi it can be partially responsible for higher asthma prevalence in the city. In addition, increased level of vehicular pollution in Delhi may have some influence, since vehicular pollution from combustion of diesel increases the prevalence of wheeze (Hoppin et al., 2004).

Air pollution and lung function

Compared with rural controls, reduced lung function growth as well as lung function deficits was found at a given age both in men and women of Delhi. Decrement of lung function was observed in 47.4% residents of Delhi compared with 22.2% of rural controls. All the measured lung function parameters, such as FVC, FEV_1, $FEF_{25-75\%}$, and PEFR, were decreased by about 10% among the citizens of Delhi. Lung development and lung function are influenced by several factors. Most important among these are birth weight, infections, nutrition and environmental factors such as air pollution. It is important to mention in this context that air pollution hampers lung development from childhood to adolescence and adulthood (Khan, 2004). Lung is affected by chronic exposures to high level of NO_2, SO_2 and PM, of which the effect is strongest for PM (Ackerman-Liebrich et al., 1997). Recent study has identified $PM_{2.5}$ as the most dangerous particulate fraction in this regard (Bernstein and Abelson, 2005). Fine particulates ($PM_{2.5}$) are ubiquitous because they are largely derived from common combustion processes such as engines of motor vehicles, power generation, burning of biomass, and manufacturing, and they are transported over long distances and readily penetrate indoors (Pope 2004b). Exposure to fine particulate matter may be an important public health concern (Pope, 2004b). Such matters that can be breathed deeply into the lungs include sulfates, nitrates, acids, metals, and carbon particles with various chemicals adsorbed onto their surfaces (Pope, 2004b). About 31% and 34% of adult males in Delhi and control group respectively were current smokers. In addition, 1.4% women of Delhi against none in control group were current smokers. Bidi smoking was common in low SES, while cigarette smoking was more prevalent in higher SES. In addition to respiratory symptoms, a large number of citizens of Delhi had reduced lung function. Decline in lung function values could be associated with City's vehicular pollution, because chronic exposure to high levels of PM_{10} causes reduction in lung function (Churg et al., 2003). In agreement with this, reductions in FVC and FEV_1 have been found in highly polluted areas of Delhi (Chhabra et al., 2001), Kolkata (Lahiri et al., 2000a,b) and Kanpur (Sharma et al., 2004). A study among traffic policemen operating in a major highway crossing in Jalgaon, Maharastra showed significant reduction

of FEV_1, FVC and PEFR. Very high level of PM_{10} (515µg/m^3) has been implicated for this as Sox and NOx levels were within standards (Ingle et al., 2005). Similar findings in traffic policemen have been reported from Thailand (Wongsurakiat et al., 1999).

Inhalation of dust is an important cause of interstitial lung disease in India (Jindal et al., 2001). Inorganic metallic dusts cause progressive pulmonary fibrosis. Breathlessness, dry cough and general constitutional symptom usually accompany pulmonary fibrosis (Jindal et al., 2001). Inorganic metals (Pb, Cd, Zn, Ni) are abundant in particulate pollution of Delhi, especially in fine fraction of PM_{10} (Balachandran et al., 2000). Particulate pollution targets respiratory bronchioles, and chronic exposure decreases FEF_{75} value, implying small airway problems (Hyde et al., 1978). Autopsy of lungs from women who were life-long residents of highly polluted Mexico City has shown that particulate matter penetrates and retained in the walls of the airways causing small airway remodeling and chronic air flow obstruction (Churg et al., 2003). It has been suggested that PM_{10} is fibrogenic in individuals exposed to higher levels for longer periods (Churg et al., 2003). This may explain in part the higher prevalence of restrictive type of lung function defect among the residents of Delhi. Reduced FVC could be due to fibrosis, edema, hemorrhage, cellular hyperplasia and heavy particle loading. It is known that the respiratory system has a large functional reserve. Therefore, a relatively diffused and extensive lung lesion might have occurred in a substantial number of citizens of Delhi with detectable change in lung function.

It has been shown that for 34 µg/m^3 rise in total suspended particulate matter from the standard, FVC is decreased by 2.25%. (Chestnut et al., 1991). A study in Kanpur with 91 adult subjects has documented decreased spirometric lung function in polluted commercial and residential areas while normal lung function was found in relatively clean area of IIT campus (Sharma et al., 2004). The peak expiratory flow rate (PEFR) was also markedly reduced in urban subjects of this study. Marked decline in morning PEFR has been shown to be associated with exposure to PM_{10} (van der Zee et al., 2000). It was estimated that 100 µg/m^3 increase of PM_{10} could reduce PEFR by 3.2 l/min (Sharma et al., 2004). Particulate pollution from combustion of organic matters has also been shown to reduce mean PEFR (Ige and Awoyemi, 2002). The main source of particles in accumulation mode (most intimately related to lung function impairment in urban air) is the coagulation of ultrafine particles (0.01–0.1 µm) present in automobile exhausts. On the other hand, increased concentration of transitional metal like iron, a component of the fine and ultrafine particle range, tended to be associated with decline in lung function in adult asthmatics (Dusseldorp et al., 1995). Likewise, heavy iron deposition in AM, as observed in urban individuals of this study, may contribute to impairment in lung function. Studies in young adults have shown that lung function decrement is associated with long-term elevated levels of particulates, and lung function decrement can be further worsened by concomitant exposure of PM_{10} with ozone (Abbey et al., 1998). Indeed, the 24-cities study in North America has shown a strong association of annual mean PM_{10}, ozone and acid aerosols with lung function (Raizenne et al., 1996). The authors reported 2.4% decrement in FVC and 2.1% decrement in FEV_1 for a rise of 17.3 µg/m^3 in annual mean PM_{10} level.

COPD is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive, with an abnormal inflammatory response of the lungs elicited by noxious particles or gases generally from tobacco smoking. COPD is a leading cause of morbidity and mortality worldwide. It is the fourth leading cause of death after heart diseases, cancer and stroke in USA and Europe. The annual mortality due to COPD in USA alone is more than one lakh per year, and COPD mortality in females has more than doubled over the last 20 years. Prevalence and mortality data greatly

underestimate the total burden of COPD because the disease is usually not diagnosed until it is clinically apparent and moderately advanced.

In the present study, 3.9% of adult individuals of Delhi had chronic obstructive pulmonary disease (COPD), compared with only 0.8% of control subjects, indicating nearly 5-times more relative risk in the city. A recent multicentric epidemiologic study of COPD diagnosis based on chronic bronchitis criteria in questionnaire survey among the residents of four cities – Bangalore, Chandigarh, Delhi and Kanpur who were above 35 years of age revealed COPD in 4.1% participants with a male: female ratio of 2.65:1. Prevalence was greater in smokers, men, elderly, lower socio-economic status and urban or mixed residence (Jindal et al., 2006). Thus the prevalence of COPD in the present study was largely similar with the findings of multicentric study. SPM and RSPM levels of Delhi were found to have significant positive correlation with COPD (rho=0.476 and 0.353 respectively; Agarwal et al., 2006).

Confounding factors of lung function:

Obesity

Besides air pollution and smoking, BMI is considered as a modifying factor for lung activity. It is important to mention in this context that the proportion of overweight and obesity in adults is increasing very rapidly in India among urban residents and high-income rural residents (Popkin et al., 1995). The percentage of obese women has been reported to be 0.1% in South Asia, 2.5% in Sub-Saharan Africa, 9.6% in Latin America and the Carribbean, 15.4% in Europe, 17.2% in the Middle East and North Africa and 20.7% in the USA (Martorell et al., 2000). Levels of obesity in countries increased sharply until a per capita gross national product is reached US dollars 1,500 (1992 value) and thereafter it changed very little. In the poor countries obesity levels were greatly concentrated among urban and higher educated women whereas in more developed countries obesity levels are equally distributed in the general population (Martorell et al., 2000). Rising national income in developing countries along with increased "westernization" have been partially attributed to rising trend in obesity (Martorell et al., 2000). The increase in fat reserves that characterizes obesity results from an energy imbalance (Bocquier et al., 2006). In India, the diet is shifting towards higher intakes of high-energy dairy products and added sugar, and India's cost of under-nutrition is declining continually and the cost for over-nutrition is increasing rapidly (Popkin et al., 2001). The higher level of obesity found in Delhi may also be due to the higher level of nutrition as well as the higher availability of junk food in Delhi compared to the control areas.

Emphysema, a clinical entity under COPD, is significantly associated with underweight conditions (BMI <18.5 kg/m^2), whereas asthma and chronic bronchitis are more prevalent in overweight (BMI ≥28 kg/m^2) persons (Guerra et al., 2002). In addition, a strong negative correlation exists between BMI and FVC, FEV_1 and PEFR (Ulger et al., 2006). Besides poor lung function, obesity is associated with metabolic disturbances, cardiovascular and pulmonary complications, predisposition to some cancers and psychosocial repercussions (Bocquier et al., 2006). Elevated BMI is associated with thromboembolic stroke in non-smoking men 55-68 years of age who were free of commonly observed conditions related to cardiovascular diseases (Abbott et al., 1994). Of the 7 million deaths from cancer worldwide in 2001, approximately 30% deaths were attributable to nine potentially modifiable risk factors one of which is BMI (Danaei et al., 2005). In high-income countries, smoking, alcohol use and overweight/obesity were the most important causes of cancer (Danaei et al., 2005). Moreover, obesity increases the risk of adult leukemias (Kasim et al., 2005).

SES

Study in England among people aged 16-79 years has shown that low social class and poor air quality are independently associated with decreased lung function (FEV_1), but not with asthma prevalence after controlling potential confounders. Importantly, social class effect was not attenuated by adjustment for air quality. The effect of low SES on lung function was more in men than in women (Wheeler and Ben-Shlomo, 2005).

Implication of lung function decrement

Extensive studies in different parts of the world have established that lung function is not only an indicator of existing pulmonary disease but also an important predictor of mortality (Bates, 1989; Lipfert, 1994). It is also considered an index of general physical robustness (Schwartz, 1989). Since apparently healthy, mostly non-smoking subjects participated in this study, impairment of lung function in 47% citizens of Delhi is a matter of great concern.

Changes in lung defense

The lung defence against inhaled particles and gaseous pollutants include innate mechanism such as aerodynamic filtration, mucociliary clearance, particle transport and detoxification by alveolar macrophages, as well as local and systemic innate and acquired antiviral immunity. In particular, alveolar macrophages (AMs) provide an innate defence mechanism against bacteria and viruses. Virus particles are ingested by phagocytosis and macrophages, like epithelial and other virus-infected cells, produce interferons that potentially inhibit viral replication. AMs also contribute to the neutralization of viral infection by removing the debris of the destroyed virus-containing cells and by presenting viral antigen to T lymphocytes. In addition, cell mediated immunity play an important role in the control of many viral infections of the respiratory tract. Many of these functions can be modulated by exposure to PM_{10}, NO_2 and other air pollutants (Chauhan and Johnston, 2003; Chauhan et al., 2005).

PM_{10} from highly polluted Mexico City was able to induce the production of inflammatory mediators TNF-alpha and IL-6 in monocytic cell lines *in vitro* (Alfaro-Monaro et al., 2002). Polycyclic aromatic hydrocarbon (PAH) fraction of diesel exhausts particles (Nel et al., 2001) and NO_2 (Chauhan et al., 1998) elicited proinflammatory effects such as persistent neutrophilic infiltration in human airways (Blomberg et al., 1999) and increased histamine release in nasal eosinophil (Schierhorn et al., 1999). Association between aeroallergens and respiratory symptoms are strong for pollen and fungi (Delfino et al., 2002). Pollutant mix act independently of aeroallergens, while pollen and fungal allergens cause allergic respiratory disease via inflammatory mechanism (Metzger et al., 1987).

Acute exposures to air pollutants result in ciliostatis in both upper and lower airways, which may prevent the nasal and bronchial mucosal cells from filtering inhaled particles such as aeroallergens, bacteria and viruses (Devalia et al., 1993). In vitro study has shown that very high concentration of NO_2 reduces ciliary beat frequency of human bronchial epithelial cells and causes ciliary damage. The results have been confirmed *in vivo* in human volunteers (Helleday et al., 1995). Air pollution also increases the number of mast cells, lymphocytes and natural killer cells in bronchoalveolar lavage fluid (BALF) (Sandstrom et al., 1990). Repeated exposures to NO_2 impair bronchial immunity by reducing total lymphocytes in peripheral blood and CD4+ and CD8+ cell ratio in BALF (Sandstrom et al., 1990). Particulate air pollutants are able to penetrate the lower airways, and the percentage of particle containing AM was found increased in subjects who lived on a main road compared with those living on a quiet residential road (Bunn et al., 2001).

Activated AM provide protection against bacterial and viral infections by a variety of mechanism including superoxide radicle-anion generation and cytokine production. Therefore it is likely that alterations in AM function are important risk factors for infections. Indeed, exposure of human AMs to pollutants for short durations resulted in functional impairment of these cells (Kienast et al., 1996). Particulate pollutants contain materials that may promote antigen presentation by monocytes, while the ability to specifically recruit T helper lymphocyte is contained in AM stimulated with PM_{10} (Baker and Soukup, 2003). Exposure of AM to PM_{10} significantly reduced inflammatory responses to viral infection (Baker and Soukup, 1999). Prior exposure of laboratory animals to diesel exhaust particles (DEP) has been shown to increase viral replication, lung inflammation and impairment in host defence mechanism (Harrod et al., 2003). DEP synergistically enhance neutrophilic lung injury related to endotoxin from gram-negative bacteria. The combustion of fuels produces a variety of outdoor pollutants such as NO_2, ozone, SO_2 and PM_{10}. There is increasing evidence to suggest that exposure to these pollutants elicits adverse health effects often at levels well below the current WHO guidelines. Viral infections can cause severe pathological abnormalities in both the upper and lower respiratory tract, the extent of epithelial damage varied with the virus type. The lower airway epithelium between the bronchial and alveolar regions is particularly susceptible. It is possible that the penetration of allergens into the epithelium could be facilitated both by epithelial shedding and by reduced ciliary clearance, resulting in easier access of allergen to antigen presenting cells leading to increased inflammation.

