CHAPTER -2

REVIEW OF LITERATURE

2.0 DEFINITION

There used to be and still is a great deal of confusion in the use of the terms chronic bronchitis, emphysema and chronic obstructive disease. The clinical term "emphysema" in USA was equivalent to chronic bronchitis in Great Britain. To add further to the confusion, it was suggested in 1961 that bronchial asthma, chronic bronchitis, and emphysema should be considered as different expression of one disease and the term "chronic non-specific lung disease (CNLD) " should be used for this. Both endogenous (host) and exogenous (environment) factors were thought to play a role (Dutch Hypothesis) (Behera, 2010). A hereditary predisposition to develop allergy and bronchial hypereactivity were considered to be important denominators of disease susceptibility. Although there are still proponents of this hypothesis, over the years with better understanding of the pathophysiologic processes of these diseases, it is clear that these entities are distinct even if they have common features like airflow obstruction, and similar manifestations like cough, wheezing and dyspnea, etc. However, sometimes in a few minorities of patients, it may not be possible to distinguish one from the other and they may co-exist (Ciba Guest Symposim Report, 1959; Aspen conference Report, 1959; Medical Research Council, 1965; Snider *et al*., 1985; American Thoracic Society, 1987; Snider *et al*., 1995).COPD (Chronic Obstructive Pulmonary Disease) is an umbrella term used to describe lung disease associated with airflow obstruction. Most generally, emphysema, and chronic bronchitis, either alone or combined, fall into this category. There is continuing debate as to whether this term also includes asthma. However, as a general rule, it is not included. Although it does have obstructive components, it is in part reversible and is more generally considered a restrictive lung disease (Behera, 2010). Many with emphysema and chronic bronchitis also have asthma or an asthmatic component to their illness as well.

2.1 ETIOLOGY

2.1.1 Smoking

Various retrospective and prospective studies have proved unequivocally that chronic bronchitis and emphysema are closely to smoking and smoking is the most important factor in the etiology of COPD (Crofton, 1989; Higgins *et al.,* 1990; USDHHS, 1984a; Sherril *et al*., 1990; Davis *et al.,* 1989).

One of the major risk factors for developing COPD is cigarette smoking (NIH Bethesda, 2001). The benefits of smoking cessation seen in the improvement in pulmonary function test results have been well documented (NIH Bethesda, 2001; Murray *et al.*, 1998) although little is known about the effects of smoking cessation on the changes in the inflammatory response in the lung. The major importance of COPD as a component of chronic morbidity has been recognized in the UK as early as the early twentieth century. Its relationship with atmospheric pollution and dust was also realized since that time. Numerous surveys have shown that much higher rates of cough and sputum in smokers than in nonsmokers with the incidence being increased with the degree of smoking. Recent studies have shown that smoking lower tar cigarettes does not necessarily reduce the prevalence of cough and sputum. However, smoking cessation usually leads to decreased symptoms and the number of exacerbations and dyspnea, at least in the early stages of the disease. The most important evidence of association of smoking with that of chronic bronchitis was first evident from the classy study by and Peto (Doll *et al.*, 1976) and (Doll *et al.*,1980). The risk of morbidity and mortality increases with the amount smoked. Heavy smokers have 2-25 times greater mortality than that in nonsmokers. Those who inhale deeply have a higher mortality. The incidence decreases after smoking early in the course of the disease. Although earlier studies were in men, subsequent studies showed that susceptibility is the same in either sex, if they smoke. A high incidence of chronic bronchitis has been reported from India in women smokers, which varied between 33 to 52 %. Both bidi and cigarette as well as reverse smoking of homemade “chuttas” are associated with the development of chronic bronchitis (Malik, 1977a; Malik 1977b; Behera *et al.*, 1984).The effects of smoking on the airways are the best and most documented in medical literature. There is an inverse relationship between cigarette consumption and expiratory flow rates. However, a significant number of patients, in spite of being active smokers show no lung function abnormalities. In the patients who smoke and are susceptible to the effect of smoke, cessation of smoking slows down the speed of pulmonary function deteriorations to that of non-smokers, but do not normalize the lung function. A prospective study of early stages of the development of COPD in London working men showed that Forced Expiratory Volume in one Second (FEV_1) falls gradually over a life time, but in many smokers clinically significant airflow obstruction never develops (Fletcher *et al.*, 1976a).In susceptible people, however, smoking causes irreversible obstructive changes. If a susceptible smoker stops smoking, he will not recover his lung function, but the average further

rates of loss of FEV_1 will revert to normal. Therefore severe, and fatal obstructive lung disease can be prevented by screening smokers lung function in early middle age if those with reduced lung function can stop smoking. Inflammatory process and chronic mucus hypersecretion do not cause chronic airflow obstruction to progress more rapidly.

2.1.2 Passive Smoking

It is increasingly being believed that passive smoking, also known as environmental tobacco smoke (ETS), or second hand smoke, is related to various respiratory disorders. The 1984 report of the Surgeon General of USA had shown a significant correlation between parental smoking and bronchitis or pneumonia in young children (USDHHS, 1984a). In older children the data is conflicting. A number of other studies have shown a small but significant decrease of lung function in children of smoking mothers. Smoking in the household has been found to be strongly associated with continuing asthma symptoms in young children. Continuing symptoms more than 3 years after an attack of acute bronchiolitis in children is significantly associated with maternal smoking. Some, but not all studies have demonstrated minor changes in adults. The effect of cigarette smoke may begin at an early age. There is an increase in respiratory illness and diminished lung function in children passively exposed to parental smoking (Tager *et al.*, 1983; Ware *et al.*,1984;Charlton, 1984).

Even infants of smoking parents have more respiratory illness than infants of nonsmokers (Collery *et al.*, 1976) and pneumonia at that age may predispose to chronic bronchitis in later life. The significance of these findings for the later development of COPD is uncertain at this moment. In the absence of smoking in adult life the effects of passive smoking may not be very important.

2.1.3 Alpha-1-Antitrypsin Deficiency

One of the most significant breakthroughs in the field of COPD in the past 30 years was the discovery of a close association between an inherited deficiency of a protein in the blood called the α1- antitrypsin (AAT). It is the only known genetic disorder that leads to COPD. AAT deficiency accounts for less than 1 percent of COPD in USA (ATS, 1995a). This deficiency is an autosomal hereditary disorder in which there is low level of α_1-antitrypsin in serum and lung, with a high risk of development of panlobular emphysema in the third to fifth decade. There is an

increased risk of development of liver disease in the childhood associated with this condition. The enzyme is synthesized and secreted by the hepatocytes and to a lesser extent by the mononuclear phagocytes , and then is released in to the blood from which it then diffuses in to the lungs (Crystal *et al*.,1990).AAT is a glycoprotein coded for by a single gene on chromosome 14 . It is a serine protease inhibitor with primary function of inhibiting neutrophil elastase. Emphysema results from an imbalance between the neutrophil elastase in the lung and the anti-elastase while the former has the capability of destroying elastin and other tissue components, the later is responsible for protecting the lung from elastase. This concept is known as the "elastase–anti elastase balance hypothesis of emphysema." The concept has been proved both in humans and animal experiments. According to the theory, either an excess of protease or a deficiency in the amount of functional activity of anti-protease (or both) can lead to the development of emphysema.

At least 75 alleles of the α_1-antitrypsin gene have been identified (Guidelines for the approach to the patient with severe hereditary Alpha-1-Antitrypsin deficiency, 1989) and categorized in to the protease inhibitor (Pi) system. These alleles can be categorized into four groups according to the α_1-antitrypsin levels in the serum (Brantly *et al.*,1988). They are:

1. Normal: This is associated with normal serum levels of α_1-antitrypsin with normal function.
2. Deficient: This is associated with serum α_1-antitrypsin levels < 35 percent of average normal levels.
3. Null: In this type there is no detectable α_1-antitrypsin protein in serum.
4. Dysfunctional: In this type α_1-antitrypsin is present but does not function normally.