Cellular lung reaction to air pollution: importance of sputum cytology

Pulmonary cytology is an accurate diagnostic modality for the evaluation of pulmonary neoplasm and non-neoplastic pulmonary diseases. Sputum, bronchial washings, and broncho-alveolar lavage (BAL) fluid provide adequate samples from the proximal and distal airways and alveoli, the portions of the lung most frequently encountered by inhaled particles and adsorbed organic and inorganic pollutants. Sputum is derived mostly from central airways of the alveolar compartment whereas BAL is obtained from more peripheral airways (Fireman, 2001). Although bronchoscopy and BAL collection provide the gold standard assessment of pulmonary cellularity, the procedure is expensive and is associated with some risk and therefore less suitable for epidemiological studies in the third world countries (Fireman, 2001). In comparison, spontaneously expectorated sputum is a safe, non-invasive and cost-effective technique for the collection of samples from the airways (Erkilic et al., 2003). The technique can identify any changes in the airways and lung that may be attributed to air pollution. Quantification of cellular morphologic integrity of ciliated tracheobroncho epithelial cells provides the most sensitive index of pulmonary damage. More importantly, sputum cytology is used for early detection of the disease process. For example, sputum cytology has been used for screening early changes in the process of lung carcinogenesis (Roby et al., 1990). Although examination of sputum cells and differential counts of each cell type from smears are time consumable, they are informative and reproducible and aid to diagnosis of several disease conditions (Lewis et al., 1998). In comparison, sputum induction is costly and its superiority over routine expectorated sputum in diagnosis of tuberculosis is debatable (Merrick et al., 1997). Considering these, cytology of spontaneously expectorated sputum has been used in the present study to analyze the pulmonary effects of vehicular pollution of Delhi.

Cytological alteration in the bronchial epithelium is a valid indicator of the carcinogenic potentiality of environmental toxins (Frost et al., 1973; Fullmer, 1968). Changes in macrophage number, morphology and function, presence of mucus and mucus spirals, and abundance of columnar epithelial cells are useful for monitoring cancer of the airways. Chronic presence of neutrophil in sputum is associated with

development of emphysema (Janoff, 1985). Cytomorphological changes of the airway epithelial cells by air pollution exposure such as smoking precede alteration in lung function. Similarly, presence of reactive bronchial cells and squamous metaplasia are predictive of an abnormal FEV_1/FVC ratio indicating obstructive lung disease (Madison et al., 1984).

Alveolar macrophage response to air pollution

An important finding of this study is the marked increase in AM number in sputum of citizens of Delhi. Exposure to particulate pollution increases in number of AM in sputum (Mylius and Gullvag, 1986, Nobutomo, 1978; Brain, 1986; Talbot et al., 1987; Hornby and Kellington, 1990; Mauderly, 1994; Kyi et al., 2000; Lahiri et al., 2000 a, b). The rise in AM count could be due to increased proliferation of resident inflammatory cells, release and migration of existing cells from reservoir within the lungs, increased production of macrophage precursors in the lung, or increased influx of circulating monocytes into the lungs (Johnston 1988; Kyi et al., 2000). Since AM are recognized as the first line of cellular defense in the lung, marked rise in these cells may indicate upregulation of lung defense in the face of greater challenge from airborne pollutants. In general there are three mechanisms by which AM play a critical role in protecting the lung from bacterial and viral infections: production of inflammatory cytokines that recruit and activate lung phagocytes, production of antimicrobial reactive oxygen species (ROS), and production of interferon, an antiviral agent. Exposure to diesel exhaust decreases the ability of AM to produce ROS and interferes with the ability of the lung to clear bacteria, depresses the ability of the lung to produce interferon facilitating viral multiplication and decreases TNF-alpha and IL-1 production (Castranova et al., 2001). Therefore DEP increases the susceptibility of the lung to infection by depressing the antimicrobial potential of AM. This inhibitory effect was shown to be due to adsorbed organic chemicals rather than the carbonaceous core of the particle (Castranova et al., 2001).

The diameter of sputum AM in residents of Delhi was appreciably increased presumably to accommodate greater particle load. This hypothesis is supported by the observation that murine AMs substantially increase their size following exposure to cigarette smoke (Hornby and Kellington, 1990). A sharp rise was observed in the frequency of multinucleated and micronucleated AM in urban subjects. Multinucleated AM could result from DNA synthesis without cell division (endomitosis) while micronucleus is formed following chromosomal breakage or mitotic apparatus disruption. Therefore, the findings can be interpreted as evidence of dysregulated AM production from their precursors following chronic exposure to higher level of ambient air pollution in Delhi.

On an average, 50-100 ingested particles were detected per AM under microscope in urban subjects compared with ≤10 particles per AM of rural individuals. Assuming that the AM of urban subjects had a mean diameter of 20 µm, the volume of an AM was calculated as 4190 µm3 whereas human AM normally has a volume of 1000 µm3 (Oberdorster et al., 1992). The nucleus and the cytoplasmic organelles like mitochondria, Golgi bodies, ribosomes and lysosomes occupy approximately 40% of the cell volume. Therefore, at most 60% of AM volume is available to accommodate the inhaled particles. Accordingly, the urban subjects of this study had a maximum of 2514 µm3 of AM cell volume available for the ingested particles. More than 50 particles was found in that space. Thus each particle occupies a volume of 50 µm3. It suggests that each particle had a diameter of less than 2.5 µm i.e. PM2.5. Considering the limitation of the resolution of the microscopic lens and the possibility of aggregation of fine particles such as from diesel exhaust, it is possible that the number of particles per AM is actually much higher than counted. In that case, the ingested particles may contain a fair proportion of ultrafine particles.

The enormous increase in particle load in the lungs is likely to cause 'overloading'. It has been estimated that macrophage overloading begins when the total volume of particles ingested by a cell reaches 60 µm3 i.e. only 6% of the estimated cell volume (Morrow, 1994). Overload of a macrophage becomes complete when the total volume of the ingested particles covers 60% of macrophage volume. The volume of ingested particle correlates with macrophage activity, greater the load lesser the activity. In case of complete overload by particles, the cells' activity with respect to mobility is reduced to zero (Morrow, 1994). Based on these reports, it is reasonable to conclude that a majority of AM of subjects occupationally exposed to vehicular pollution of Delhi had lost their mobility to a large extent for their greater ingestion of airborne particles. Particle overloading of macrophages and their subsequent inability to move triggers the release of proinflammatory cytokines from particle-laden AM leading to acute and chronic inflammation of the lung (Morrow, 1988).

Activated AMs produce and release a myriad of enzymes and regulatory cytokines. It has been reported that the initiation of acute lung injury is associated with the activation of alveolar macrophages (Wang et al., 1999).

Sputum neutrophilia and eosinophilia

A large number of urban subjects had airway inflammation, as evident from sputum neutrophilia and eosinophilia. Influx of neutrophils has been reported following exposure to PM_{10} (Salvi et al., 1999; Ghio et al., 2000). Therefore, the changes can be attributed to Delhi's high PM_{10} level. Particulate pollution such as cigarette smoke increases the number of inflammatory cells in BAL fluid (Johnston 1988; Kyi et al., 2000). Breathing of polluted air with high levels of PM_{10} and SO_2 has been shown to be associated with increased release of WBC and their precursors from the bone marrow and elevated number of band cells in peripheral blood (Athens, 1993; Tan et al., 2000). A band cell is an immature neutrophil recognized by an incomplete separation of the lobes of the nucleus that is characteristic of mature granulocytes. These cells are commonly found in the marrow and form 2-6% of the normal circulating WBC. An increase in their number indicated that the marrow has been stimulated to increase the release of PMN cells. Elevated percentages of myelocytes, metamyelocytes and band cells have been recorded in peripheral blood of urban subjects, suggesting granulopoietic stimulation of bone marrow. Like the present study, stimulated production of neutrophilic cells from the bone marrow has been shown during air pollution exposures (Athens, 1993; Tan et al., 2000).

Sputum neutrophils are more phagocytic than peripheral blood neutrophils (Alexis et al., 2000). Accumulation of immature neutrophils in the lungs could be helpful for better antimicrobial defense because granules of immature neutrophils contain greater defensin levels than the mature ones (Klut et al., 2000). Defensin plays a major role in cytotoxicity against bacteria, fungi and some enveloped viruses (Lehrer et al., 1993; Ganz and Lehrer, 1995). The driving force behind neutrophil recruitment is the stimulation of cytokines like interleukin-1 and TNF-□ and the chemokines viz. interleukin-8 (IL-8) produced by an array of cells including AM and airway epithelial cells (Fahy et al., 1995). The observed neutrophilia and eosinophilia could be linked to lung function decrement because an inverse relationship exists between FEV1, FEV1/FVC ratio and sputum neutrophil and eosinophils counts (Sagel et al., 2002; Birring et al., 2002). In fact, neutrophil accumulation in the lung is a prominent feature of COPD (Williams and Jose, 2001; Birring et al., 2002). Highest percentage of neutrophils is usually found in sputum of patients with worst airflow obstruction, suggesting that neutrophilic inflammation in small airways is the key factor in the pathogenesis of COPD (Merrick et al., 1997; Green et al., 2002). Sputum

eosinophilia, on the other hand, could partially explain cough because sputum eosinophils count > 3% is usually associated with eosinophilic bronchitis, wheeze and chronic cough (Ayik et al., 2003). Sputum eosinophilia is a common finding in smokers with bronchial allergy (Maestrelli et al., 1996; Komori et al., 2001) and in patients with COPD (Brightling et al., 2002).

Increased number of lymphocytes

Lymphocytes are present in increased numbers in sputum of urban subjects. In contrast to bacterial infection, host responses to viruses, fungi and mycobacteria are more complex and require the phagocytic cells as well as antibody-mediated and cell-mediated immunity. While neutrophils play a role in host defense against fungal and mycobacterial pathogens, the recruitment and activation of macrophages and T lymphocytes are essential for effective clearance of these organisms from the lung. Macrophages engulf, process and present antigen to reactivate T-helper cells, which, in turn, release cytokines resulting in enhanced phagocytic and cytotoxic activities. The T-helper cells also stimulate B cells to produce antibody, which further facilitate microbial phagocytosis. Cytotoxic (CD8+) T cells mediate cellular cytotoxicity crucial for host defense against viral agents. The presence of lymphocytes in increased numbers, therefore, probably indicates adverse lung reaction to a greater load of inhaled pathogens.

Air pollution and airway epithelial cells

Several cytological changes were found in sputum of the residents of Delhi. Sputum cytology was employed because it is a cost-effective and useful technique to assess possible adverse effects of chronic expoure to vehicular pollution on respiratory mucous membrane (Gluck et al., 2003). Customs officers who were chronically exposed to diesel engine emissions from trucks (diesel exhaust particles 31-60 µg/m^3, and B(a)P 10-15 ng/m^3) had rhinitis and chronic inflammatory changes in nasal epithelia (Gluck et al., 2003). As in the present study, the investigators found increased prevalence of cellular changes like metaplasia and dysplasia in exposed subjects, and explained these changes as due to genotoxic insults of mutagens present in diesel exhausts (Gluck et al., 2003). Exposure of airways to environmental toxins (ozone, endotoxin, cigarette smoke) or allergens induces proliferation of epithelial cells. Depending on the type of exposure, existing and newly formed cells can differentiate into mucus-producing cells resulting in mucous cell metaplasia. During recovery, the epithelium reduces the number of epithelial cells by inducing apoptosis to return to original state (Tesfaigzi, 2002).

Sheets of ciliated and non-ciliated columnar epithelial cells were often observed in sputum of the residents of Delhi. These cells line the respiratory surface of the upper airways. They are usually absent in sputum or present as isolated cells. The appearance of these cells in large sheets, therefore, suggests damage to the respiratory epithelium following chronic pollution exposure. The change could be detrimental to respiratory physiology because it will affect the efficacy of the mucociliary escalator for pollutant disposal. Moreover it will facilitate entry of the microorganisms into the lung tissues thereby enhancing pulmonary infection. Curschmann's spiral, found in sputum of Delhi's non-smokers, is a matter of concern because it is usually associated with COPD. The finding is possibly associated with city's vehicular pollution, because traffic policeman of Italy who never smoked had increased number of Curschmann's spiral in sputum (Cenci et al., 1998; Alderisio et al., 2006), and the authors have causally associated its presence with urban air pollution exposure.

Multinucleated ciliated epithelial cells, metaplasia and dysplasia of epithelial cells that has been seen in some residents of Delhi are present more often in subjects with high risk of cancer development

(Chalone et al., 1973). In epithelial carcinogenesis, progressive accumulation of molecular lesions, such as activation of oncogenes, inactivation of tumor suppressor genes and methylation of promoter is paralleled by a morphological journey of the cells from normality via progressively more severe abnormalities (metaplasia-dysplasia-carcinoma-in-situ) to malignancy. Metaplasia is thus the initial morphological change in this journey towards neoplasia (Grubb, 1994). Squamous metaplasia usually develops as an adaptive response to toxic insults and the cells behave differently from that of normal airway epithelium. Metaplasia often leads to dysplasia when cell turnover becomes more rapid (Snead et al., 2003). Although it is rarely seen in sputum samples of non-smokers, metaplasia with atypia, a hallmark of cytological change in the lower respiratory tract after carcinogen exposure (Kamei et al., 1993), and a risk factor for lung cancer (Vine et al., 1990) has been frequently recorded in occupationally exposed subjects of this study. Metaplasia of squamous epithelial cells is an indicator of predisposition to chronic obstructive pulmonary disease (Madison et al., 1984) and lung cancer (Djuricic and Plamenac, 1999). Increased prevalence of squamous metaplasia in urban subjects of this study therefore signifies a greater risk of lung diseases including cancer.

Enhanced release of elastase

Human AM produces several protein-degrading enzymes like elastase, collagenase–I, gelatinase–B, stromelysin–I and matrilysin (Shapiro, 1999). Elastase is capable of destroying elastin, a fibrous protein widely distributed in elastic tissues of the lung. The enzyme is important for antimicrobial activity, but its excess production may cause lung damage. AM and neutrophils are the major sources of elastase in the lung. Besides tissue degradation, AM elastase helps recruit macrophages within the alveolus causing an increase in AM number. Elastase disintegrates the elastin molecules into smaller fragments, and these fragments attract monocytes to leave the bloodstream and migrate to the alveolus as AM (Huninghake et al., 1981). The macrophage-recruiting role of elastase has been demonstrated in laboratory animals in which elastase-lacking mice failed to recruit macrophage in their lungs in response to cigarette smoke (Bedard et al., 1993). Elevated elastase production and release coupled with massive increase in AM number indicate a profound increase in elastase activity in urban subjects of this study. Prolonged and elevated secretion of this enzyme by activated AM may lead to breakdown of the elastic tissue of the lung and injury to pulmonary blood vessels. The consequence would be reduction in the number of alveolus and depletion of surface area for gas exchange as in case of emphysema. An association between AM with the chain of events linking smoking and emphysema is well recognized (Janoff, 1985, Clark et al., 1998). Mice lacking AM elastase did not develop emphysema in response to long-term cigarette smoke exposure (Hautamaki et al., 1997). Neutrophil elastase, on the other hand, is capable of causing eosinophils degranulation in atopic asthmatic subjects (Liu et al., 1999). Thus neutrophil elastase may be one of the several agents responsible for asthmatic attack.