The normal and deficient α_1-antitrypsin alleles are designed from A to Z on the basis of their electrophoretic mobility. The family of normal α_1-antitrypsin alleles is referred to as M (M1, M2, and M3) and are found in approximately 90 percent of the population. The most deficiency allele associated with emphysema is the Z allele .The α_1-antitrypsin phenotype, therefore, is made up of the two parental alleles and is referred to as Pi phenotype. α_1-antitrypsin variants are inherited as co dominant alleles. The most common phenotype is PiMM (PiM2M3) and the most common deficient phenotype associated with a high risk for the disease is PiZZ. The

specific mutations responsible for many forms of α_1-antitrypsin deficiency have been identified. The abnormal Z allele is associated with replacement glutamic acid by lysine at position 342 as a result of a single base mutation from GAG to AAG. This substitution results in alteration of the three dimensional configuration of the molecule. Thus, it aggregates in the rough endoplasmic reticulum of the hepatocyte and consequently a decreased secretion of AAT occurs from the liver to about 15 percent of the normal (Weinberger, 1993; Crystal *et al.*., 1989).The normal values of serum AAT are 150 to 350 mg / dl or 20 to 48μM. Levels of 80 mg /dl(11 μM) have been considered to be the threshold serum level above which the quantity is sufficient to protect the lung and below which the individual has an increased chance of emphysema compared with the general population . PiZZ homozygotes have levels of 2.5 to 7 μM (mean ,16% of normal), and PiSS homozygotes have levels of 13 to 33 μM (mean ,52% of normal).In contrast , individuals who are heterozygous have reduced concentrations with the extent of reduction depending on the phenotype. Thus PiSZ will have values of 8 to 19 μM (mean, 37% of normal) and PiMZ will have values of 12 to 35 μM (mean, 57% of normal). They do not appear to be at increased risk for COPD, in family studies and in surveys in some population of COPD patients ,however there is an increased frequency of heterozygotes (Feld ,1989). There is some evidence that MS heterozygotes may have an increased frequency of non specific airways hyperactivity (Townley *et al.*, 1990).Subjects with Pi null phenotype ,by definition ,have serum values of 0. The deficient null alleles are very rare, together representing less than 1 percent of all α_1-antitrypsin alleles. They are at a very high risk of developing emphysema since there is no α_1-antitrypsin to protect their lungs. Epidemiological studies indicate that a threshold value of 11 αM or about 35 percent of the average normal level, is sufficient to protect the lungs. It follows therefore, that individuals who are at greater risk are PiZZ homozygotes, the null homozygotes, and, occasionally PiSZ heterozygotes .PiSS and other heterozygotes like PiMZ , and PiMS ,do not appear to be at increased risk. Severe AAT deficiency lead to premature emphysema of the panacinar type with more severe affection at the bases, and is often associated with chronic bronchitis and occasionally with bronchiectasis (Snider *et al*., 1989). Individuals with a PiZZ phenotype who smoke cigarettes are at increased risk, become symptomatic earlier with dyspnea occurring at a median age of about 40 years .The same will be about 53 years in cases of nonsmokers. Smokers with AAT deficiency and COPD will have a life expectancy that is approximately 10 years less than the nonsmokers with

this condition. The rate of decline of FEV_1 is also more in them with a decline in excess of 100 ml / year. Severity of lung disease varies considerably. Patients who are detected on population surveys only live longer to the age of 80 or 90 years. Airflow obstruction occurs more in men. Other risk factors are asthma, recurrent respiratory infections and familial factors (Silverman *et al.*,1989).Liver disease associated with α_1-antitrypsin deficiency is less common than emphysema and occurs in less than 10 percent of the PiZZ phenotypes, the most common manifestation in neonatal hepatitis. Other presentations include unexplained chronic liver disease in older children, and cirrhosis and hepatoma in adults (Erikson *et al.*,1986). Many Indian studies have tried to examine the role of alpha -1-antitrypsin deficiency in the causation of COPD and is summarized by Malik *et al.* (1977c).The heterozygote state (intermediate) was found to be 10.3 to 23.3 percent and homozygous (severe) state in 2.8 to 20 percent of cases of COPD.

2.1.4 Air Pollution

The interrelationship of COPD and pollution associated with the London "smogs" that became notorious in the later parts of 19^{th} century through the early 1960s is well reported in literature .Incomplete combustion of coal with emission of black smoke and sulfur dioxide and production of tar with sulfuric acid resulted in a smoke / fog /SO_2 mixture that was associated with a marked increases in sudden deaths ,hospital admissions, illness in bronchitis patients with reduction in lung function and urban excess of morbidity and mortality from chronic airway disease (Reid,1964; Chretien *et al.*,1989; Crapo *et al*.,1992). Subsequently many cross sectional studies suggest that air pollution of whatever origin is responsible for respiratory symptoms and reduced lung function (Crofton *et al.*,1981).Surveys conducted in Lancaster, Burbank ,Long Beach ,Mumbai ,Netherlands and many other places , compared the importance and nature of air pollution with lung function. The town of Lancaster was considered a clean area as opposed to Burbank , known for its pollution due to its oxidants , and to the town of Long Beach , characterized by the presence of petrochemical plants causing pollution due to particles , hydrocarbons , and sulfur dioxide .The percentage of patients with diminished airflows was significantly higher in the polluted areas of Burbank and Long Beach than the Lancaster area .Another longitudinal survey in Netherlands compared the expiratory flows of subjects in rural areas free of air pollution and another area, Vlaardingen ,polluted by refineries releasing sulfur

monoxide and smoke .It was demonstrated the mean reduction in vital capacity and the mean expiratory flow in ml / year was higher in the polluted than in the non polluted area . A study from Mumbai has reported a high prevalence of cough , dyspnea , or both in nonsmokers (13%) and was attributed to the industrial pollution (Kamat *et al.*,1992).

2.1.5 Occupation

The university of the smoking habit in working men and women and its strong casual role in the genesis of COPD has tended to overshadow the potential contribution of occupational exposure. However, recent evidence from cross sectional and longitudinal studies, both community based , and work place related , points to a casual role in association with or independently of cigarette smoking (Becklake,1989a ;Sharp *et al.*,1973). Occupational exposures to dusts alone or to dusts and in association with fumes an vapors are independent risk factor for the development of COPD (Becklake, 1989a). There are also reports of occupational exposure in which smoking has the effect of amplifying the exposure risk. In a longitudinal study over 12 years period in 575 men, aged 30 to 54 years ,working in the Paris area, the yearly reduction of FEV_1 was reported to be significantly higher, by 10 to 20 ml per year, in the subjects exposed to occupational air pollutants whatever their smoking level was.

2.1.6 Bronchial Hyper responsiveness

With normal aging process there is a fall in FEV_1 of about 25 ml / year in healthy nonsmokers over the age of 30 years. In smokers the fall is about 50 ml / year. However, certain smokers show a markedly decline in FEV_1 of more than 50 ml / year and these are the subgroups who later on develop COPD. Although factors which result in such an accelerated rate of decline in lung functions are not known, Dutch investigators have proposed a "Dutch hypothesis"(Orie *et al.*.,1961) which suggested that an allergic constitution might predispose chronic smokers to severe chronic airflow obstruction. These investigators proposed that an "asthmatic constitution" underlay the development of chronic airflow limitation . This asthmatic constitution consist of a predisposition to atopic disease, eosinophilia , and airway hyperresponsiveness . According to the hypothesis , smoking is only an extrinsic factor that is superimposed on this constitutional susceptibility leading on to chronic airflow limitation .This view has subsequently been found favor with many investigators who believe that hyperreactivity is a contributing factor in the

causation of COPD (Connor *et al.*,1989;Pande,1989;Koyama *et al.*,1994;Pande *et al.*,1992;Suri *et al.*,1992;Taskin *et al.,* 1992).It has been established that smokers have a higher level of IgE levels (Annesi *et al.*,1992) and there is a correlation of eosinophilia in the peripheral blood with that of smoking (Burrows *et al.*,1980). Smokers have a high prevalence of certain markers of atopy compared to controls.

Nonspecific hyperreactivity of the airways is reported in smokers in many studies although it is possible role in the development is unclear (ATS, 1995b).This reactivity might occur from the airway inflammation that typically is seen in smoking related COPD . In the Lung Health Study, non specific airway hyperreactivity was noted in 85.1 percent of women than men (58.9%) . While 46.6 percent of women responded to 5 mg / ml or less of methacholine , only 23.9 percent of men responded to the same dose , a number almost half of that of women . In both sexes, degree of airflow obstruction was highly correlated with severity of airflow obstruction but not with the age (Taskin *et al.*, 1992).

2.1.7 Demographic and Other Variables

There is higher prevalence of respiratory symptoms in men. Mortality rates for COPD are higher in whites than in non-whites .Morbidity and mortality rates are inversely related to socioeconomic status and are higher in blue collar than white-collar workers (ATS,1995a).COPD may also aggregates in families suggesting a genetic link(Kaufmann *et al.*,1983).It is suggested that dietary fish intake perhaps reduces the susceptibility to COPD as is evident that asthma is extremely low in Eskimoes .Britton reported that a high dietary intake of ω-3 fatty acids may protect cigarette smokers against COPD (Britton,1995).

2.2 World Wide Information

The World Health Organization (WHO) estimates that COPD as a single cause of death shares 4th and 5th places with HIV/AIDS (after coronary heart disease, cerebrovascular disease and acute respiratory infection).The WHO estimates that in 2000, 2.74 million people died of COPD worldwide. In 1990, a study by the World Bank and WHO ranked COPD 12th as a burden of disease; by 2020, it is estimated that COPD will be ranked 5th. According to the WHO, passive smoking carries serious risks, especially for children and those chronically exposed. The WHO

estimates that passive smoking is associated with a 10 to 43 percent increase in risk of COPD in adults [(COPD International (Some Alarming Statistics, Worldwide information) (http://www.ingen-tech.com/PDFbin/COPD_International.pdf)]. Although cigarette smoking is the primary cause of COPD, the WHO estimates that there are 400,000 deaths per year from exposure to biomass fuels. In Algeria, the prevalence of tuberculosis and acute respiratory infection has decreased since 1965, but an increase in chronic respiratory diseases (asthma and COPD) has been observed in the last decade. COPD is estimated to be 6.2 percent in 11 Asian countries surveyed by the Asian Pacific Society of Respiratory Diseases. The use of biomass fuels, especially in the rural areas, contributes towards a higher prevalence of COPD in some of these countries and suggests that COPD may be significantly greater in this region of the world than previously estimated. [(Conditions by Death" http://www.wrongdiagnosis.com/lists/deaths_printer.htm and National Institutes of Health, "Estimates of Funding for Various Diseases, Conditions, Research.Areas,"March 8,2005[http://www.nih.gov/news/fundingresearchareas.htm)].In China, where it is estimated that over 50 percent of the men smoke, chronic respiratory diseases are the 4th leading cause of death in large urban areas, but the first leading cause of death in rural areas. In China, smoking rates among women remain low (estimated at 6 percent), although the prevalence of COPD in men and women is about the same. This points to the importance of risk factors other than smoking as a cause for COPD in Chinese women. In Malaysia, respiratory illness is the primary cause of visits to health clinics and outpatient hospital clinics. It is estimated that 50 percent of the male population smokes, with higher rates in the rural areas than the urban areas. [(COPD International (Some Alarming Statistics, Worldwide information) (http://www.ingen-tech.com/PDFbin/COPD International.pdf)].

COPD is the fourth leading cause of death in the U.S. and is projected to be the third leading cause of death for both males and females by the year 2020.The NHBLI reports 12.1 million adults 25 and older were diagnosed in 2001 [(COPD International (Some Alarming Statistics, United States information) (http://www.ingen-tech.com/PDFbin/COPD_International.pdf)]. It is estimated that there may currently be 16 million people in the United States currently diagnosed with COPD. It is estimated that there may be as many as an additional 14 million or more in the United States still undiagnosed, as they are in the beginning stages and have little to minimal symptoms and have not sought health care yet [(COPD International (Some Alarming Statistics,

United States information) (http://www.ingen-tech.com/PDFbin/COPD International.pdf)].Men are 7 times more likely to be diagnosed with emphysema then women, though the prevalence in women is on a steady increase and this number is lowering with each year. People over the age of 50 are more likely to be considered disabled, however, the damage started years before [(COPD International (Some Alarming Statistics, Worldwide information) (http://www.ingen-tech.com/PDFbin/COPD International.pdf)].About 1.5 million emergency department visits by adults 25 and older were made for COPD in 2000.

More emergency department visits for COPD were made by adult females than adult males (898,000 vs. 651,000).About 726,000 hospitalizations for COPD occurred in 2000. More females than males were hospitalized for COPD (404,000 vs..322,000).[(COPD.International.(Some.Alarming.Statistics,Worldwide.information)(http://www.ingen-tech.com/PDFbin/COPD International.pdf)].

According to the Center for Disease Control (CDC), there was 124,816 deaths in the US in 2002.It is the only major disease with an increasing death rate, rising 16%. [(Conditions by Death"http://www.wrongdiagnosis.com/lists/deaths_ printer.htm and National Institutes of Health, "Estimates of Funding for Various Diseases,.Conditions,.Research.Areas,"March 8,2005 [http://www.nih.gov/news/fundingresearchareas.htm)]; (Cary *et al*.,1999).

2.2.1 COPD in India

COPD in India has been recognized and investigated with the help of small surveys conducted in different populations for the last 40 years. Prevalence rates varying from about 2 to 22 percent in men and from 1.2 to 19% in women have been shown in different reports (Reddy *et al.*, 2004).The number of published reports are small (Pande *et al*., 1997; Nigam *et al*., 1982; Joshi *et al*., 1975; Thiruvengadam *et al*., 1977; Malik *et al.*, 1986; Vishwanathan *et al.*, 1977;Radha *et al*., 1977; Bhattacharya *et al*.,1975).

Review studies on prevalence of COPD in India by Jindal et al, were reported in the three time periods of up to 1970, between 1971-1990 and after 1994 (Jindal *et al*.,2001).Most of these studies were conducted with the help of an interview or a questionnaire while a few had used peak expiratory assessment (PEF) as well. A median prevalence of 5% in men and 2.7% in

women was calculated which accounted for a total burden of 8.15 million male and 4.21 million female patients in a population of 944.5 million in 1996 (Jindal *et al*., 2001).

2.2.2 WHO/ GOLD estimates

According to WHO estimates (2007), 210 million people had COPD and 3 million people died from COPD in 2005, accounting for 5% of all deaths globally. The WHO predicts that the total deaths from COPD will increase by 30% over the next 10 years and that COPD will become the third leading cause of death worldwide by 2030, after heart disease and stroke (World Health Organisation, 2008 (http://www.who.int/mediacentre/factsheets/fs315/en/index.html.); Halbert *et al.*, 2006).

It has been postulated that the increasing mortality of COPD is driven by the continued high use of tobacco in many countries and the changing age structure of populations in developing countries (GOLD, 2010). COPD morbidity and mortality vary across countries, but in general are related to the prevalence of tobacco smoking (GOLD, 2010). However in many developing countries, air pollution arising from the burning of biomass fuel or wood stoves has also been identified as a leading cause of COPD. The prevalence of COPD among lower income populations was highlighted by the statistic that almost 90% of the COPD deaths reported in 2005 came from countries of low to mid income. Fig. 1 illustrates the worldwide prevalence of COPD (GOLD, 2010).

2.3 DIAGNOSIS

The preliminary diagnosis of suspected COPD is based clearly on

- Risk exposure (eg. Cigarette smoking ,occupational, urban and indoor pollution)
- Symptoms such as chronic cough and phlegm, and exercise dyspnoea
- Signs such as modification of thorax configuration, changes in lung sounds etc. (Celli *et al*., 2004).

The diagnosis of COPD needs to be confirmed by spirometry. Independently whether the term obstruction or limitation is used, all of the national and international guidelines on ‘assessment &

management of COPD' indicate the measurement of FEV_1 (Forced Expiratory Volume in one second) as the landmark parameter of respiratory function.