Mucus hypersecretion

Mucus hypersecretion is a prominent manifestation of chronic airway diseases and contributes to their morbidity and mortality by plugging airway and causing recurrent infections (Shao and Nadel, 2005). Airway mucus hypersecretion is a serious and presently untreatable symptom of COPD (Kim and Nadel, 2004). A special type of epithelial cell known as goblet cell produces mucus. Goblet cells are non–ciliated mucus-producing epithelial cells usually shed singly or in loose clusters in sputum. They are seldom seen in abundance in routine smears except in certain diseases like asthma and chronic bronchitis. However, a large number of these cells along with sheets of mucus was found in sputum of apparently healthy, never smokers of Delhi. The reason could be chronic exposure to

high level of air pollution and related airway stress that increase goblet cell number and the quantum of mucus production as a part of pulmonary defense to trap and dispose off inhaled particles and microorganisms (Rogers, 1994).

Goblet cells are situated in the epithelium of the conducting airways, often with their apical surfaces protruding into the lumen, a location which fits them for a rapid response to inhaled airway insults (Rogers, 1994). In animal studies, Wistar rats exposed to diesel emissions containing variable concentrations of NO_2 and particles exhibited increase in the number of mucus-producing goblet cells, infiltration of the airways and alveoli with AM, PMN, mast cells and plasma cells, and development of alveolar holes, an early marker of alveolar destruction (Kato et al., 2000). The observed cellular changes in the lung appeared to be due to the activity of particles, rather than NO_2, because elimination of particles from diesel emissions led to reduction of morphological changes (Kato et al., 2000). In a subsequent study, proliferation of goblet cells, increased production of mucus in the airway, and infiltration of submucosa by inflammatory cells such as AM, mast cells and plasma cells were found following exposure of rats to polluted roadside air of Kawasaki city for 60 consecutive weeks (Kato and Kagawa, 2003).

Role of neutrophils in mucus production

Human neutrophil elastase (HNE) exists in high concentrations in airway secretions of subjects with COPD and induces overproduction of MUC5AC mucin, a major component of airway mucus. HNE induces MUC5AC induction through generation of ROS, induction of TNF-alpha-converting enzyme, and activation of PKC and epidermal growth factor receptor (EGFR) in human airway cells (Shao and Nadel, 2005). EGFR expression and activation have been implicated in mucin production by goblet cells. Activated PMN recruited to the airways and their secreted product play several key roles in EFGR-dependent mucin hypersecretion. The secretion of active products by PMN is carefully regulated. The local release of PMN elastase requires close contact between the PMN and another cell, mediated by surface adhesion molecule, thus limiting proteolysis to the immediate pericellular environment. In the airway lumen, PMN undergoes apoptosis and are cleared by macrophage without releasing their intracellular content. In contrast, PMN that dies by necrosis disgorge proteases and ROS into the lumen. In COPD, PMN necrosis occurs within lumen, causing release of high concentration of eosinophil and ROS in lumen. Thus, inflammatory cells (PMN), molecules (elastase and ROS), signaling pathway (EGFR) and cellular processes (PMN necrois) contribute to mucus hypersecretion in COPD (Kim and Nadel, 2004).

Iron disposition in AM

Association between chronic exposure to Delhi's air pollution and microscopic hemorrhage inside the lung was investigated by enumerating siderophages. Generally smokers contain in their sputum AM with iron deposits (Smith and Raso, 1999; Mateos et al., 1998). However, non-smokers of Delhi also recorded abundance of siderophages in sputum. Hence, reason other than smoking appears to be responsible for the change. The possible sources of high level of iron in AM are chronic inhalation of iron-rich dust as observed in iron-foundry workers (Plamenac et al., 1974), phagocytosis of moribund cells including erythrocytes (Richter, 1978), and uptake of iron from iron-binding proteins eosinophils and lactoferrin via the eosinophils receptors (Mateos et al., 1998). Inhalation of metallic iron may not be a significant contributor in Delhi (Balachandran et al., 2000). A likely possibility is that the iron in AM is derived from hemoglobin of phagocytosed erythrocytes as in case of patients with Goodpasture's disease (Mateos et

al., 1998). Hemosiderin-laden macrophages are a common finding in bronchoalveolar lavage (BAL) of patients with alveolar bleeding (Schnabel et al., 1999). Likewise, abundance of siderophages in sputum of pollution-exposed individuals of Delhi may suggest covert pulmonary hemorrhage. However, there is no direct proof to support that inhaled particles damage the lung vasculature or red cells leading to microscopic hemorrhage and subsequent phagocytosis of erythrocytes by AM. But it has been suggested that some components of PM_{10} may affect the lung endothelial cells or erythrocytes causing sequestration of red cells in the circulation (Seaton et al., 1999). Moreover, premature destruction of red cells has been indicated in persons chronically exposed to traffic-related air pollution (Giovagnoli et al., 1999).

The AMs have only a limited ability to metabolize hemoglobin (Custer et al., 1982) and the cells lack heme oxygenase, the enzyme required for liberation of iron from hemoglobin (McGowan et al., 1986). However, heme can induce the synthesis of this enzyme (Mateos et al., 1998). It is possible, therefore, that heme oxygenase is induced in AM of pollution-exposed individuals actively engaged in phagocytosis of the moribund cells like that in patients with Goodpasture's disease (Mateos et al., 1998). Besides erythrocytes, the AM may acquire iron and ferritin from alveolar cells destroyed during infection and inflammation of the lung (Wesselius et al., 1996). It is important to mention in this context that eosinophils are synthesized within the human alveolus by T lymphocytes, especially the CD4+ cells (Thompson et al., 1991).

A higher Golde's score was found among the residents of Delhi suggesting occult alveolar hemorrhage. Alveolar hemorrhage is associated with thrombocytopenia (platelet count less than 50,000 per microliter) abnormal coagulation, renal failure and history of heavy smoking, but not with pneumonia (De Lassence et al., 1995). The changes could be related to hemorrhage because intranasal instillation of blood in mice resulted in marked rise of siderophage in BAL within 3 days with a peak at day 7 and it persisted for 2 months (Epstein et al., 2001). Alveolar hemorrhage is a characteristic feature of a rare, pulmonary veno-occlusive disease (POVD; Rabiller et al., 2006). But the participants of this study were apparently healthy, without evidence of gross pulmonary impairment. Thus, the possibility of POVD may be excluded. Thrombocytopenia and invasive fungal infections are associated with severe hemorrhage in the lung that leads to an increased number of siderophages in the BAL (Kahn et al., 1987). Several fungi were detected in sputum of a large number of Delhi's individuals that can be instrumental in part for high Golde score, suggesting pulmonary hemorrhage.

The consequence of iron overload in AM

The accumulated iron is likely to be retained for the entire life of the cell because AMs lack the mechanism of iron release for recycling (Lee and Herbert, 1999). This could be detrimental to the lung because excessive accumulation of iron, especially in unbound, 'free' form, facilitates the generation of potentially toxic hydroxyl radicals from less reactive superoxide and hydrogen peroxide via Fenton and Haber-Weiss reaction (Mateos et al., 1998). The consequence would be tissue injury. Fibrosis of the lung is another possibility because iron has the ability to stimulate fibrogenesis (Britton et al., 1994). Moreover, iron, at least in certain forms, promotes the development of cancer (Hueper and Payne, 1962).

Mechanism of air pollution-related lung injury: ultrafine particle and oxidative stress

Ultrafine particles (UFP) in vehicular exhausts are extremely important for pulmonary toxicity of vehicular emissions, as they induce a greater inflammatory response per given mass than larger particles

(Oberdorster, 2000). Exposure of rats to a concentration of $5x10^5$ UFP per cm^3 of air for 10-20 minutes resulted in the development of severe pulmonary inflammation and hemorrhage within 4 hours and high mortality afterwards (Oberdorster, 1995). Lung lavage fluid of these animals illustrated neutrophilia (up to 80% of total cells) and increased lysosomal and cytoplasmic enzymes. UFP-exposed animals showed a high degree of alveolar and interstitial edema following disruption of the epithelial and endothelial cell layers, and upregulation of pro-inflammatory cytokines such as IL-6, IL-1A, Il-1B, TNF-□ and antioxidants such as Mn-SOD suggesting that the lung injuries were due to severe oxidative stress (Oberdorster, 1995, 2000). Therefore, UFP could be a significant contributor to pulmonary inflammation observed in a large number of residents of Delhi. Deposition of UFP in the respiratory tract occurs by diffusion process, and human nose is highly equipped to collect inhaled UFP by this process in the nasal compartment (Cheng et al., 1991, Swift et al., 1992). The translocation of UFP deposited in the lung epithelial, interstitial and endothelial sites appear to be rapid because these tiny particles escape AM surveillance, which is otherwise very efficient for larger particles (Hahn et al., 1977).

The changes in airway epithelial cells of the residents of Delhi can be attributed to pollution related oxidative stress. This hypothesis is supported by animal studies where smoke exposure caused loss of epithelium and exposure of the basement membrane of trachea (Dubick et al., 2002). Thiobarbituric acid reactive substances (TBARS), a measure of oxidative stress, was 2-3 times higher in lung and liver of smoke-exposed rats, suggesting that the cellular changes in the lungs of smoke-exposed rats were due to oxidative stress (Dubick et al., 2002). There is extensive evidence that air pollutants cause lung disease by generating oxidative stress. PM_{10}, $PM_{2.5}$ and transition metals including iron are known to mediate the oxidative stress (Kadiiska et al., 1997; Jimenez et al., 2000). There is a link between oxidative stress and inflammation in the lung via activation of oxidative stress-responsive transcription factor nuclear factor kappa beta, which control pro-inflammatory genes in the cells (Rahman and MacNee 1998).

Protection by antioxidants

Several lung diseases are associated with oxidative stress evoked by environmental pollutants. It is important to mention in this context that dietary factors and nutrients including fruits and vegetables, antioxidant vitamins such as vitamin C, vitamin E,

beta carotene and other carotenoids, vitamin A, fatty acids and some minerals such as sodium, magnesium and selenium confer protection against oxidative process and inflammatory response responsible for the genesis of these diseases (Romieu, 2005).

Hematological effects of air pollution

Recent evidence suggests that effect of inhaled particles is not merely restricted to the lungs. Ambient particles are known to exert significant effect on several hematologic parameters after inhalation (Gordon et al., 1998). Inhaled ultrafine particles may directly enter the blood circulation resulting in translocation of air pollutant to extra-pulmonary sites (Oberdorster, 2001). Therefore the hematologic profile of the citizens of Delhi were examined and compared to the findings with that of the rural control. A small, but consistent rise was found in hemoglobin and RBC level among the residents of Delhi. This could be associated with chronic air pollution exposures, because exposure to particles ($PM_{2.5}$) can induce oxidative stress and increase RBC and hemoglobin levels by enhancing hypoxia-inducible factor-1 –mediated erythropoietin production particularly in women (Sorensen et al., 2003). An increase in RBC would cause an increase in blood viscosity, which is a risk factor for cardiovascular events (Donaldson et al., 2001). 'Target' cells are erythrocytes with increased surface-to-area ratio. They

are rare in normal, healthy individuals. Presence of these cells in excess in peripheral blood is associated with, among others, thalassemia and liver toxicity related to cholesterol metabolism (Dacie and Lewis, 1975). The overall frequency of thalassemia carriers in Indian population is 4-6%. Since a much higher percentage of Delhiites had target cells, it is unlikely that the finding is attributable to thalassemia trait. Instead, it may indicate a change in liver function in association with cumulative pollution exposure.

The residents of Delhi had increased number of total leukocytes in their circulation than the rural controls. They had significantly increased number eosinophils and monocytes besides elevated neutrophil numbers while lymphocyte count remained relatively unaltered. The urban subjects showed marked increase in the number of immature neutrophils in circulation in the form of band cells, myelocytes and metamyelocytes. Band cells, recognized by an incomplete separation of nuclear lobes, are found in the bone marrow and form 2 to 6% of normal circulating neutrophilic leukocytes. An increase in their number in blood indicates that the marrow is been stimulated to increase the release of polymorphonuclear (PMN) cells (Athens et al., 1993). Rise in the number of these immature neutrophils in peripheral blood could result from increased rate of granulocyte production in the bone marrow and their premature release into the bloodstream. Current studies in laboratory animals and human volunteers have conclusively established that particulate air pollution enhances the rate of granulocyte production in the bone marrow and their release into the circulating blood. Immature cells released from the marrow following air pollution exposure marginate more readily in the lung and have greater tissue damaging capabilities than do mature PMN (Terrisema et al., 1997). Therefore it is possible that these immature cells released from the marrow have an important role on the pathogenesis of cardiopulmonary mortality and morbidity associated with air pollution. Indeed, Weiss (1995) and colleagues have shown that an increase in WBC count is the predictor of total mortality.

Following exposure of rats to ambient particulate matter the percentage of neutrophils in peripheral blood was significantly elevated while the percentage of lymphocytes was significantly decreased (Gordon et al., 1998). Rise in PM_{10} level results in granulocytosis in bone marrow therefore releasing band cells into the circulation (Tan et al., 2000). Exposure of human volunteers to diesel exhaust for only one hour produced a marked increase in the number of neutrophils in the peripheral blood (Salvi et al., 1999). Levels of exercise can also influence peripheral WBC counts, stress and health related events such as acute infections. The control and exposed subjects of this study were controlled for this variation in lifestyle stresses. The subjects were of the same sex, similar age, working outdoors and living in the same environment. The absence of these confounding influences on WBC kinetics and the clear temporal relationship between the band cell count and the indices of air pollution strongly suggest the elevation in band cell count can be attributable to high pollution load.

Peripheral blood of urban subjects had more platelets. Moreover, a significantly increased percentage of these platelets expressed P-selectin, an activation marker. P-selectin plays an important pro-inflammatory role in mediating interactions among neutrophils, platelets, and the vascular endothelium (Zimmerman et al., 1992). P-selectin exposed on the activated platelet membrane binds its counter receptor, the P-selectin glycoprotein ligand 1 (PSGL1) present on leukocytes and endothelial cells, resulting in platelet-leukocyte and platelet-endothelial cell interactions (Palabrica et al., 1992) , leading to fibrin formation via induction of tissue factor on monocytes, release of superoxide anion and proinflammatory cytokines from monocytes and neutrophils, and modulation of the coagulant properties of endothelium (Nagata et al., 2000; Celi et al., 1994). The net result could be a sharp rise in the risk of cardiovascular diseases (Rider et al., 2001; Blann et al., 2004). Indeed, in patients with peripheral artery disease and deep vein thrombosis the platelets are hyperactive and circulate in an activated (P-selectin-expressing) state

(Robless et al., 2003; Kappelmayer et al., 2004). Therefore, rise in the number of activated platelets in citizens of Delhi appeared to be a potential cardiovascular risk.