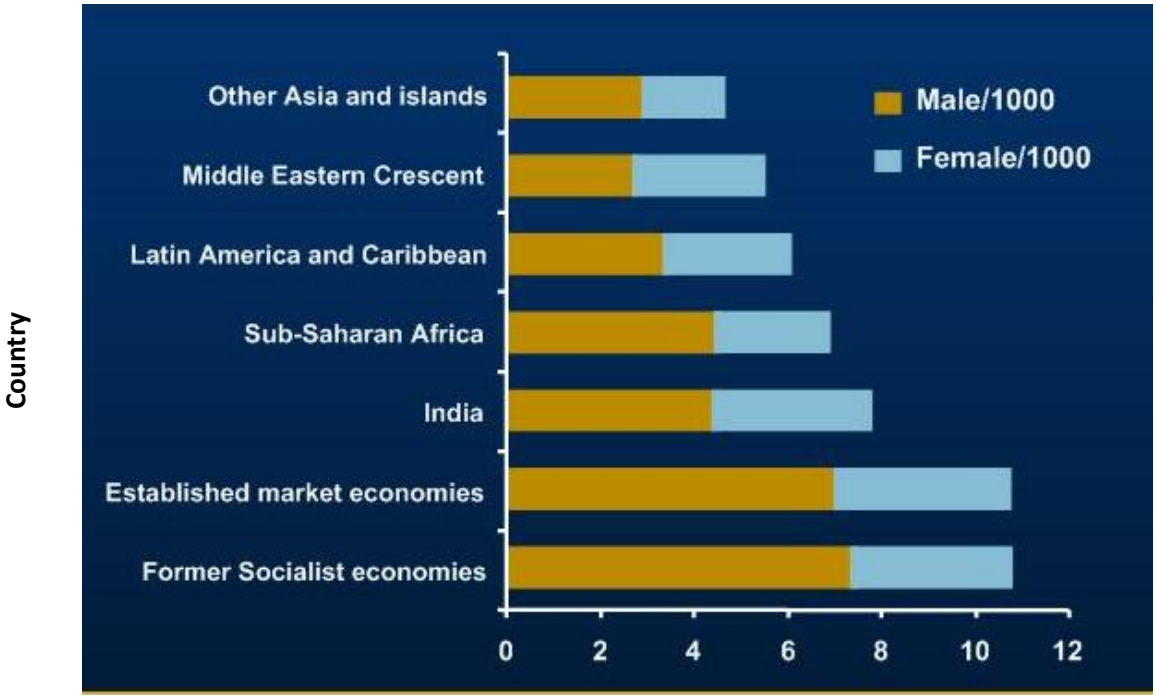
- To make diagnosis of COPD
- To stage severity of the disease and
- To assess its progression

A value of the FEV_1/FVC ratio < 0.7 after bronchodilator indicates existing COPD, independent of age , gender, ethnicity, height, weight and so on (Celli *et al*., 2004; Pauwels *et al*., 2004).

Pulmonary function measures are the most important phenotypes of COPD. These pulmonary function measures predict the development of lung diseases (Green *et al.*, 1974; Litonjua *et al.*,1976).

2.4 DUST INDUCED RESPIRATORY TOXICITY

Among the most prevalent occupational lung diseases in the world are those induced by inhalation of mineral dusts such as asbestos, silica and coal dust (Mossman *et al*., 1990).



Prevalence of COPD/1000 of the population(http://www.goldcopd.com)

Fig 1: Worldwide Prevalence of COPD (GOLD, 2009)

Inhalation of coal dust may cause a variety of lung diseases including simple Coal Worker's Pneumoconiosis (CWP) (Collins & Gilchrist, 1928) progressive massive fibrosis (PMF), (Cochrane,1973) chronic bronchitis (Rae *et al.*, 1970) lung function loss (Love & Miller,1982), and emphysema (Cummins,1936).

The interaction among anthropogenic activities, such as coal mining, air, water and soil quality are highly complex. Extensive epidemiological studies and controlled clinical trials have clearly established an association between asthma and several air pollutants such as SO_2 , NO_2, ozone and particulate matter (Gauvin *et al.*,1999).There is also general agreement that these pollutants are unlikely to cause asthma but rather act as potent irritants in exacerbating the airway inflammatory reactivity (Koren, 1995).It is thus critical, when evaluating the potential effects of air toxics on respiratory disorders or other, health end points, to determine where the exposure occurs, the contribution of each pollutant and its anthropogenic origin as well as to define the target population.

Chronic Obstructive Pulmonary Disease (COPD) is the consequence of an abnormal inflammatory response to inhalation of noxious agents such as cigarette smoking (the main etiological agent), occupational exposure, and environmental (Barnes, 2004). Exacerbations of COPD are known to result from increased levels of air pollutants, specifically particulate air pollution (Mac Nee, 2002).Particulate air pollution causes oxidative stress in the airways (Donaldson *et al.*, 1997).Oxidative stress occurs when Reactive Oxygen Species (ROS) is produced in excess of the antioxidant defense mechanisms and results in harmful effects, including damage to lipids, proteins, and DNA. There is an increasing evidence that oxidative stress is an important feature in COPD (Repine *et al*, 1997; Macnee , 2001; Henricks 2001).

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including neutrophils, eosinophils, macrophages and epithelial cells (Macnee ,2001).

Superoxide anions are generated by NADPH oxidase and this is converted to hydrogen peroxide by superoxide dismutase hydrogen peroxide is then dismuted to water by catalase. Superoxide anion and hydrogen peroxide may interact in the presence of free iron to form the highly reactive

hydroxyl radical. Superoxide anion may also combine with NO to form peroxynitrate, which also generates hydroxyl radical (Beckman *et al*.,1996).

Oxidative stress leads to the oxidation of arachidonic acid and the formation of new series of prostanoid mediators called isoprostanes, which may exert significant functional effects including bronchioconstriction and plasma exudation (Morrow, 2000; Okazawa,1997).

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract (Cantin *et al*., 1990).The major intracellular antioxidants in the airways are catalase, superoxide dismutase and glutathione, formed by the enzyme gamma-glutamyl cysteine synthetase and glutathione synthetase. Oxidant stress activates the inducible enzyme heme oxygenase-1,converting heme and hemin to biliverdin with the formation of carbon monoxide (Choi *et al.*,1996). Biliverdin is converted via bilirubin reductase to bilirubin, which is a potential antioxidant. HO-1 is expressed in human airways and carbon monoxide is production is increased in COPD (Lim *et al.*, 2000; Montuschi , 2001).

In the lung, intracellular antioxidants are expressed at relatively low levels and are not induced by oxidative stress, whereas major antioxidants are extracellular (Comhairs *et al.*, 2002).Extracellular antioxidants particularly glutathione peroxidase are markedly up-regulated in response to cigarette smoke and oxidative stress. The glutathione system is the major antioxidant mechanism in the airways. The increased oxidative stress in the airways of COPD patients may play an important pathophysiological role in the disease by amplifying the inflammatory response in COPD. Oxidative stress may also impair the function of antiproteases such as α_1 – antitrypsin (α_1 AT) and SLPI, and thereby accelerates the breakdown of elastin in lung parenchyma (Taggart *et al.*,2000).Normally proteases are counteracted by an excess of endogenous antiproteases .The major inhibitors of serine proteases are α_1 AT in the lung parenchyma and airway epithelium derived SLPI in the airways (Rooney *et al.*, 2001).The best described deficiency that results in early onset emphysema is the ZZ type on which a single amino acid substitution (Gly342-Lys) results in structural alterations in α_1 AT resulting in the failure of its normal posttranslational modification and secretion by hepatocytes leading to very low plasma concentrations (Carp *et al*.,1978).

Coal is the most polluting fossil fuel. There is adverse health, environmental and economic consequences associated with the use of coal. In fact, Indian coal is one of the dirtiest coals in the world. Its high ash content and lack of infrastructure to clean it early in the process creates a huge environmental risk for India. Coal is the most polluting fuel in terms of the greenhouse gases, and it already accounts for 65% of the CO_2 emissions in India. In addition to harmful carbon dioxide gas emissions, "black carbon" (fine char particles) is produced during the burning of coal. Although many Indian coals have low ash content, yet some of them contain high amounts of sulphur (e.g., the coals of northeastern India).High temperature conversion of coals results in the emission of nitrogen and sulfur in the coal as NOx and SO_2. These gases are responsible for smog (Borah, 2010).

2.5 COAL RESERVES IN INDIA

India is the 3rd largest coal-producing country, and has the fourth largest reserve of coal in the world (viz. approximately 197 billion tons).Coal deposits in India occur mostly in thick seams and at shallow depths. The reserve for non-coking coal is about 167.45 billion tons (i.e., 85% of the total reserve) while the coking coal reserve is 29.55 billons tons. Indian coals have high ash content (15-45%), and they are of low calorific value. With the present rate of extraction of approximately 0.8 million tons per day, the reserves are likely to last over the next hundred years. At present, India has 48 coal washeries having a total capacity of 102 million tons per annum. Of these washeries, 19 are owned by Coal India Limited (CIL).The use of beneficiated coal has gained acceptance in the steel and power plants located at a distance from the pithead.CIL is the largest company in the world in terms of coal production. It contributes to almost 85% of coal production in India (Borah , 2010).