Air pollution and the cardiovascular risk

Although the adverse health impact of air pollution on cardiovascular system is well recognized, the pathophysiology is not clearly understood. Heart rate and the entire cardiovascular system are under the constant influence of both sympathetic and parasympathetic innervations from the autonomic nervous system. Parasympathetic effect is conveyed through vagus nerves originating from the CNS while sympathetic nerve originates from spinal chord. Increase in parasympathetic activity slows the heart rate whereas sympathetic activity increases the heart rate by directly affecting the sinus node. Normal resting heart beats 55-70 times per minute. In case of stress there is an increase in sympathetic discharge leading to an increased heart rate. Various conditions such as hypertension, diabetes and COPD are associated with altered autonomic function due to increased activation or withdrawal of parasympathetic or both (Zareba et al., 2001). Air pollution is associated with decreased heart rate variability, implying impaired autonomic nervous activity and increased risk of cardiac events associated with imbalance of sympathetic and parasympathetic system (Zareba et al., 2001). Biologic or psychologic stress induces a cascade of events by increasing sympathetic activation, which may increase myocardial vulnerability leading to ventricular tachyarrythymias, aggravation of ischemia or heart failure and death (Zareba et al., 2001). The stress from pollution exposure and consequent high levels of circulating catecholamines may cause myocardial damage (Wood et al., 1991). Air pollution as a stressor can trigger this chain of events, especially in vulnerable subjects such as the elderly and patience with heart problem or COPD (Zareba et al., 2001). Stable atherosclerotic plaque may narrow the arterial lumen and cause stable angina pectoris but are relatively harmless.

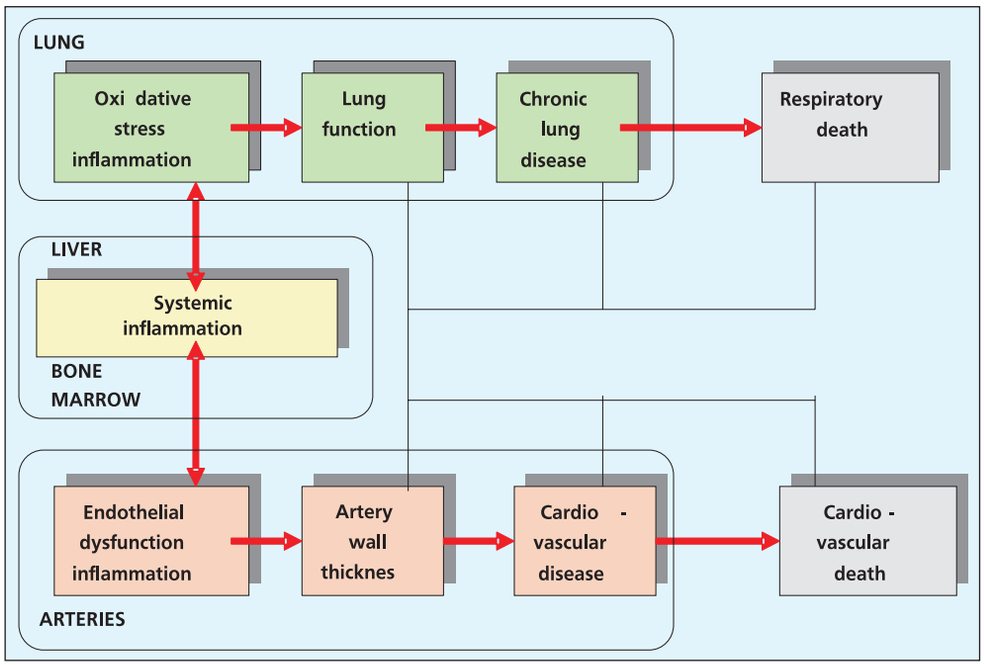


Figure 10.1 Interrelated pulmonary, systemic, and vascular chronic inflammatory responses to air pollution. Lung function and artery wall thickness are examples of markers of the continuous chronic process from health to disease. (Thin lines denote correlations established in epidemiological studies (adapted from Air pollution: from lung to heart by N. Künzli and I.B. Tager)

Due to compensatory enlargement of artery with atherosclerosis lumenar diameter of the vessel remains normal until approximately 40% is occupied by plaque. It is the large unstable plaques that rupture and form occlusive thrombosis, resulting in MI and unstable angina. A thin fibrous scrap infiltrated by macrophages characterizes vulnerable plaques prone to rupture. Inflammatory process are important in the pathogenesis of acute MI and accumulation of mast cells develop at the site of atheromatous lesions or rupture. Smoke exposure increases the risk of both MI and ischemic stroke due to occlusion of the carotid arteries (Smith et al., 2000).

Systolic and diastolic hypertension was observed in 33.9% and 21.3% individuals of Delhi. Moreover, systolic and diastolic pre-hypertension was present in 23.9% and 10.7% respectively. Our findings are in general agreement with the recent report from urban West Bengal showing systolic and diastolic hypertension in 40.9% (21.3% in this study) and 29.3% (33.9% in Delhi) subjects respectively (Das et al., 2005). Like the present study, they also found greater prevalence of hypertension in women (Das et al., 2005). Our findings also agree with the WHO report in which 65% overall prevalence of hypertension among elderly (age>60 year) in India and Bangladesh has been reported (WHO, 2002). Hypertension is emerging as a major health problem in India, and the problem is more severe in urban than in rural subjects (Gupta, 1997). Hypertension is a multifactorial disease with stress, obesity and socio-economic status having a srong contribution. In the early 50's, hypertension prevalence ranged from 1-4% in Indian cities like Kolkata, Kanpur and Mumbai. The prevalence increased to 11-and14% in mid 80's to 90's in Jaipur and Ludhiana respectively. There has been a significant increase in systolic than in diastolic BP (Gupta, 1997).

Hypertension was more prevalent among women, and in persons with high BMI and in high SES in Delhi. Arterial hypertension is influenced by some non-modifiable and modifiable factors. Non-modifiable factors such as age, female gender, and family history of hypertension increases the risk of increased SBP and DBP. Among the modifiable factors, lower physical activity, body weight gain, excess sodium chloride intake (> 5-7 g/day), and alcohol consumption increase BP. Higher prevalence of hypertension has been noted in upper socio-economic groups in countries at a transitional stage of economics. In contrast, in developed countries with affluent economics, greater prevalence is found in lower socio-economic groups. In women, hormonal (estrogen-progesterone) contraceptives cause a distinct increase in blood pressure (Antezana et al., 1996).

Air pollutants, especially the particulates from road traffic, are known risk factors for serious cardiac problems including myocardial infarction. Evidence in support of this statement comes from studies conducted in Europe in which increased risk of myocardial infarction has been reported in professional drivers, such as bus, truck and taxi drivers (Bigert et al., 2003). Pande and his co-workers (2002) conducted a 2-year (January 1997-December 1998) time-series analysis in Delhi. They found ambient level of pollutant exceeded the national air quality standard on most of the days over the two-year period of study. Emergency room visits for acute coronary events at All India Institute of Medical Sciences increased by 24.3% on account of higher than acceptable level of air pollutants. It was concluded that there is a considerable burden of cardiopulmonary diseases in Delhi due to high level of ambient air pollution (Pande et al., 2002). In addition to air pollution, unfavorable life style, social factors, and duration of work were found to be potential risk factors. Likewise, exposure to fine particulate pollution ($PM_{2.5}$) is associated with increased cardiovascular mortality (Samet et al., 2000; Pope et al., 2002).

Exposure to air pollution has been shown to cause arterial vasoconstriction and alter autonomic balance (Gold et al., 2000; Devlin et al., 2003). Inflammation of the airways and alveoli is considered as a risk

factor for cardiovascular problems. Increase in the number of neutrophils in BAL fluid in dogs has been reported to be associated with disturbances in cardiac rhythm (Godleski et al., 1997a).

Remarkable increase in the number of P-selectin- expressing activated platelets in the circulation of the residents of Delhi may indicate increased risk of thrombosis. This is supported by reports showing association between raised P-selectin expression in platelets with deep vein thrombosis (Yang et al., 2002), and acute myocardial infarction (Matsumoto et al., 2002). In addition, over-expression of P-selectin in platelets may promote systemic alterations because up-regulation of platelet P-selectin may accompany liver injury (Massaguer et al., 2002), eosinophil syndromes (Sirolli et al., 2002), and depression (Walsh et al., 2002). In fact, a significantly higher prevalence of depression was found among the residents of Delhi. Incidentally, a close relationship exists between cardiovascular disease and depression (Walsh et al., 2002).

Air pollution and diabetes

Diabetes is a highly prevalence chronic illness: 5.1% of US population older than 20 years of age has diagnosed diabetes and an additional 2.7% has undiagnosed diabetes (Harris et al., 1998). A 7.9% prevalence of diabetes was found in Delhi compared with 1.9% in rural controls. A recent multicentric study has concluded that the prevalence of diabetes mellitus in Indian population is 3.3% (Sadikot et al., 2004). In agreement with our observation, the study reported a significantly higher prevalence in urban subjects (4.6% vs. 1.9% in rural). Till date, there is no evidence to suggest that air pollution causes diabetes. But, a number of studies have demonstrated that diabetics are more susceptible to adverse health effects of air pollution than persons without diabetes. For example, a 10mg/m^3 increase in PM_{10} was associated with 2.01% (95% CI 1.4-2.62%) increase in hospital admissions for heart diseases in persons with diabetes, but only 0.94% (95% CI 0.61-1.28%) increase in persons without diabetes (Zanobetti et al., 2000a, 2002).

Airborne particles are associated with reduced heart rate variability, increased plasma C-reactive protein, increased fibrinogen and white cell counts (Peters et al., 2001; Schwartz 2001; Salvi et al., 1999). These indicators of cardiovascular vulnerability are affected by diabetes as well. It raises the question whether other cardiovascular risk factors generally associated with diabetes are also affected by airborne particles. For example, diabetics have increased risk of vascular injury. Whether particulate exposure has similar effect is an area of intense current research. Some preliminary reports have strengthened these assumptions, as urban particles increased endothelin I and III concentrations in plasma (Vincent et al., 2001) and atherosclerotic lesions in coronary arteries (Suwa et al., 2001).

Besides heart problems, diabetes increases PM_{10}-induced hospital admissions for COPD in older people and the risk of pneumonia in younger people (Zanobetti et al., 2001). These findings indicate that the interaction of particulate pollution with diabetes is a matter of serious concern and deserves more attention due to its large public health implication in India, termed as the diabetic capital of the world.

Immunology, lymphocyte subtypes and lymphocyte activation

A depletion of CD4+ T-helper cells and B cells, but increase in the number of natural killer (NK) cells in peripheral blood of the residents of Delhi was found, suggesting change in body's defense against infections. In agreement with the present finding, significant increase in the percentage of NK cells has been reported among residents of highly polluted Teplice district of Czech Republic compared

to relatively less polluted district of Prachatice (Dostal et al., 2000; Hertz-Picciotto et al., 2002). In a separate study in Czech Republic, women from highly polluted city had lower percentages of CD4+ and lower ratios of CD4+: CD8+ than women from less polluted cities. Higher percentages of NK cells and depleted proportions of T cells were also found in cord blood of these women from polluted areas (Hertz-Picciotto et al., 2002). Thus, higher levels of air pollution affect the immune system of pregnant women and their fetuses.

The change in immune status can be attributed, at least in part, to Delhi's vehicular pollution. Human volunteers after exposure to diesel exhausts showed fall in CD4+ T cells and B cells in peripheral blood due to migration of these cells from circulation to the airways (Salvi et al., 1999). Decrement of CD4+ cells but increment of CD8+ cells has been reported in non-smoking urban policemen in Italy (Boscolo et al., 2000). Chronic inhalation of diesel exhaust particles has been shown to increase the susceptibility of lung towards infection by depressing the antimicrobial potential of alveolar macrophages (Kastranova et al., 2001). On the other hand, substantial decline in circulating CD4+ T cells has been reported in workers exposed to aromatic amines in Japan (Araki et al., 1993). Similarly, Chinese shoe workers exposed to low level of benzene (<1 ppm) showed decline in CD4+ T cells, CD4+/CD8+ ratio, and the number of B cells in peripheral blood (Lan et al., 2004). In laboratory animals also, exposure to industrial air pollutants caused depletion of B and T cells (Kozlowska et al., 1996).

In a cross-sectional survey in 17 Central European cities the annual average $PM_{2.5}$ and from 12-38 µg/m^3 PM_{10}-$PM_{2.5}$ [Coarse]). It was found that the number of B, CD4+, CD8+ and NK cells of children aged between 9 –11 years increased with increasing concentration of PM after adjusting for age, gender, passive smoking and recent respiratory illness. Lymphocyte total count and total IgG also increased in these children, suggesting activation of the cellular and humoral immune system in response to long term exposure to airborne particles (Leonardi et al., 2000).

Exposure to ultrafine particles along with exercise in normal healthy subjects reduces the expression of CD18 on monocytes and PMN. Also there was concentration- related reduction in the number of monocytes, basophils and eosinophils in peripheral blood and increased expression of lymphocyte activation marker CD25 (Frampton et al., 2006). Particle exposure also reduced the percentage of CD4+ T cells thus inhalation of elemental carbon UFPs alters peripheral blood distribution and expression of adhesion molecules implying increased retention of leucocytes in pulmonary vascular bed (Frampton et al., 2006).

Besides air pollution, physical or psychological stress can trigger changes in lymphocyte subpopulations, and the changes are possibly mediated by catecholamines via beta 2-adreno receptors of lymphocytes (Schedlowski et al., 1996; Benschop et al., 1998). It is important to mention in this context that a significant rise in plasma catecholamine level has been recorded in urban subjects of this study. Alternatively, the mechanism may involve direct antigenic stimulation by air pollutants. For example, alteration of lymphocyte subsets has been shown following smoking (Hockertz et al., 1994) and occupational exposures (Vojdani et al., 1992; Daniel et al., 1995). On the other hand, change in lymphocyte subset could be elicited by benzene, because chronic exposure to benzene caused alteration of T lymphocyte subsets (Irons et al., 2005). In any case, the present study showed changes in immune defense among the citizens of Kolkata that could make them more vulnerable to bacterial and viral infections. In agreement with this, downregulation of pulmonary immunity to infection by Listeria monocytogenes has been observed in rats following exposure to diesel exhaust particles (Yang et al., 2001, Yin et al., 2005).

Air pollution and metabolic changes, Liver function

Cumulative exposures to traffic-related air pollution in laboratory animals cause biochemical changes in the liver. Exposure of rats to diesel exhausts particles (0.3 mg/m^3) 12h per day for 4 weeks significantly increased P4501A1 and P450 1B1 metabolizing enzymes in lung and liver. The induced P4501B1 and P4501B1 may catalyze the genotoxic activation of diesel exhausts particles (Hatanaka et al., 2001). In a subsequent comparative study among 111 traffic policemen and 118 office workers, in Rome, higher AST, ALT values were found in the traffic policemen, suggesting impairment of liver function (Tomao et al., 2002). It has been suggested that urban air pollution, even within admissible level, contains chemical that cause liver damage (Tomao et al., 2002), and the change can be attributed, at least in part, to city's vehicular pollution.

Genotoxic effect of air pollution

The citizens of Delhi illustrated greater prevalence of micronucleus formation in buccal and airway epithelial cells and DNA damage in peripheral blood lymphocytes, suggesting genetic damage. Micronuclei (MN) are formed in cells as a result of breakage in chromosome. The chromosomal fragments that cannot be attached to spindle apparatus due to the absence of centromere in the break away portion during cell division are excluded from the nucleus. These chromosomal fragments stay in the cytoplasm as MN. Thus, presence of MN is an indication of chromosomal damage.

The assessment of MN in exfoliated cells is a useful tool to study the degree of cytogenetic damage in target tissues by human carcinogens (Stich and Rosin, 1984, Stich et al., 1982; Belien et al., 1995). The population in general is exposed to a variety of carcinogens and co-carcinogens present in ambient air that can act in an additive, synergistic or antagonistic manner. A study in China has shown marked rise in MN frequency in lymphocytes of traffic policemen who worked outdoors than their colleagues who worked in offices. It was also reported that the enhanced MN count was attributed to their smoking habit (Zhao et al., 1998). Smoking and chewing appeared to have additive effect on MN formation. But nonsmokers and nonchewers of the present study also showed elevated MN count. The results may suggest chronic inhalation of Delhi's air elicits genotoxic changes in the exposed cells. Like the present study, significant increase in MN frequency has been demonstrated in buccal epithelial cells of traffic police (Karahalil et al., 1999; Maffei, 2005), and taxi drivers and engine repair workers of Turkey (Karahalil et al., 1999). It is conceivable that carcinogens present in air induces chromosomal damage to the dividing basal cells of the buccal epithelium resulting in the production of micronuclei in the daughter cells which migrate up through the epithelium and are exfoliated. But an increase in the frequency of MN does not necessarily indicate the formation of a precancerous lesion or a carcinoma because the aberrant cell may be destroyed through apoptosis. But elevated frequency of MN does indicate increased probability of carcinogenesis. In agreement with this, increased risk for oral cancer among urban individuals has been reported (Stich et al., 1982).

Compared with rural controls, we found significantly higher frequency of DNA damage in lymphocytes in citizens of Delhi. The change can be linked to higher level of air pollution in the city, because air pollution is a conglomerate of many organic chemicals, some of which are potentially genotoxic. In agreement with the present finding, particulate pollutant (PM_{10}) from Mexico City has been shown to cause DNA damage in Comet assay (Alfaro-Monaro et al., 2002). DNA damage may lead to several diseases including cancer. The most notable examples of genotoxic and cancer-causing compounds in

urban air are benzene and benzo(a)pyrene that come primarily from road traffic. Burgaz and co-workers (2002) showed that occupational exposure to urban air pollutants leads to induction of cytogenetic damage in lymphocytes of traffic policemen and taxi drivers. Excess DNA damage in lymphocytes has been recorded in bus manufacturing workers (Zhu et al., 2001), elevator manufacturing workers (Lam et al., 2002), rubber factory workers (Somorovska et al., 1999) and gasoline service attendants (Navasumrit, 2005).

Mechanism of pollution-mediated cellular injury: Oxidative stress

Oxidative stress imposed by pollutants affects certain pathways involved in DNA damage and the antioxidative defense system (Nia et al., 2001). Thus, the damage gets multiplied whenever there is an oxidative stress. Our results also show significant depletion of superoxide dismutase and total antioxidant level in blood of the citizens of Delhi, suggesting decreased antioxidant activity. It implies that although the Delhiites are more exposed to genotoxic air pollutants, their antioxidant machinery has been down regulated, rather than enhanced, to combat the oxidative stress. In conformity with our findings, depletion of antioxidant proteins, such as catalase, SOD, and glutathione peroxidase, has been observed in traffic policemen of Hyderabad (Suresh et al., 2000). Thus, chronic expoure to vehicular pollution causes decrease in the antioxidant level and increase in oxidative stress. Reactive oxygen species, which are generated during reduction of O_2, are able to break DNA strands, to destroy proteins, and to induce the process of lipid peroxidation (Doelman et al., 1990). The radicals can attack DNA bases or deoxyribose residues to produce damaged bases or strand breaks (Ward et al., 1987). The presence of a methylene group between two double bonds renders the fatty acid sensitive to oxidation (Porter, 1986). The oxidized products are either reduced by glutathione peroxidases to unreactive fatty acid alcohols or they react with metals to produce a variety of products, such as epoxides and aldehydes which themselves are reactive and mutagenic (Schauenstein and Esterbauer, 1978). Thus, oxygen radicals can oxidize lipid or protein molecules to generate intermediates that react with DNA to form adduct (Esterbauer et al., 1990). This could be a reason for excess genetic damage in these subjects.

Air pollution and cancer

Urban air pollution consists of a complex mixture of organic compounds, many of which are potentially cancer causing for humans. An association has been found between high level of urban air pollution and lung cancer. Diesel exhausts particles (DEP) are classified as probably carcinogenic for humans by the International Agency for Research on Cancer (IARC, 1988). Some occupational groups, such as professional drivers working in urban areas, are exposed to a high level of ambient pollution (Guillemin et al., 1992). An increased risk of several types of cancer, including cancer of the lungs, urinary bladder and gastrointestinal tract, was observed among men working as drivers of passenger car, bus, and truck in urban areas (Hansen et al., 1998; Balarajan and McDowal, 1988; Risch et al., 1988; Guberman et al., 1992; Steenland et al., 1992; Barbone et al., 1995; Jacobsson et al., 1997; Soll-Johanning et al., 1998;; Gustavsson et al., 2000). Greater prevalence of lung cancer has also been reported among traffic policemen in China (Zhu et al., 2003). The yearly excess of lung cancer due to air pollution in Europe and the USA has been estimated as 30-150 cases per million people (Hemminki and Pershagen, 1994). Squamous cell carcinoma is still commonest, but adenocarcinoma is rapidly increasing. In addition to smoking, occupational and environmental exposures to carcinogens, indoor air pollution and dietary factor are important (Behera and Balamugesh, 2004).

Benzo(a)pyrene (B(a)P), benzene in breathing air

Delhi had 31.2 ng/m^3 total PAHs in its air during winter, which is comparable with that of three cities of the USA: 4.2-64 ng/m^3 in Los Angeles, 10-160 ng/m^3 in Houston, and 12-110 ng/m^3 in Elizabeth (Naumova et al., 2002). However, a more recent study in Roxbury near Boston has reported a remarkably lower level of 8ng/m^3 median concentration (range 4-57 ng/m^3) of particle-bound PAHs at the side of the road (Levy et al., 2003). The concentrations of PAHs in vehicular exhausts in Delhi varied with the vehicle type. It was 50.76 mg/g for bus, 57.72 mg/g for truck, 27.27 for car, 28.61 for autorickshaw and 29.81 for 2-wheelers (Khillare et al., 2005a,b). The authors found higher concentration of PAHs in scooter exhaust than in car and auto rickshaw exhausts.

The concentration of B(a)P, a carcinogenic PAH, in five residential areas of Delhi during winter of 2004-2005 was 3.13 ng/m^3 with a lowest concentration of 1.98 ng/m^3 in Shahzada Bagh and highest of 4.60 ng/m^3 in Shahdara. Traffic intersection area at ITO had even more- 7.31 ng/m^3. The overall concentration of B(a)P in Delhi's air was 3.82 ng/m^3. In contrast, cities of the USA and Europe had a mean B(a)P level of 0.1-1.0 ng/m^3. For example, 9-year mean of B(a)P in San Francisco's Bay area was 0.4 ng/m^3 (Flessel et al., 1991), 1.0 ng/m^3 of B(a)P was found in ambient air of Berlin (Fromme et al., 2004), and 0.37 ng/m^3 and 0.12 ng/m^3 of B(a)P were found Italy during winter and summer respectively (Minoia et al., 1997). Higher values (4-61 ng/m^3) are usually found in occupational settings such as in rubber factory (Barnski et al., 1992). A study among ng/m^3 which was significantly greater than controls (0.16 ng/m^3) and the values were not significantly different from ambient B(a)P level (Piccardo et al., 2004).

Delhi had a mean of 21.4 µg/m3 of benzene in air in 2005: 12.4 µg/m3 in residential locations and 34.6 µg/m3 in roadside areas. Compared to other Asian cities, benzene level in Delhi's air was significantly higher. For example, Fuji in Japan had 0.78-3.17 µg/m3 of benzene in summer and 1.35-6.04 µg/m3 in winter (Amagai et al., 2002). The concentration of benzene in urban air of Korea was 6.8 µg/m3 (Jo et al., 2003). However, a higher mean level of 20.4 µg/m3 (range15.4-27.9 µg/m3) has been reported in Athens, Greece (Chatzis et al., 2005). Similarly, higher levels of ambient benzene in residential/commercial areas (average 70 µg/m3) and in petrol pumps (1100µg/m3) have been reported in Bangalore (Raghavan and Basavaiah, 2005). In contrast, toluene level in Delhi was comparable with that of Korea (29.1 µg/m3 ; Jo et al., 2003) and lower than the current level in Greece (47-84 µg/m3; Alexopulos et al., 2006). Therefore, the levels of B(a)P and benzene are substantially higher in Delhi's air compared with Europe, US and Asian cities. Both of these chemicals are potentially cancer causing, and there is no threshold for their cancer-causing potential. Thus, there is no safe limit for B(a)P or benzene, and efforts should be made by all concerned to lower the levels of these carcinogens in breathing air as far as possible.

Meteorological influence on air pollution

Meteorological factors have considerable influence on air pollution. A study in Cairo has emphasized the importance of relative humidity on the concentration of urban air pollutants. Low humidity (less than or equal to 40%) increases the levels of NO2 and ozone while high (>80%) humidity increases the concentration of PM_{10}, CO, and SO2. Moreover, low wind speed was found to be associated with higher levels of vehicular pollution while high wind speed is associated with elevated crustal pollution (Elminir, 2005). Incidentally, PM_{10} in urban areas are mainly anthropogenic in origin whereas in rural areas its source is mainly natural such as resuspended soil dust associated with traffic on unpaved roads

and soil dusts from agricultural activities (Celis et al., 2006). In a study in Delhi during a 4-year period of 2000-2003, Agarwal et al., (2006) have concluded that winter months had greater expoure risk as pollutant often trapped in the lower layers of atmosphere resulting in high concentrations. They also found a significant negative correlation between meteorological factor such as temperature with COPD (r = -0.318). A study in Chile has shown that mean PM_{10} level during the cold season was twice that of warm season. Moreover, carbonaceous substances are the most abundant components of PM_{10} during winter; whereas while crustal material is the most abundant component of PM_{10} during summer (Celis et al., 2006).

Biomonitoring and health impact of benzene

Benzene is an important constituent of vehicular pollution. Benzene is well known for its neurotoxicity besides hematologic toxicity including leukemia and lymphoma (Wallace 1984, 1989; Midzenski et al., 1992; Farris et al., 1993; Ritchie, 2001; Burbacher, 1993). Long-term occupational exposure to high concentrations of benzene is reported to be associated with leukemogenesis (Snyder and Kalf, 1994). From the late 1980s on, proposals were made and adopted in many countries to lower the occupational exposure limit value for benzene from 10 to 1ppm (8-hr time-weighted average [TWA]). In some countries, even lower values have been adopted (e.g., 0.5 ppm in Sweden) or proposed (0.3 ppm in USA). Benzene exposure, calculated from urinary t,t-MA levels, in urban subjects varied between 1.4 ppm and 5.7 ppm respectively. The t,t-MA values closely resembled actual measured benzene concentration of 5.5 ppm (17.6 $\mu g/m^3$) in ambient air. Since measurement of t,t-MA is easier and cost-effective than benzene measurement; it can be used for all future biomonitoring study for benzene in the country.

Medical treatments are usually very expensive and often ineffective for benzene-induced blood diseases like aplastic anemia and leukemias. Therefore, emphasis should be given on early detection of reversible health effects of benzene through medical surveillance system. Medical surveillance, a secondary preventive measure in occupational health control strategies, is based on detection of preclinical abnormalities before a worker generally seek medical attention. Since assessment of benzene toxicity by sensitive procedures like bone marrow biopsy is difficult to perform routinely for technical and economic reasons especially in the developing countries, peripheral blood indices, though not sensitive enough, serve as the ideal parameters for surveillance program on benzene exposure (Bogadi-Sare et al., 2003). In this context, present findings like rise in band neutrophils in circulation, increase in MCV, depletion of B lymphocytes and overexpression of P-selectin on platelet surface can be used as valuable monitoring endpoints for health surveillance of cumulative benzene exposures in the country.

Air pollution and the brain

Increased prevalence of neurobehavioral symptoms among the residents of Delhi could be the fall-out of possible neurological changes, because behavior is the outcome of multiple mechanisms within the central nervous system (CNS) and changes are sensitive indicators of nervous system dysfunction. Besides, a sharp increase has been demonstrated in plasma epinephrine and norepinephrine and depletion of dopamine and cholinesterase among the residents of Delhi, suggesting neurological alterations in these subjects. Stress is an important factor causing such changes. The changes could be partially attributed to city's air pollution, because exposure of rats to ambient particles increases the concentration of norepinehrine in brain, and corticosterone in serum (Sirivelu et al., 2006). Several other studies have

shown brain damage following sustained exposures to high level of air pollution. In a pioneering study, Calderon-Garciduenas et al., (2004) have demonstrated inflammation (elevated level of COX-2) and Alzheimer's-like pathology (accumulation of beta-amyloid) in autopsy of brain of persons who lived in cities with severe air pollution such as in Mexico City. It has been argued that particulate pollutants can act on the brain directly to stimulate the stress axis that predisposes individuals to respiratory diseases (Sirivelu et al., 2006).

It is now well recognized that air pollution can adversely affect psychological health of the people. Exposures to increased levels of some air pollutants are associated with psychiatric symptoms, including anxiety and changes in mood, cognition and behavior (Lundberg, 1996). Toxic air pollutants including carbon monoxide interfere with the development and adult functioning of the CNS causing impairment of memory, learning ability, attention and concentration (Amitai et al., 1998). It is now well established that ultrafine particles present in combustion products cross the alveolar-capillary barrier, reach the blood stream, and influence the activities of all vital organs including the CNS. Therefore, it is an interesting proposition to explore whether cumulative exposures to high background air pollution level mediate changes in CNS function that, in turn, result in neurobehavioral symptoms. It has been proposed that inflammation of the lung by UFP could lead to systemic responses (Oberdorster, 2000). Animal studies have established that pulmonary inflammation affects behavior and work performance up to 40%, and the effect starts immediately after exposure and lasts for several days (Oberdorster, 2000).

The lower level of blood cholinesterase in citizens of Delhi raises the possibility that they could be exposed to higher level of cholinesterase-inhibiting pesticides viz. organophosphates and carbamates. It is not unlikely because vegetables available in markets in North India are highly contaminated with pesticides. Besides, urban people consume lots of soft drinks that are alleged to contain high level of pesticides.