The development of coalfields in Assam was initiated in the year 1870 (Akala,1995) and is now operated in the name North Eastern Coalfields, Coal India Limited (NECF-CIL), Margherita.There are about 1.00 billion tonnes coal reserves estimated in this coal bearing zone of North-East (NE) India, which is 0.5% of the country's total reserve of about 200 billion tones (Chaoji,2002).

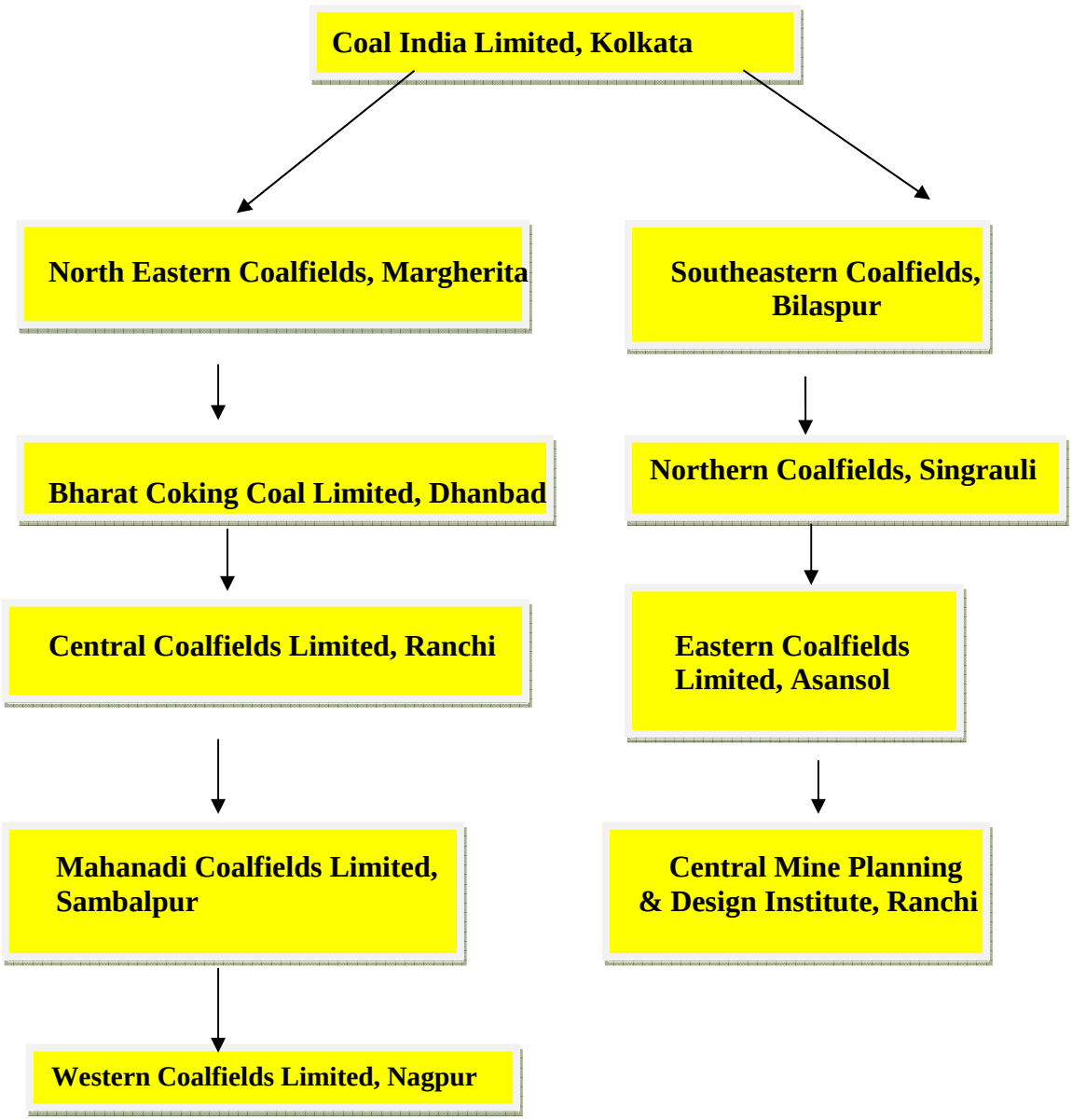


Fig 2: Coal India Limited and its subsidiaries.

2.6 COAL MINE COVERAGE IN NORTH-EAST INDIA

The coal mine coverage in North-East India along with annual production is as follows:

State	***Production/annum (million tons 2009)**
Assam	375.40
Arunachal Pradesh	90.23
Meghalaya	459.00
Nagaland	19.94

Table 2: Coal Mine Coverage in North-East India (2009)

*****Data from geological Survey of India, Central Mine Planning & Design Institute, Mineral Exploration Corporation Limited, Singareni Colliery Company Limited, DGM, Maharashtra & DGM, Chhatisgarh.

2.7 COALS OF NORTHEASTERN INDIA

The northeastern region of India is comprised of eight states viz. Arunachal Pradesh, Assam, Meghalaya, Mizoram, Manipur Nagaland, Tripura and Sikkim. Northeastern coalfield (NEC) (a unit of Coal India Limited) is engaged in carrying out coal mining operations in this region. Coal is produced by both open-cast and underground mining methods from the coal-bearing strata, which have been tectonically folded with the coal seams dipping from 30-60 degrees. The coal reserves to a depth of 600 meters aggregate to about 945 million tons. The coal available in this region is tertiary coal, which is unique in character by virtue of its low ash content, and thus of high value.

The coal bearing tertiary sediments of the northeastern India range from Paleocene to Oligicene geological epochs. These epochs belong to the tertiary geological period which ranges from 1.8 to 65 million years ago. The tertiary period of geological age has been divided into several divisions. The Paleocene epoch spans the period 55-65 million years ago. On the other hand, the Oligocene epoch refers to the period between 22 and 39 million years ago. The coal deposits of Meghalaya, and the Mikir and North Cachar hills of Assam generally contain thin seams of Eocene age (38 to 54 million years ago). They were formed under stable shelf condition in peripheral platform areas.The coal deposits of Oligicene epoch occur in a narrow, linear belt of over-thrusts known as the "belt of Schuppen", which extends from Nagaland to Arunachal Pradesh through Assam. They were deposited in near-shore, deltaic wet forest swamps, and in marshy environments close to geosynclinals trough.The coal seams have attained considerable thichness in the Makum and Namchik-Namphuk coalfields.

The coals of northeast India are high in volatile matter [38-57% on dry mineral-matter-free (dmf) basis], sulfur (1-10% dmf basis) and hydrogen (4-9% dmf basis) contents. The carbon content of these coal ranges from 68 to 85% (dmf basis).The coals of northeastern India have high sulfur content.In a particular seam, total pyritic and organic sulfur content increases from the floor to the roof of the seam. Upper seams generally contain more sulfur than the seams lying below.More than 80% of the sulfur present in these coals is organic in nature.Therefore, it is not possible to remove this sulfur by mechanical means.Free burning of these coals causes environmental pollution (Borah,2010).

2.8 MECHANISMS AND MEDIATORS IN COAL DUST INDUCED TOXICITY

Most of the occupational lung diseases in the world, are induced by inhalation of mineral dusts such as asbestos, silica and coal dust (Mossman *et al*., 1990;Van Sprundel,1990;Oxmen *et al.*,1993;Meredith and Mc Donald,1994).

Inhalation of coal dust may cause a variety of lung diseases , including simple coal workers' pneumoconiosis (CWP) (Collis and Gilchrist, 1928), progressive massive fibrosis (PMF); (Cochrane,1973), chronic bronchitis (Rae *et al*.,1970; Rogan *et al*.,1973;Soutar and Hurley,1986), lung function loss (Love and Miller,1982; Attfield,1985; Wouters *et al*.,1994),and

emphysema (Cummins,1936; Lyons *et al*.,1981).Mechanisms to explain the fate ofparticles in the respiratory tract have been reviewed by several authors (Lippman,1994; Morrow, 1992).