Since plasma NE is used as a measure of sympathetic activity (Landsberg and Young, 1994), stimulation of the sympathetic activity in citizens of Delhi is envisaged. But the mechanism by which both E and NE levels are increased in these subjects is currently unknown. Carbon monoxide can be excluded, as it has no significant effect on plasma CA levels (Zevin et al., 2001). Alternatively, it can be attributed to more strenuous and stressful urban life because strenuous exercise and mental stress cause significant increase in both plasma E and NE levels (Kilburn, 1994). Emotional or physical stress can increase plasma E up to 8-fold, whereas physical stress elevates plasma NE level more acutely than emotional stress (Klein, 2001). The net impact of elevated plasma CA level could be alteration of the activity of all major organ systems. CA stimulates vasoconstriction and increases heart rate and cardiac output. Cardiac stimulation increases myocardial oxygen consumption, a major factor in the pathogenesis of myocardial ischemia. Indeed, rise in plasma NE is considered as a potential cardiovascular risk (Landsberg and Young, 1994). Rise in plasma E and NE could also alter immune function by increasing the number of natural killer cells, T-suppressor/cytotoxic cells and total WBC, and by depleting T-helper cells and B cells in peripheral blood (Imrich et al., 2004). In essence, the study has demonstrated stimulation of sympathetic nervous system and increased prevalence of several neurobehavioral symptoms in residents of Delhi. Stress of urban living could have played a role in eliciting neurobehavioral problems as observed among the participants from Delhi. The consequence of these findings could be far reaching, because the changes may interfere with mental health of the people (Fiedler et al., 1996).

Adaptation to air pollution

One of the oft-repeated questions is whether people can adapt themselves with air pollution. Almost 80 years ago, Drinker (1927) observed that repeated exposures to metal such as zinc and cadmium in workplace induce a state of tolerance for subsequent exposures. Subsequent studies have confirmed the initial finding by documenting adaptation after exposure to ozone (van Bree et al., 1993; Dodge et al., 1994) and ultrafine polymer fumes. Such adaptation was age dependent, because the risk of oxidative lung injury from ultrafine carbon particles in combination with ozone was greater in aged individuals than young ones (Oberdorster, 2003). As a possible mechanism of adaptation, increase in antioxidant proteins including metallothionein, which protect the lungs from toxic effects of subsequent exposures to high concentrations of these pollutants, has been proposed (Hart et al., 1989).

In summary, high level of air pollution in Delhi was associated with higher incidence of upper and lower respiratory symptoms; greater prevalence of lung function decrement; very high alveolar macrophage count in sputum suggesting higher particle load; changes in sputum cytology indicating adverse cellular reaction of the airways; presence of iron laden AM in sputum that may indicate covert pulmonary hemorrhage; increased secretions of elastase by AM and neutrophils suggesting inflammation and damage to the bronchial and alveolar wall; alterations in lymphocyte subtype implying changes in immunity; greater prevalence of altered blood cell morphology indicating hematological changes; increased prevalence of micronucleus in buccal and airway epithelial cells and DNA damage in lymphocyte illustrating genotoxicity, and alterations in plasma cholinesterase and catecholamine levels and higher prevalence of neurobehavioral symptoms indicating neurobehavioral complications mediated by air pollution. Fortunately, most of these health impairments are reversible. Therefore, concerted efforts should be made by all concerned for reduction of particulate air pollution in Delhi in order to safeguard the physical and mental health of the citizens.

CHAPTER-11.0

SUMMARY AND RECOMMENDATIONS

11.1 SUMMARY

Air pollution from road transport has become an almost inescapable part of urban life throughout the world. Studies have revealed that exposures to high level of air pollution has adverse effects on human health like premature deaths due to diseases of the heart and lungs, aggravates preexisting cardio-pulmonary diseases, mediates development of respiratory and cardiac problems, exacerbates asthma attacks, causes suppression of body's immune defense to fight against infections, inflicts damage to the DNA that may lead to several diseases including cancer, affects child's growth and development which frequently cause reduced birth weight and congenital defects, and influences the activity of the brain which often manifests itself by neurobehavioral changes such as depression, anxiety and impaired memory. In essence, living in an environment of high air pollution for long can affect a person's physical and mental health. One of the main objectives of Air Act is to protect human health from air pollution. Keeping this in mind Central Pollution Control Board initiated a detailed study on health impact of air pollution on human health with Chittaranjan National Cancer Institute. The study was carried out through questionnaire survey and study of detailed physiological parameters. The major findings of the study are summarized as below:

11.1.1 Measurement of ambient air quality of Delhi

a. Mean concentration of total suspended particular matter (SPM) in Delhi's air during 2002-2005 was 370 µg/m3 in residential areas, 396 µg/m3 in industrial areas, and 514 µg/m3 in traffic intersection point at ITO.

b. Mean concentrations of the respirable suspended particulate matter (RSPM, particulate matter with less than 10 µm diameter, PM10) during this period were 142, 165, and 250 µg/m3 in residential, industrial, and traffic intersection point respectively.

c. Mean concentrations of sulfur dioxide (SO2) and nitrogen dioxide (NO2) in Delhi's air during 2002-2005 were 10 and 47 µg/m3 respectively. In the control areas the concentrations of SO2 and NO2 were 5.6 and 30.3 µg/m3 respectively. The levels of these two pollutants were within the Standard in Delhi as well as in control areas.

d. A small decline in the concentrations of SPM and RSPM in ambient air has been recorded in residential areas of Delhi during 2002-05.

f. The average concentration of benzo(a)pyrene in ambient air of Delhi was 3.70 ng/m3 during December 2004 and January 2005. The concentration was highest at ITO (7.31 ng/m3).

g. Benzene levels were 7.8 µg/m3 in residential areas of Delhi in 2004. Highest concentration was found in traffic intersection point at ITO.

11.1.2 Questionnaire survey for prevalence of respiratory symptoms

a. One or more respiratory symptoms were present in 33.2% residents of Delhi and 19.6% of control subjects. Respiratory symptoms were 1.7-times more prevalent in Delhi indicating breathing problem

b. Respiratory symptoms were grossly divided into two: Upper respiratory symptoms (URS), such as sinusitis, runny or stuffy nose, sneezing, sore throat and common cold with fever, and lower respiratory symptoms (LRS), such as chronic dry cough, recurrent sputum-producing cough, wheezing breath, breathlessness on exertion, and chest pain or tightness. URS was present in 21.5% residents of Delhi compared with 14.7% control subjects. Therefore, Delhiites had 1.5-times greater prevalence of URS indicating increased viral infection.

11.1.3 Assessment of lung function by spirometry

a. Lung function was reduced in 40.3% individuals of Delhi compared with 20.1% in control group. Residents of Delhi showed increased prevalence of restrictive (22.5% vs. 11.4% in control), obstructive (10.7% vs. 6.6%), as well as combined (both obstructive and restrictive) type of lung functions deficits (7.1% vs. 2.0%) indication labored air intake, labored expiration and both respectively.

b. Lung function reduction was more prevalent in women than in men both in rural and urban settings. In Delhi, 41.9% women had lung function deficits against 39.8% of men. In control group, 24.9% women had lung function deficit than 18.5% of men.

c. Chronic obstructive pulmonary disease (COPD) was detected in 3.9% residents of Delhi against 0.8% of controls indicating lung obstruction. Current smokers had significantly higher prevalence of COPD than ex-smokers and never-smokers. 3.1% of non-smokers of Delhi who participated in this study had COPD compared with only 0.2% of non-smokers with COPD in control group.

d. Nearly 10% adult individuals of Delhi were obese, against 1.2% of controls. Another 27.3% Delhiites were overweight against 7.1% of controls. Obesity in Delhi was more prevalent in women than in men (15.4% vs. 7.9% $p<0.001$).

e. Greatest prevalence of reduced lung function was recorded in obese subjects as 46.4% of obese and 43.4% of overweight citizens of Delhi had reduced lung function. Underweight individuals also had poor lung function as 44.6% of them had reduced lung function in Delhi. Overweight and obese subjects had increased prevalence of obstructive type of lung function deficits, while underweight persons had predominantly restrictive type of lung function reduction.

f. Decrement in lung function was more prevalent in North (43.8%), Central (41.1%) and West Delhi (40.9%), where the RSPM level was relative high compared with relatively cleaner East (37.0%) and South Delhi (27.4%).

11.1.4 Assessment of cellular lung reaction to Delhi's air pollution

a. A greater percentage of urban subjects produced sputum representative of lower airways compared with their rural counterparts (93.4% vs.80%). This could be attributed to higher level of air pollution in Delhi as particulate pollution increases mucus production and sputum expectoration.

b. Sputum samples from the residents of Delhi contained significantly increased number of all the cell types like the absolute number of neutrophils and lymphocytes were increased by 1.6-fold each, eosinophils number by 5.5-fold, alveolar macrophage (AM) number by 1.8-fold and epithelial cells by 1.8-fold. Sputum cytology indicates allergy and inflammatory changes in the lung among the residents of Delhi.

c. Sputum of Delhi's citizens contained 12.9±2.6 alveolar macrophage per hpf in contrast to 6.9±1.6 AM/hpf in controls, which were heavily loaded with particles indicating high particulate exposure.

d. Metaplasia (15.9% of non-smokers) and dysplasia (3.0% individuals) of airway epithelial cells were more frequent in Delhi's residents which are risk factors for cancer in the exposed tissues.

e. The citizens of Delhi also had greater prevalence of several cytological changes in sputum like presence of ciliocytophthoria (3.0% vs. 0.7% in control), aggregates of columnar epithelial cells (6.9% vs. 2.5%), koilocytes (2.7% vs. 1.1%), Charcot-Leyden crystals (0.6% vs.0%), goblet cell hyperplasia (6.4% vs. 2.0%) and mucus plugs (39.7% vs. 14.3%), spores and conidium of the

fungus Alternaria (23.1% vs. 12.3%). The findings suggest greater chances of influenza and HPV infection, injury to the airway wall, greater exposure to fungal bioaerosols and hypersecretion of mucus that may obstruct the airways.

f. The rise in AM count and percentage of individual with high AM count (≥10 AM/hpf) in Delhi significantly correlated ($p<0.001$) with the city's particulate pollution level. For instance, AM/hpf value of Central Delhi having very high PM10 level was 16.7 compared to 11.9 of South Delhi, which was less polluted.

g. The number of iron-laden macrophages (siderophages) was significantly increased in sputum of the citizens of Delhi suggesting covert pulmonary hemorrhage in the lungs.

h. A considerable rise in elastase activity in both alveolar macrophages and neutrophil was found among residents of Delhi which emphasizes greater risk of damage to the bronchial and alveolar walls that may lead to emphysema.

11.1.5 Hematological, immunological, metabolic and cardiovascular changes associated with air pollution

a. Overall, 36.1% residents of Delhi had hypertension. Thus the prevalence of hypertension was nearly 4-times higher in Delhi. Both systolic and diastolic hypertension was present in 16.7% citizens of Delhi against 2.6% of controls which is a risk factor for cardiovascular diseases.

b. Hypertension prevalence was marginally higher in men both in Delhi (36.5% vs. 34.5%) and in controls (10.1% vs. 8.3%).

c. RSPM (PM_{10}) level in ambient air was positively correlated with both systolic and diastolic blood and the risk factors for hypertension were high socio-economic status, elevated RSPM level, and obesity.

d. Systolic and diastolic blood pressure was negatively correlated with FVC, FEV_1, $FEF_{25-75\%}$, and PEFR. Therefore, rise in blood pressure appears to be risk factor for reduced lung function.

e. Greater prevalence of several hematological abnormalities like target cells, toxic granulation, anisocytosis, poikilocytosis, hypochromic RBC, immature neutrophils, metamyelocytes and giant platelets were found in the individuals from Delhi in comparison to the control population indicate altered liver function, increased bacterial infection and cardiovascular risk.

f. Platelet P-selectin was remarkably unregulated (2.8-times more activated platelets in circulation than controls) in residents of Delhi, suggesting hyper activation of circulating platelets and cardiovascular risk.

g. A significant reduction in the percentage of CD4+ T-helper cells and CD19+ B cells and concomitant increase in the percentage of CD8+ T-cytotoxic cells and CD56+ natural killer (NK) cells was found among the residents of Delhi indicating altered immunity and increased risk of infection.

h. Liver function was altered in 5.6% residents of Delhi compared with 2.4% of controls. On the other hand, kidney function was impaired in 2.6% citizens of Delhi compared with 1.2% of controls.

i. Diabetes (random blood glucose >200mg/dl) was recorded in 7.4% residents of Delhi compared with 1.9% of controls. Prevalence of diabetes in Delhi was more in men than in women (7.9% vs. 5.4%).

j. 30% of erythrocyte superoxide dismutase level, and 76% reduction in total antioxidant status were observed among the citizens of Delhi, compared with rural controls. The findings imply

down-regulation of body's antioxidant defense and consequent rise in the risk of oxidative stress-mediated cellular injury among the citizens of Delhi. The change could be attributed to city's high level of particulate air pollution, because a significant negative correlation was found between PM_{10} level and superoxide dismutase and total antioxidant status (rho values –0.257, and –0.470 respectively, $p<0.05$).

11.1.6 Biomonitoring of benzene exposure and genotoxicty

a. High concentration of t,t-MA was found among the subjects of Delhi indicating benzene exposure (326 µg/g creatinine in auto rickshaw and taxi drivers, 218 µg/g creatinine in Delhi's office employees vs 102µg/g creatinine of t,t-MA in control subjects)

b. Delhi's non-smokers had 2.3-times more micronucleus than control non-smokers (3.03 vs.1.34 MN/1000 cells, $p<0.001$), which may indicate chromosomal damage.

c. RSPM (Respirable Suspended Particulate Matter) level of the city positively correlated with MN formation in buccal (rho=0.4, $p<0.1$) as well as airway epithelial cells (rho=0.44, $p<0.05$). A strong correlation (rho=0.77, $p<0.001$) was also found between benzene level in ambient air and MN frequency in buccal and airway epithelial cells. B(a)P concentration in breathing air of Delhi also showed a significant, positive correlation but the strength of this correlation was weaker than elicited by benzene (rho=0.33 and 0.41 for buccal and airway cells, $p<0.05$).

11.1.7 Neurobehavioral symptoms

a. About 16% citizens of Delhi showed severe depression compared with 2.4% control subjects.

b. Delhi's residents had increased prevalence of other neurobehavioral symptoms like anxiety, burning sensation in extremities, inability to concentrate, transient loss of memory, and palpitation.

c. PM_{10} level was found to be positively associated with increased prevalence of transient loss of memory, burning sensation in extremities, and depression. Benzene exposure was positively associated with transient loss of memory, inability to concentrate and anxiety

d. Delhi residents had elevated epinephrine (E), norepinephrine (NE) and a decline in plasma dopamine (DA) levels in blood plasma than their rural counterparts indicating a stress effect. Elevated t,t-MA excretion was found to be associated with rise in plasma E and NE

e. The residents of Delhi had depleted level of AChE in plasma, suggesting alteration in cholinergic neurotransmission.

11.1.8 Improvement of respiratory health following intermittent exposures to cleaner air

Stay in a cleaner indoor environment for 8-10 hours a day reduces the prevalence and magnitude of health impairments associated with chronic exposures to air pollution.

11.2 SUMMARY OF THE SALIENT FINDINGS OF THE STUDY

11.2.1 Air quality and benzene exposure

Parameter	Control	Delhi	Magnitude of change
PM_{10} (µg/m^3)	82.5±14.2	136.8±16.5	1.6-fold rise
SO_2(µg/m^3)	5.6±2.2	9.4±1.3	1.6- fold rise
NO_2 (µg/m^3)	30.3±5.2	43.4±5.9	1.4- fold rise
Benzene (µg/m^3)	2.2±0.6	12.4±12.7	5.6-fold rise
t,t-MA in urine (µg/g creatinine)	102	218	2.1-fold rise

11.2 .2 Prevalence (%) of respiratory symptom complex (RSC), asthma and reduced lung function

Parameter	Control	Delhi	Magnitude of change	Significance
Total RSC	19.6	33.2	1.7- fold rise	Breathing problem
URS	14.7	21.5	1.4- fold rise	Increased viral infection
LRS	12.7	22.3	1.7- fold rise	Increased bacterial infection
Current asthma	3.9	7.6	1.9- fold rise	Hypersensitivity
Doctor-diagnosed asthma	2.1	3.6	1.7-fold rise	Hypersensitivity
Lung function reduction	20.1	40.3	2- fold rise	Short of breath
Restrictive type	11.4	22.5	1.9- fold rise	Laboured air intake
Obstructive type	6.6	1.7	1.6- fold rise	Laboured expiration
Combined type	2.0	7.1	3.5-fold rise	Laboured inspiration & expiration
COPD	0.8	3.9	4.9-fold rise	Serious lung obstruction

11.2.3 Changes in sputum cytology

Parameter	Control	Delhi	Magnitude of change	Significance
AM /hpf	6.9	12.9	1.8- fold rise	High particulate exposure
Eosinophil /hpf	0.6	3.3	5.5- fold rise	Bronchial allergy
Lymphocyte /hpf	2.9	4.7	1.6- fold rise	Viral infection
Neutrophil /hpf	29.8	48.1	1.6- fold rise	Inflammation
Metaplasia (%)	3.2	15.9	4.9- fold rise	Cancer risk
Dysplasia (%)	0.7	3.0	4.3-fold rise	Cancer risk
AM diameter (µm)	16.2	27.8	1.7- fold rise	High particulate exposure

11.2.4 Changes in sputum cytology in percentage of subjects

Parameter	Control	Delhi	Magnitude of change	Significance
Ciliocytophthoria	0.7	3.0*	4.3-fold rise	Viral infection
Epithelial cell aggregates	2.5	6.9*	2.7-fold rise	Injury to airway wall
Goblet cell hyperplasia	2.0	6.4*	3.2-fold rise	Mucus hypersecretion, obstructive lung
Mucus strands	14.3	39.7*	2.8-fold rise	Obstructive lung
Curschmann spiral	0.7	1.6	2.3-fold rise	Obstructive lung
Koilocyte	1.1	2.7*	2.4-fold rise	HPV infection
Charcot -Leyden crystal	0	0.6*	Marked rise	Respiratory allergy
Presence of Alternaria	12.3	23.1*	1.9-fold rise	Respiratory allergy

11.2.5 Alveolar macrophage activity (% +ve cells)

Parameter	Control	Delhi	Magnitude of change	Significance
Elastase-positive AM/hpf	2.9	9.4	3.2- fold rise	Emphysema
Neutrophil with high elastase activity (%)	41	64	1.6-fold rise	Emphysema
Siderophage/hpf	0.6	3.7	6.2- fold rise	Hemorrhage in lung
Golde score	12	63	5.2- fold rise	Hemorrhage in lung

11.2.6 Hematological and cardiovascular changes (% of individuals)

Parameter	Control	Delhi	Magnitude of change	Significance
'Target' RBC	3.3	11.7	3.5- fold rise	Altered liver function
Toxic granulation in PMN	15.8	34.5	2.2- fold rise	Increased bacterial infection
Giant platelet	2.6	4.8	1.8- fold rise	Cardiovascular risk
P-selectin +ve platelet (%)	1.6	3.4	2.1- fold rise	Cardiovascular risk
Hypertension	9.5	36.1	3.8-fold rise	Cardiovascular risk
Obesity	1.2	9.8	8.2-fold rise	Cardiac and pulmonary risk

11.2.7 Metabolic change

Parameter	Control	Delhi	Magnitude of change	Significance
Hyperglycemia (%)	1.9	7.4	3.9- fold rise	Diabetes
Raised serum ALT, AST (%)	1.2	4.6	3.8- fold rise	Change in liver function
Raised serum urea, creatinine (%)	1.2	2.6	2.1- fold rise	Change in kidney function
Superoxide dismutase (U/ml)	255.3	179.4	1.4- fold decline	Depleted antioxidant status, cell injury
Total antioxidant (mmol/L)	1.93	0.46	4.2- fold decline	Depleted antioxidant status, cell injury

11.2.8 Immunotoxicity

Parameter	Control	Delhi	Magnitude of change	Significance
CD4+ T cell /µl of blood	939	795	1.2- fold decline	Altered immunity and increased risk of infection
CD8+ T cell /µl	562	719	1.3- fold rise	do
CD19+ B cell /µl	456	329	1.4- fold decline	do
CD56+ NK cell /µl	243	475	1.9- fold rise	do

11.2.9 Genotoxicity

Parameter	Control	Delhi	Magnitude of change	Significance
MN / 1000 buccal epithelial cells	1.15	2.46	2.1- fold rise	Chromosome breakage
MN / 1000 airway epithelial cells	1.35	2.92	2.1- fold rise	Chromosome breakage
Comet-positive lymphocyte (%)	18.5	33.8	1.8- fold rise	DNA damage
Tail length of Comet µm)	1.7	2.8	1.6- fold rise	Greater magnitude of DNA damage

11.2.10 Neurotoxicity

Parameter	Control	Delhi	Magnitude of change	Significance
Norepinephrine (nmol/l)	2.34	3.71	1.6- fold rise	Stress effect
Epinephrine (nmol/l)	0.31	0.51	1.6- fold rise	-do-
Dopamine (nmol/l)	2.73	2.13	1.3- fold decline	-do-
Cholinesterase (U/l)	6325.1	3603	1.7- fold decline	Alteration in cholinergic neurotransmission
Depression (% of individuals)	35.4	69.0	1.9- fold rise	Depression

11.3 RECOMMENDATIONS

11.3.1 Air quality monitoring

a. There has been a rapid expansion of Delhi in recent times. Therefore the number of air quality monitoring stations should be increased from the present 10 to keep pace with city's growth. More stations are needed particularly in hot spots where the vehicular and industrial emissions are high. Similarly, monitoring stations at areas with low background air pollution level such as in the extreme south of the city may be considered.

b. Besides increasing the number of monitoring stations, the performance of the existing stations should be overhauled and streamlined ensuring strict quality control. It should be ensured that all the existing stations of CPCB and NEERI are operational for sampling for a mandatory minimum period of 104 days/ year. The collected air quality data should be comprehensively and statistically analyzed to get an insight into temporal and spatial trends.

c. Urban air pollution causes five times as many deaths and illnesses as malaria and is almost the largest contributor of regional burden of diseases in South Asia. Information collected till date suggests that the main air pollutant of concern in relation to public health in India is the particulate matter. More recent epidemiological studies have identified that PM_{10} and $PM_{2.5}$ are the principal mediators of health effects of air pollution. There is a general consensus today of mitigation measures targeted on fine particles ($PM_{2.5}$), the most serious pollutant in relation to health in South Asia. Therefore, a review is needed as to which pollutant should be monitored at a regular basis. Monitoring of $PM_{2.5}$ at regular basis is recommended. To save cost, time and energy, monitoring of TSP may be discontinued instead.

d. Source apportionment of airborne pollutants is weak in India. For example, re-suspension of dust may contribute significantly but it is rarely quantified. Current results show that there are a number of sources, rather than a single dominant source, for $PM_{2.5.}$ Contribution of different sources in relation to season may vary across the cities. Emphasis should be given on these aspects. Similarly there is no reliable emission inventory for PM_{10}, and when available, they often differed considerably for the same city. Efforts should be made to bridge the gaps in our understanding in these respects.

e. Till date, attention has been focused almost entirely on controlling emissions from road traffic. Obviously, this is important. But the contributions of other significant sources of pollution, for example, small stationary sources like refuse burning, emissions from small-scale industries, and household use of biomass are not known with any degree of certainty. Efforts should be made at all levels to control these sources through an effective urban air quality management strategy.

f. Ozone is one of the most harmful pollutants. It is not directly emitted but it is formed in complex photochemistry of oxides of nitrogen and hydrocarbons in the atmosphere. Only a limited amount of information is available for photochemical air pollution in Indian cities. Regular monitoring of ozone is recommended in other cities. Meteorological variables like wind speed should be taken into account during monitoring days. Besides, ozone levels have diurnal variation; therefore it should be measured at different times of the day to get an overall picture.

g. Volatile organic compounds such as benzene, and polycyclic aromatic hydrocarbons such as benz(a) pyrene are potential cancer-causing agents present in ambient air and their main source is vehicular exhaust, which is the principle contributor of urban air pollution in India. Considering their health impact coupled with absence of a threshold level for their carcinogenicity, these two compounds should be monitored regularly in order to minimize their emissions to protect public health.

h. Recent published works have identified transition metals adsorbed on particulates as the main source of oxidative stress that mediate most of the health effects of air pollution. Therefore, regular monitoring of iron, copper, nickel, chromium and manganese is recommended in urban air. In addition, special emphasis should be given on the concentration of heavy metals particularly lead for its neurotoxicity.

i. Estimation of various aeroallergens including pollen and fungal spores and their relationship with asthma and other forms of bronchial allergy should be undertaken in the city at regular intervals.

j. Since people on average spend two third of their daily time indoor, indoor air quality has profound effect on human health. In fact 'sick building syndrome' is a growing concern worldwide. Besides smoking, indoor air quality in Indian households is affected by emissions from burning biomass and kerosene during cooking, emissions from mosquito-repellants, from molds, and from cooking oil vapors during cooking at high temperatures. Reports are scanty on air pollution level in indoor air and contribution of each potential source in Indian households. Therefore indoor air quality needs to be monitored periodically.

11.3.2 Reduction of vehicular emissions

a. The use of CNG and LPG in all classes of vehicles, both private and public, should be encouraged in Delhi and in other cities with high level of vehicular pollution.

b. The authority should explore the avenues of improvement in quality of automotive fuels, lubricating oils, and vehicle types.

c. The use of cetane enhancer and detergent additive in diesel fuel may be encouraged to combat the build-up of detrimental deposits of fuel injectors that is expected to reduce emissions and increase fuel economy and extended component life.

11.3.3 Health impact

a. An important lacuna is the absence of facilities for regular monitoring of public health in relation to air pollution exposures despite the fact that protection of public health is the ultimate goal of air quality monitoring. Regular monitoring of public health may be carried out.

b. The concentrations of air-borne pollutants in different parts of India have been measured by various organizations but in most cases these studies were not linked to health of the exposed subjects. How air pollution affects public health and which fraction or component need to be controlled is important for reduction of air pollution in order to safeguard health of the citizens. Unfortunately, data on health effects of air pollution from Indian cities is scanty, and investigation on the health effects of chronic, long-term exposures to ambient air pollution with special reference to particulate matter is almost lacking. More epidemiological studies on health impact

of air pollution in urban as well as rural India with special reference to chronic exposures may be undertaken. In this context, it is recommended that the epidemiological study may be conducted at regular intervals to detect and analyze the health effects of air pollution.

c. Recent studies in Europe and the United States have emphasized the need to explore the effect of ambient air pollution on the cardiovascular system because most people die due to air pollution exposures from diseases of the heart. Unfortunately, very little is known about this problem in India. Therefore, efforts need to be made to investigate the effects of chronic air pollution exposures on the cardiovascular system of the Indian population.

d. Biological markers of pollution exposure and effect are now readily available. Notable examples are alveolar macrophage response and sputum cytology for respiratory toxicity, platelet activity, fibrinogen and C-reactive protein for cardiovascular effects, urinary excretion of trans,trans-muconic acid for benzene exposure, and micronucleus assay for genotoxicity. It is recommended that these biomarkers in air pollution-related epidemiological studies in India.

11.3.4 Public education and awareness

a. Mass awareness campaigns involving local bodies, voluntary organizations, students, trade unions and others may be initiated educating people about the health impact of air pollution. Moreover, air quality management and air pollution mitigation measures taken up by the government at the state or central level may be widely promoted through educational and information programmmes.

b. Environmental Health subject may be introduced in medical education syllabus in India.

c. Mass transportation should be strengthened in Delhi. Introduction of metro railway service is a right step in the right direction. On the other hand, people should be advised to maintain their vehicles properly. At the same time they should be encouraged to walk or use bicycles for traveling short distances, and to share vehicles for long distances.

d. In many cases pollution victims are not beneficiary of the polluting facility, such as a pedestrian is affected by the exhaust fumes of a moving car. 'The polluter should pay' philosophy should be introduced in India as well.

e. Consumer awareness should be promoted about air quality benefits by opting to eco-friendly products such as use of water-based acrylic paints that contains less VOCs.

d. From the economic point of view, the total cost of control measures should be measured in terms of total benefits to the society. The monetary benefits of reducing illness and premature mortality associated with a small change in air pollution exposure is important to estimate the value of unit reduction in each pollutant that can serve as an input for a cost-benefit analysis of air pollution mitigation programs. Besides, it helps in calculating the relative benefits from controlling one pollutant versus another.

11.3.5 Better air quality management

a. The state and central pollution control boards may continue to collaborate in the ongoing development and implementation of monitoring, data analysis, modeling, prediction and reporting, R&D, and health impact studies. Whenever necessary, they may continue to invite expertise from

the universities, basic and biomedical research institutes, non-government organizations and private consultants. For data interpretation, modeling and prediction, GIS based methodologies may be adopted.

b. Personal exposure estimates, which are most important for evaluation of health impact of air pollution, are almost non-existent in India. The monitoring agencies should consider this aspect. Admittedly, it is a tough proposition to implement. But someone should start it somewhere, because it is necessary.

c. In many cities hot spots are highly influenced by surrounding buildings. Furthermore, due to lack of space, many new developments of infrastructure and housing need to be realized in very small areas, with high risk of creating new hot spots of air pollution. An improvement in our understanding regarding the effect of buildings on hot spot concentrations is needed.

d. For integrating local policies regarding air, water, soil and waste, cities require information on how pollutants travel between environmental media. More knowledge about this would be beneficial directly at the local level and indirectly by making it more feasible to develop consistent regional legislation among the South Asian countries.