Important factors include, intrinsic (chemical, morphological) properties of the dust as well as host factors including lung volume,breathing rate and depth.Animal studies as well studies of human biopsies have been the basis of continuing debate on, crucial parameters of particle toxicity,which includes aspects such as particle durability and leaching, particle deposition and translocation,and particle clearance via mucociliary and interstitial (lymph nodes) clearance routes(Bolton *et al.*,1983;Morrow, 1992). Upon deposition,coal dusts principally reach two main target cells,i.e. macrophages and epithelial cells.As a consequence,other cells as well as interstitial components, may be affected following coal dust deposition ,due to particulate translocation as well mediators released by the primary target cells.

Mechanisms of coal dust toxicity can be arbitrarily subdivided into two major pathways, involving the production of reactive oxygen species and related antioxidant protection. Both pathways are based on the key concepts of macrophage activation and lung inflammation, and are considered to be crucial mediators in the respiratory effects that are observed in chronic exposure to mineral dusts.

From the time it was realized that activation of phagocytes leads to generation of superoxide anion (Babior *et al*., 1973) ROS have been implicated in a variety of diseases. The most important effects of ROS in the lung may include:

- Damage to cell membranes by means of lipid peroxidation processes
- Oxidation of proteins and
- Damage to the DNA of target cells.

Lipid peroxidation is a chain reaction process with unsaturated membrane fatty acids resulting in the formation of lipid radicals, and may lead to cell damage and tissue remodelling. Reactions of ROS with proteins may lead to inactivation of enzymes involved in cell metabolism or in the modification of intra or extracellular structural components. Oxidative DNA damage may have various consequences ranging from cell death and tissue destruction to cell or tissue proliferation (Janssen *et al.*, 1993).In addition to these interactions with DNA , ROS may also act as regulators of intracellular signaling cascades and transcription factors of a variety of genes

including those of proinflammatory cytokines, adhesion molecules , and proto- oncogenes (Schreck *et al*.,1992; Suzuki *et al*.,1997).

Enzymatic antioxidant mechanisms as well as non enzymatic antioxidants are present in all lung compartments to cope with the toxic effects of ROS. The role of ROS and antioxidant defense mechanisms have been extensively reviewed by several authors (Suzuki *et al*.,1997; .Janssen *et al.*,1993; Kehrer, 1993;Halliwell and Cross,1994).

2.8.1 Coal Dust and the Formation of Reactive Oxygen Species (ROS)

A role for ROS has been considered in the pathogenesis of a variety of lung diseases (Gross 1997; Chvapli and Peng ,1975;Cantin and Crystal,1985;Cantin *et al*.,1987).

Basically two mechanisms by which mineral exposure causes formation of ROS *in vivo* have been proposed.

1. The formation of ROS by intrinsic properties of the particles, i.e. non-cellular mechanisms and
2. The excessive formation of ROS by the oxidative burst of macrophages and

neutrophils activated during particle phagocytosis and persistent inflammation.

2.8.2 Non-Cellular Mechanisms

Grinding i.e. cleavage of coal dust is believed to cause generation of radicles on fresh surfaces (Vallyathan *et al.*, 1988b; Dallal *et al*., 1995).

The iron present on the surface of asbestos fibres may be involved in the formation of hydroxyl radicals via the Fenton-reaction (Weitzman and Graceffa, 1984; Goodlick and Kane, 1986, Zalma *et al.*; 1987).

The iron content may also play an important role in the toxicity of coal dust (Tourmann and Kaufmann, 1994; Dallal *et al*., 1995). Hydroxyproline, which is considered as a marker of fibrosis (Murray and Laurent, 1988) has been found correlated to iron content in the lungs of coal miners (Ghio and Quigley,1994).Fenton-reaction type formation of hydroxyl radicals was found to be positively correlated to iron content of coal dust (Dalal *et al*.,1995).Electron spin

resonance (ESR) studies with coal dust have shown that hydroxyl radical formation was 8-fold higher in the coal dust samples. Coal dust may contain stable radicals can generate ROS in biological fluids, and may as such cause direct oxidative damage through non-cellular mechanisms (Roel *et al*.,1999).

2.8.3 Cell Mediated Generation of ROS

Indirect generation of radicals in the lung is considered to be controlled by alveolar macrophages .Macrophages incubated with mineral dusts produce excess amounts of oxygen radicals (Kamp *et al.*,1992) as well as chemoattractant factors for other inflammatory cells including monocytes and neutrophils which in turn may produce ROS and amplify local radical formation. Animal inhalation studies with various mineral particles including coal dust have provided further support for recruited neutrophils as a major source of ROS in dust – inflammed lungs (Kusaka *et al*.,1990; Driscoll *et al.*,1991).Physical and chemical properties of particles were found to be related to the extent to which ROS are generated from phagocytic cells(Hansen and Mossman,1987), and very large fiber shaped particles can cause extracellular burst by incomplete or 'frustated' phagocytosis by phagocytic cells(Mossman *et al.*,1987; Mossman and Marsh,1991). Crucial support for the involvement of ROS in humans exposed to coal dust was initially based on observations from bronchoalveolar lavage studies. Alveolar macrophages of dust exposed subjects produced increased amounts of oxygen radicals compared to non-exposed subjects (Voisin *et al*., 1985; Rom *et al*., 1987).

2.8.4 The Role of ROS in Coal Dust Induced Lung Disorders

Several independent studies have provided further support for the significant role of ROS in lung disorders associated with coal dust exposure. Following the observation in bronchoalveolar lavage macrophages of subjects exposed to coal dust (Voisin *et al.*, 1985; Rom *et al*.,1987), oxidant generating capacities of alveolar macrophages was also studied in relation to pneumoconiotic lung disease ,and comparison of macrophage ROS production in simple (Coal Workers Pneumoconiosis) CWP versus (Progressive massive fibrosis) PMF yielded a correlation with disease severity (Wallart *et al*., 1990). Furthermore ESR analysis of dust recovered lymph nodes showed higher amounts of stable coal radicals in lung biopsies of exposed subjects compared to controls, and the amount of these radicals was also related to CWP disease severity

(Dalal *et al.*, 1991). Further support for increased generation of hydroxyl radicals in the course of pneumoconiosis was also obtained from an *in vivo* silica model of fibrosis (Schapira *et al*., 1995). Intratracheal administration of either silica or coal dust in rats has also been reported to enhance $NO^{\cdot}$ production from alveolar lavage macrophages of these animals (Blackford *et al*.,1997) ROS are also considered to play a crucial role in the pathogenesis of emphysema by oxidative damage to antiproteases reducing its activity reduced antiprotease activity may cause exaggerated lung tissue destruction by proteases (Janoff and Carp,1982), and therefore it has been suggested that dust mediated oxidant generation may be involved in the typical focal emphysema observed in coal workers (Rom,1990).However in miners with emphysema α-1-antitrypsin levels were not altered (Rom,1990).

2.8.5 Antioxidants and Coal Dust Induced Lung Disorders

Studies using radical scavengers or antioxidants have underscored the significance of ROS in dust-elicited lung toxicity including pulmonary fibrosis and genotoxicity (Goodglick and Kane, 1986; Mossman *et al*., 1986; Voisin *et al*., 1985; Dong *et al*., 1994; Driscoll *et al*., 1997).

Based on these observations, adverse oxidative effects have been suggested to be related to and impaired balance between ROS and antioxidant defense status, and upregulation of antioxidant enzymes is considered as a marker of oxidative stress (Borm *et al*., 1986; Janssen *et al*., 1994). Red blood cells contain large amounts of antioxidants and are considered as powerful antioxidant carriers (Toth *et al*., 1984; Van Asbeck *et al*., 1985).

Consequently, altered levels of red cell antioxidants (GSH, catalase, SOD) found in CWP are considered to reflect the significance of radical mechanisms in coal dust exposure and pneumoconiosis in humans (Borm *et al*.,1986; Engelan *et al*., 1990; Evelo *et al*.,1993; Perrin-Nadiff *et al*.,1996).

Longitudinal analysis of blood antioxidant status in coal workers showed that red cell antioxidant enzymes (GST, SOD, catalase) were related to lung function decline, and red cell GSH levels were significantly reduced in chronic bronchitis (Schins *et al*., 1997).Other investigators showed that antioxidant capacity was reduced in asthmatics and subjects with COPD (Rahman *et al*.,1996; Schins *et al*., 1997).

2.8.6 Particulate Air Pollution

Particulate air pollution may impair the ventilation in COPD patients by causing airway narrowing and increasing the work of breathing (Anderson *et al*., 1990). Particles may be deposited in the extra thoracic airways (mouth, nose, larynx), in airways of the trachiobronchial regions and in the alveolar regions where gas exchange takes place (Moller *et al*., 2008). The respiratory tract deposition patterns depend on particle size and distribution within the inspired air .Biologic effects may be a function also of particle number, composition, and the total surface area of the particle. Various factors have been shown to influence particle deposition ,such as age ,ventilation patterns and the presence of obstructive or inflammatory airway disease(Kim *et al*.,1997).Higher ventilation increases total deposition ,and obstructive airway disease, such as chronic bronchitis ,emphysema and asthma results in increased deposition in the lower respiratory tract (Brown *et al*.,2002).Retention of particles is a function of deposition site and clearance, of particles which again may be impaired in persons with COPD. Chronic effects may also arise from recurring cycles of pulmonary injury and repair (Moller *et al.*,2008).Studies have shown that high doses of particles can trigger oxidative and the induction of inflammation, increased blood coagulation, impaired cellular defence , and modulation of immune system (Kreyling *et al.*,2004; Oberdoster *et al*.,2002).

Respiratory diseases have a distinct role in the health of miners, with important implications for morbidity and mortality (Miller *et al.*, 1985; Morgan *et al.*, 1980).

Respiratory symptoms may be early manifestations of acquired respiratory diseases, and examining such symptoms among miners can be helpful during health surveillance of these dust-exposed workers. Various studies from industrialized countries have documented the relationship between exposures to coal dust and increased respiratory symptoms. Both longitudinal and cross-sectional studies (Rae *et al*., 1971; Rogan *et al.*, 1973; Marine *et al*., 1988; Carta *et al*., 1996; Henneberger *et al.*, 1997;Wang *et al.*,1999) have shown that symptoms of persistent cough and phlegm production, breathlessness and wheezing relate significantly with individual cumulative exposure to respirable mixed coal dust.

The British Pneumoconiosis Field Research among 30,000 miners showed that coal dust contributes to the development of respiratory symptoms at an early age (Ashford *et al.*,1961).US

Coal Mine Health and Safety Act in 1969 set the legal respirable mixed coal dust standard for coal mines in the in the US at 3mg/m^3 ,with a reduction to 2mg/ m^3 in 1973.Despite these standards, studies in the United States showed statistically significant associations between cumulative exposure to respirable dust and respiratory symptoms for miners joining the industry after 1970 (Seixas *et al*.,1969). Henneberger and Attfield (Henneberger *et al*., 1997) showed a high prevalence of dyspnoea and wheezing for coal miners joining the industry in the United States before 1970. This study suggested that respiratory symptoms might provide an early warning related to prior exposure and might be followed by impairment in lung functioning.

2.9 GENETIC EPIDEMIOLOGY OF PULMONARY FUNCTION

It is believed that Chronic Obstructive Pulmonary Disease (COPD) is both environmental and genetic (Snider *et al*., 1989; Higgins *et al*., 1989); however specific genetic factors in the development of COPD have not been clearly identified; except for protease inhibitor types.Alpha1-antitrypsin deficiency is rare in the general population (Horne *et al*., 1984; Horne *et al*., 1992) and accounts for less than 2% of the cases of COPD (Cohen *et al*., 1980; Snider *et al*., 1995). Fewer than 1 in 1600 U.S. Caucasians and even fewer non-Caucasians are estimated to have the Pi-ZZ genotype, so only a small minority of cases of COPD can be attributed to severe API deficiency related to this genotype (Walter *et al*., 2000).Much less is known about the possible role of other protease inhibitor genes in the pathogenesis of COPD. The protease inhibitor α1-antichymotrypsin (ACT) is the major physiologic inhibitor of cathepsin G, an elastolytic protease produced by neutrophils, and has been observed to be protective against pancreatitis lung-injury in rats (O'Donovan *et al*., 1995).The gene encoding ACT is located on chromosome 14, in close proximity to the API gene (Rollini *et al*., 1997). α 2-macroglobulin is a serum non-specific antiprotease that appears in the sputum during infections (Burnett *et al*., 1991).Genetic variation in the ACT structure has been associated with premature lung disease (Poller *et al*., 1993; Barnes *et al*.,1999) but these findings have not been consistently replicated and the role of α 2-macroglobulin in lung disease remains unclear (Sandford *et al*., 1997).Other antiproteases including serum leukoprotease inhibitor and elafin, have been proposed as playing a role in COPD pathogenesis but their importance in the development of COPD remains speculative. Other proteases of potential importance in the pathogenesis of COPD include other matrix metalloproteinases and lyosomal cathepsins; there are no data, however, to indicate that

genetics controlling the levels of these proteases differ between emphysema patients and controls. Additionally, there are no human data linking mutations of the genes encoding these proteases to the development of COPD. The molecular genetics of COPD has been recently reviewed by Barnes (Barnes, 1999).

Antiprotease inhibitor deficiency is the only known genetic disorder unequivocally implicated as a cause of emphysema, although only a small proportion of COPD cases can be attributed to this condition.API, an antiprotease that inhibits the activity of neutruphil elastase and other proteolytic enzymes, is a serum protease primarily synthesized in the liver, mononuclear phagocytes, neutrophils, bowel and kidney (WHO,1997).The gene encoding API, located on chromosomal segment 14q32.1, is highly pleomorphic, and over 75 distinct alleles have been described. The Pi-M allele and its subtypes are the most common, with a gene frequency of approximately 900 in 1000; persons with Pi-M genotype have normal serum levels of normally functioning API. 5% of Scandinavians, 4% of Britains, 1 to 2% of southern Europeans, and 2-3% of the heterogeneous white population in the United States are MZ (carriers) heterozygotes.

The Pi-Z allele has a point mutation in exon V that impairs secretion of synthesized API protein (Yoshida *et al*., 1976) and Pi –ZZ homozygotes have extremely low serum levels of normally functioning API. The Pi-ZZ genotype is the most common genotype among persons with severe API deficiency. The Pi-S allele is associated with reduced serum levels of API but to a lesser extent than Pi-Z, and persons with the Pi-SS genotype have serum API levels intermediate between those of persons with Pi-MM and Pi-ZZ. Non-expressing null alleles are associated with the absence of API synthesis , and the Pi-null null and Pi-Z null genotypes are also associated with severe API deficiency. The API phenotype, assessed by the distinct electrophoretic patterns associated with different alleles, is often used as a surrogate for genotype. Persons with severe API deficiency (usually Pi-ZZ, rarely Pi-Z null or Pi-null null) often develop severe emphysema at a relatively early stage, and this is the major cause of morbidity and mortality in this condition (Larsson *et al.*, 1978). Pulmonary development and growth in early life appear normal, and lung function remains in the normal range in adolescence and the beginning of young adulthood. Early in adulthood, persons with severe API deficiency begin to develop emphysema due to lack of inhibition of lung connective tissue degradation by neutrophil elastase and other proteases.

Even among never smokers, longitudinal studies demonstrate excessive loss (47-86ml/year) of FEV_1 (Piitulainen *et al.*, 1999) compared to historical controls (20-30ml/year).

The World Health Organization (WHO) recommends that all individuals with COPD as well as adults and adolescents with asthma be tested.

- It is also suggested that testing be done on most patients with chronic or recurrent respiratory symptoms (dyspnea , cough, wheezing) at least once.
- It has been suggested that carrier screening should be performed on people in high- risk populations.

2.10 IMMUNOLOGIC FACTORS

Other immunologic factors have been reported to be associated with COPD. Various immunoglobulin deficiencies of IgG subtypes and IgA have been described in association with COPD (Webb *et al.*, 1974; Bjorkander *et al.*,1985; Oxelius *et al.*,1986a; Oxelius *et al.*,1986b; O'Keefe *et al.*, 1991).Vitamin D binding protein is a multifunctional protein that , in addition to binding Vitamin D, enhances the effects of chemotactic factors (Kew *et al.*,1995; Piquette *et al.*,1994; Metcalf *et al.*,1991; Kew *et al.*,1988). Isoforms of the protein have been found with decreased or increased frequency (Horne *et al.*, 1990; Schellenberg *et al.*,1998) in COPD patients, although results have been inconsistent (Kaufmann *et al.*,1983). A variety of other inflammatory mediators such as the role of lymphocyte response, and mediators thereof , are under investigation. Lymphocytes appear to respond through TH-1(Majori *et al.*,1999) mechanisms, and work on describing the mediators of this response has begun (Kemeny *et al.*,1999; Jeffery *et al.*, 1999).Variation in certain mediators of inflammation, including tumour necrosis factor alpha and interleukin (IL)-8 (Keatings *et al.*,1997), have also been found in COPD subpopulations, and IL-4 receptor blockade has been associated with a reduction in neutrophil survival (Lee *et al.*, 1999).