CHAPTER-12.0

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CHAPTER-13.0

GLOSSARY

Abatement	A process to reduce, remove, or discontinue a nuisance disease vector, or pollutant
Acute	Short and severe
Acute illness	A brief but serious episode of illness
Adverse reaction	Undesirable or unwanted side effect as a consequence of a preventive, diagnostic, or therapeutic procedure
Aeroallergens	Airborne antigens like pollen, house dust, mite etc. that cause allergic response in sensitive individuals
Aerosol	Atomized particles suspended in the air that are small having diameters ranging from 1/100 µm to 1µm
Air	The gaseous mixture, which makes up the atmosphere surrounding the earth. It consists of approximately 78% nitrogen, 20% oxygen, 0.04% carbon dioxide and traces of ozone, neon, helium etc., and a variable amount of water vapour
Air pollution	Deterioration of the quality of air for the presence of impurities
Air quality standards	Maximum allowable concentration of air pollutants. Concentrations that exceed these limits are considered harmful Primary standards are those needed to protect public health. Secondary standards protect against other effects such as damage to materials.
Allergen	Substance or agent that causes allergy
Alveolus	The terminal sac like structures of the lung, which provide vast surface area for gaseous exchange. A large number of blood vessels surround them
Allergy	An altered or exaggerated susceptibility to various foreign substances which are harmless to great majority of individuals. Asthma is an allergic condition
Alveolar macrophages	A type of defense cell present inside the lungs. The cells readily engulf inhaled particles and microorganisms
Ambient	Surrounding conditions of the environment, usually referring to air quality and pollutant levels
Ambient level	The level of pollutant in the general ground level atmosphere, i.e. to which people may be exposed
Antibody	Specific proteins produced in the blood as a reaction to foreign substance. Antibodies bind to a specific antigen that elicits its production causing its destruction. Thus antibodies provide protection against infectious disease
Antigen	A substance (protein, polysaccharide, glycolipid, tissue transplant, etc.) that is capable of inducing antibody production. Antibodies are formed against an antigen and are capable of binding to it

Aplastic anemia	A type of anemia caused by the failure of the bone marrow to generate new blood cells of all types.
Asthma	Intermittent narrowing of the airways causing shortness of breath and wheezing. Usually the muscle of the bronchi walls contract and excess mucus collects restricting flow of air
Bacteria	A group of micro-organisms some of which are pathogenic to man
Benign	Innocent. A term used to denote opposite of malignant
B- lymphocyte	A subpopulation of lymphocytes, which produces antibodies
Bilirubin	A pigment derived from the breakdown of hemoglobin from red blood cells
Biomarker	Observable endpoints indicating the process leading to the genesis of a disease.
Biomarker of exposure	Measure of a parent compound or the unique response attributable to that compound in biological samples following exposure
Biomarker of effect	Quantifiable response of an organism that can directly be linked to the exposure
Biomarker of susceptibility	The measure of the capability of an individual to respond to exposure of an environmental toxicant
Bronchiotasis	Abnormal widening of the larger airways in the lung (bronchi) causing persistent cough with large amount of sputum
Bronchi	The two tubes into which trachea divides at its lower end
Bronchitis	Inflammation of the bronchus may be acute or chronic
Cilia	Fine hair like strands. Cilia line the airways and trap foreign particles as the air pass through them
Carcinogen	A cancer causing substance
Carcinogenesis	The production of cancer or malignant cells
Cancer	Abnormal, unorganized and unregulated growth of cells in an organism
Cardiovascular disease	One or all of the diseases of the heart and blood vessels
Cardiac	Pertaining to the heart
Case-Control Study	A study of persons with a particular disease in comparison to a reference or control group, which is otherwise similar to the study group
Cell	The building blocks of all living organism.
Cellular immune response	Type of immune response characterized by generation of cytotoxic T- cells.
Cholesterol	A fatty material found in tissues and blood

Chromosome	Thread-like bodies found in cell nucleus which carry hereditary factors, the genes. The number being constant in each species e.g. in man 46 in each cell except in mature ovum and sperm where the number is 23
Chronic	Lingering, lasting, opposed to acute
Chronic Disease	An adverse health condition lasting for 3 months or more
Confidence Interval (CI)	The calculated range of numbers in a data set which, with a specified degree of probability (e.g., 95%), includes the true values of variables such as the mean, proportion, or rate of that set of numbers. The upper and the lower boundaries of the confidence interval are called confidence limits.
Confounding Variable (Confounder)	A factor or variable that can potentially cause or prevent the outcome or disease being studied (the dependent variable), and must be taken into account or it will prevent reaching a conclusion regarding the impact of independent variable, or hypothesized cause of the outcome disease being investigated.
Chronic obstructive pulmonary disease	Progressive damage of the lungs usually leading to shortness of breath and wheezing. (often mean chronic bronchitis and emphysema)
Cost-Benefit Analysis	A system of analysis that attempts to weigh the cost of some policy such as pollution control, directly against the economic gain.
Cost-Effectiveness Analysis	A form of economic evaluation where all costs are expressed in monetary terms, but the consequences are expressed in non-monetary terms, such as life years gained, cases detected, or cases prevented.
Cough	A reflex in response to irritation or infection in the respiratory tract, which helps to clear irritant or blockages from the airways.
Cross-sectional study	A study of the relationship between diseases with other variables of interest in a defined population at a particular point of time. This establishes the disease prevalence and the presence of the characteristic being studied (e.g., smoking or toxic exposure) in the diseased compared to the non-diseased person in the group.
Cytology	Microscopic study of the cells
Dependent Variable	An outcome or manifestation we seek to account for by the influence of an independent variable(s) or intermediate factor(s) in a hypothesized relationship being studied
Developed and Developing Countries	Countries are defined as to their level of development by per capita GNP by the World Bank. Those with per capita GNP of $765 or less in the year 1998 are defined as low income economies or least developed countries. Those between $765 – 3,035 and $ 3,035 – 9,386 are considered lower and upper income developing countries respectively. Countries with per capita GNP of $ 9,386 or more are defined as developed (high income economies)
Disease	Disease is physiological and psychological dysfunction

DNA	Deoxyribonucleic acid. It is present in cell nucleus. Mitochondria also contains some DNA. It carries genetic information from parents to their progeny and therefore accounts for the continuity of species
Dyspnea	Difficult or labored breathing
Elastase	A connective tissue degrading enzyme
Emission standards	Legal limits of the quantities of air pollutants permitted to be emitted from the exhaust of a source
Emphysema	Alveolar distension often accompanying chronic bronchitis. A common problem encountered in smokers
Environment	All factors external to the individual that may affect its health behavior or well-being. Includes physical, biological, social economic and other factors
Enzyme	Protein molecule which acts as a catalyst in the chemical reactions within the body
Eosinophil	A type of white blood cell containing granules staining in acid dyes such as eosin. They form 2-5% of white blood cells in man, but increased manifold in allergy and parasitic infections
Epidemiology	The study of the distribution and determination of health and its disorders
Epithelium	The surface layer of cells below which lies the basement membrane
Erythrocyte	Hemoglobin-containing red cells present in blood
Etiology	The origin or causes of a disease, health condition or risk factors
Exfoliation	The scaling off of tissues in layers
Expiration	The act of breathing out air from the lungs
Exposure	Quantity and duration of contact between a person and a harmful agent. Exposure may be continuous, periodic or episodic
Extrapolation	The prediction of points on a graph outside the range of observation
FEV_1	Forced expiratory volume in one second. The volume of air that can be expired in one second
Fibrosis	The formation of excessive fibrous tissue in a structure
Fungus	Plants including microscopic organisms capable of producing disease in man.
FVC	Forced vital capacity. The volume of air expired forcefully expiration following maximum inspiration
Gene	The basic unit of heredity composed of molecules of deoxyribonucleic acid (DNA) and located on the chromosomes of each cell. Genes govern every single structural and functional characteristic of an individual
Gland	An organ or structure capable of making internal or external secretions

Goblet cells	Secretory cells, shaped like a goblet, found in the mucous membrane
Granulocyte	Any cell containing granules, e.g. neutrophils, eosinophils and basophils
Green house effect	The effect produced by certain gases such as carbon dioxide or water vapor that causes warming of the earth's atmosphere by absorption of infrared radiation.
Health	WHO states "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity"
Hemoglobin	The respiratory pigment in red blood cells. It is composed of iron containing substance "heme" combined with protein globin.
Hemorrhage	Escape of blood from a vessel.
Hemosiderin	A form of storage iron in tissue.
Hygiene	The science dealing with the maintenance of health.
Hyperactivity	Excessive activity
Hyperplasia	Excessive formation of cells
Hypersensitivity	Type of immune response, which has an adverse impact. Also known as allergy
Immune response	Reaction of the body's defense system to insults by foreign substances Lymphocytes, macrophages, neutrophils, eosinophils and basophils play important role in mediating immune response
Immune system	A network of organs and cells responsible for generating immune response
Immunity	Individual's resistance to infection. Resistance to a disease by the host may be natural, passive or acquired
Infection	Invasion of the body by disease-causing organism, with or without manifestation of the disease.
Inhalation	The breathing in of air or other vapor
Inhalable particles	Particles which may be breathe in. "Inhalability" is defined technically as the orientation – averaged aspiration efficiency for the human head (also termed inspirable)
Inspiration	Drawing of air into the lungs
Inflammation	The reaction of living tissue to injury, infection or irritation, characterized by pain, swelling , redness and heat
Intracellular	Within the cell, opposite to extracellular
Leucocytes	White blood cells engaged in body's defense against infection
Leukemia	Cancer in bone marrow cells

Lipid	Fat
Lungs	The two main organs of respiration which occupy the greater portion of the thoracic cavity
Lung function test	A test used to detect airflow problems in the lungs
Lymphocytes	A kind of white blood cells that protect the body against diseases by direct killing or by producing antibodies
Macrophages	Phagocytic cells widely distributed in the body. Plays important role in body's defense, disposal of debris, and repair of the injured tissues
Metaplasia	A reversible cellular change in which one cell type is replaced by another adult cell type
Mucus	A clear, sticky lubricant secreted by glands in the mucus membranes that line the body cavity
Mutagen	An agent that produces mutation, ie. genetic change
Mutation	A change or alteration in the genes of a living cell. As a result the characters of the cell change
Mortality	The death rate; the ratio of the total number of deaths to the total population
Mucosa	A mucous membrane
Natural killer cells (NK)	Type of leukocytes that preferentially inactivate virus-infected or cancerous cells
Neutrophils	Granular white blood cells with the properties of chemotaxis, adherence to immune complexes and phagocytosis: also produce antimicrobial substances including oxidants and proteases
Particulate matter	Particles of solid or liquid matter in the air, including nontoxic materials (soot, dust, dirt), heavy metals (e.g., lead), and toxic materials (as asbestos, suspended sulfates, nitrates)
Pathogen	An organism, toxin, or other agent capable of causing human, animal, or plant disease
Photochemical smog	Smog caused by the formation of particles due to a chemical reaction driven by sunlight
PM_{10}	Particulate matter less than 10 μ m aerodynamic diameter
Pollutant	Any substance that renders the atmosphere or water foul or noxious or a health hazard.
Pollution	The impairment of the quality of some portion of the environment by the addition of harmful impurities
ppb	Parts per billion, 1 part by volume in 10^9

ppm	Parts per million, 1 part by volume in 106
Prevalence (Rate)	The total number of all individuals who have a disease including new and previous cases in a given population at a designated time (point prevalence) or time period (period prevalence), expressed usually as a rate per 1000 persons during a year
Relative Risk	The relation of risk of disease or death among the exposed, as compared to that of an otherwise comparable nonexposed population group
Respirable particles	Particles which can penetrate to the unciliated regions of the lung Those characteristics or behavioral pattern known to increase the risk of disease.
Risk factors	Those characteristics or behavioral patterns known to increase the risk of disease
Sampling	A sample is a subset of population selected to be as representative as possible of the total population. A sample may be random or non-random, representative or non-representative. The major categories include: cluster sample, a group of persons not individually selected, i.e., all person in city block; grab sample, A simple survey among people who happen by or show up at a service offered, such as a street fair, from which no general conclusions can be drawn; probability (random) sample, where all individuals have an equal or known chance of being selected, or if stratified, subgroups may be assigned greater weight in the design; simple random sample, All person in the group are assigned a number, and the selection of the sample is according to a random numbers table, until the needed sample size is achieved; stratified random sample, where the population is divide into subgroups, and each of these is sampled randomly; and systematic sample, where the sample is selected on the basis of a predetermined method, such as alphabetic order or birth dates
Secondary particle or aerosol	Particles may be formed when two volatile and non-condensable vapor species react to give rise to a product with a very low vapor pressure. Such a product is described as a secondary particle to distinguish it from those arising from the reaction of liquids or solids
Sinusitis	Inflammation of any of the sinuses leading to headache and tenderness in the face
Spirometer	An instrument used to measure the volume (in litres) that one can inhale or exhale over a period of time .The results can indicate whether the airways are narrowed due to lung disease
Smog	A term often used to describe a mixture of smog and fog. Also used to describe photochemical air pollution, or smoky fog the word is used loosely to describe visible air pollution
Smog precursor	An air pollutant that can undergo chemical reaction in the presence of sunlight

Smoke	Particulate matter, <15μ m diameter, derived from the incomplete combustion fuels. Or an aerosol that is usually produced by combustion or decomposition process
Stress	Response to challenge (" fright- fight- flight") of changes in the status quo of a individual causing overt or hidden psychological pressure that may manifest themselves in overt psychological symptomatology or in physical illness
Suppressor T- cells (Ts)	A subpopulation of T- lymphocytes that suppresses immune response
Susceptible	A person with insufficient resistance or with associated risk factors to a particular pathogenic agent or process, so that there is real danger to this person contracting the specific disease if or when exposed to the agent
Symptom	An organic or physiologic manifestation of a disease of which the patient is usually aware and complains of it
Synergism	A condition in which a whole effect is greater than the sum of its parts
Target cells	Abnormally flat red cells with a central mass of hemoglobin surrounded by a ring of pallor and an outer ring of hemoglobin. They are commonly associated with liver disease, impaired or absent splenic function (hyposplenism) and hemoglobinopathies
T- Lymphocytes	A subpopulation of lymphocytes that matures under the influence of thymus
Threshold level	The minimal dose of a toxic substance that causes harmful effects.
Total suspended particulate matter	A term describing the gravimetrically determined mass loading of air borne particles, most commonly associated with use of the US high volume air sampler in which particles are collected to filter for weighing
Toxic substance	Any substance whose physiological action is harmful to health
Tuberculosis	A bacterial infection (Mycobacterium tuberculosis) that most often affects the lungs. Usually transmitted by airborne droplets
Urbanization	A demographic process characterized by movement of people from rural to urban settlements
Ultra fine particles	Particles of less than 100nm diameter
Virus	Essentially a capsule of protein that contains either DNA or RNA as genetic material. There is debate as to if it is a living organism or a chemical entity. However, it has powers of reproducing when it invades a living cell