Genotype	Amount of AAT produced (percent of normal)	Possible Health Concerns	Population Incidence
MM	normal (100%)	None	86.5%
MZ (Carrier)	somewhat reduced (60%)	usually none	3.9%
ZZ	significantly reduced (7%)	significant risk for lung and/ or liver disease	0.05%
FM	slightly reduced (97%)	None	0.4%
FS	somewhat reduced (66%)		0.05%
SS	somewhat reduced (71%)	None	0.1%
SZ	reduced (39%)	slightly increased risk for lung disease	0.3%
MS	within the normal range (81%)	None	8.0%
Null/null	none (0%)	lung disease only	< 0.7%

Table 3: Levels of serum alpha 1 antrypsin and respective genotypes. [Handbook of Genetic Counselling (http://en.wikibooks.org/wiki/Handbook of Genetic Counseling)].

A positive association between pulmonary surfactant and airways diameter has been described ; surfactant proteins were observed to inhibit pulmonary inflammation (Hohlfeld *et al.*, 1997). Production of particular surfactant proteins is inhibited by tobacco smoking (Honda *et al.*, 1996).Linking of these observations in the pathogenesis of COPD has yet to be reported. As genetic polymorphisms influencing expression or function of these and other components of the inflammatory cascade are discovered, their possible roles in the differential sensitivities in the pathogenesis of the disease will be more clear.

An association between the presence of blood type A and COPD has been reported (Beaty *et al.*, 1984; Cohen *et al.*, 1980; Cohen *et al.*, 1977) but others have failed to confirm this relationship

(Higgins *et al.*,1982;Vestbo *et al.*, 1993).Many but not all people secrete the ABO blood group antigen into the upper and lower respiratory tract, and secretor status has inconsistently been associated with airflow limitation (Sandford *et al.*,1997; Barnes, 1999; Abboud *et al.*,1982). Lewis blood group and Lewis antigen secretor status have also been reported to be associated with airflow limitation (Home *et al.*, 1985; Kaufmann *et al.*, 1996).In the absence of a consistent association with COPD, or a clear biologic rationale for such an association, the roles, if any, of blood antigen specificity and secretor status in the pathogenesis of COPD remain uncertain.

2.11 GENE-ENVIRONMENT INTERACTION

A model for understanding the development of the disease has emerged, however , from investigations of the major environmental risk factor for COPD (tobacco smoking) and of the most clearly understood genetic risk factor (Antiprotease deficiency).In this model of COPD pathogenesis, the maintenance of a healthy lung structure and function depends on the ability of homeostatic mechanisms to protect the airways and lung parenchyma from both environmental insults and the inflammatory responses to these insults (Snider *et al.*, 1989).Chronic airflow obstruction can develop because of a high degree of chronic inflammation or other injurious processes, a deficiency in protective mechanisms, or both. Lung antiproteases, which modulate proteolytic enzymes released by inflammatory cells and thereby prevent damage at sites of inflammatory response, appear to be crucial in this balance (Kuhn,1986).It is generally agreed that many lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) have polygenic inheritance, and that the association of a specific genotype or genotypes with the disease is likely to vary between populations. Furthermore, it is recognized that the etiology of various lung diseases involves complex interplay between genetic background and exposure to multiple environmental stimuli, and understanding the mechanisms through which the genes and environment interact represents a major challenge. Experimental approaches and challenges must be overcome to indentify disease genes for asthma, COPD and chronic bronchitis, and occupational lung diseases (Ross *et al.*,2004; Mapp *et al.*,2005).In particular common polymorphisms in CD14, glutathione –S-transferase, and tumour necrosis factor alpha have been found to be important in gene-environment interaction and asthma pathogenesis. An understanding of the gene-environment interactions in complex lung diseases is essential to the development of new strategies for lung disease prevention and treatment (Kleeberger *et al.*,

2005). Antioxidants and other less well understood protective mechanisms may also be important in preserving normal lung function in the face of a lifetime of exposure to potentially injurious environmental factors.

Genetic factors likely affect susceptibility to oxidative injury. Metabolic processes that are up regulated in response to proinflammatory stimulants (including electron transport in the mitochondria, enzymatic activity such as cyclooxygenase, and phagocytic activity in the white blood cells) generate part of the oxidative burden to lung. Oxidants, may then damage the airways, resulting in chronic airflow obstruction. Lower levels of antioxidants have been correlated with airflow obstruction (Linden *et al*., 1993).Oxidative balance has been implicated in the pathogenesis of COPD, but the role of the host in generating oxidative stress and balancing protective mechanisms needs further investigation. Genetically controlled antioxidant defense systems may also play an important role in determining susceptibility, both to free radicals released by the inflammatory cells and to oxidants inhaled from the environment. The lungs possess several antioxidant defense mechanisms, including the reducing capabilities of iron and other metals , non-enzymatic antioxidants (including Vitamin E and C), the enzymatic scavengers (including glutathione thiol, superoxide dismutase, and microsomal epoxide hydrolase), which are under genetic control. The observations that the enzymatic antioxidants are under genetic control and that allelic variation amongst them alters their abilities to reduce free radicals (Koyama *et al.*, 1998; Smith *et al*.,1997) suggest that genetic factors may place some persons at greater risk for oxidant injury. The understanding of the cellular and biochemical pathogenesis of COPD remains rudimentary, it is increasingly evident that the host response to environmental factors is subject to genetic variation.

While active cigarette smoking is clearly important, only 25% of continuous smokers were found to develop COPD (Lokke *et al*., 2006) suggesting that individual susceptibility or genetic factors may play a role.Alpha-1-antitrypsin deficiency is a well recognized factor for COPD (Laurell *et al.*, 1963). The GSTs, a superfamily of enzymes consisting of alpha, mu, pi, theta, kappa, zeta, sigma, omega and delta families, are critical in the conversion of many reactive electrophilic compounds to less reactive metabolites which are excreted as glutathione conjugates. Several common variants of GSTs have been well characterized and are associated with certain respiratory diseases (Hayes, *et al.*, 2000).

The GSTM1 and GSTT1 genes are located on chromosomes 1p13 and 22q11.2, respectively and are actively engaged in the detoxification of cigarette smoke and environmental xenobiotics (Mehrotra *et al.*, 2010).These genes are expressed in the respiratory tract and have common functional variant alleles that result in either a total absence or a substantial change in enzyme activity.

Several studies have been conducted on the role of polymorphism of genes regulating the GST enzyme, including GST theta 1(GSTT1), GST mu 1(GSTM1) in chronic obstructive lung disease, with controversial results (Harrison *et al*., 1997; Baranova *et al.*,1997; Yim *et al.*,2000;Yim *et al*.,2002; Cantlay *et al.*, 1994; Ishii *et al.*,1999; Cheng, *et al.*, 2004; Lu *et al*.,2002; Xiao *et al.*,2004).

In order to find out whether polymorphisms of the antioxidant genes are associated with the development of lung function decline in the people living in and around the vicinity of open cast coal mines, functional polymorphisms for the GSTT1,GSTMI have to be examined.

While the environmental determinants of pulmonary function have been extensively studied, such as smoking and ambient air pollution-the genetic determinants have recently received increasing attention. Genetic epidemiological studies of pulmonary function are of potential importance in understanding normal pulmonary function and the etiology and prevention of COPD and other respiratory diseases (Yue, 1999). Heritability is a population-specific parameter and is affected by the environment in which the population developed. In addition, if there is an interaction between genotype and environment—for example, smoking may alter the genetic effects of pulmonary function—it is almost impossible to separate the genetic variance and environmental variance completely. Because of these limitations, heritability estimation should be explained with caution (Yue, 1999).

Khoury *et al*., 1986, has documented gene-environment interaction in COPD. Another study has suggested that a gene-environment interaction may influence pulmonary function (Cannings *et al*., 1978).The pulmonary function phenotype expression of a gene may therefore depend on environmental variables such as smoking. Ignoring gene-environment interactions may result in underestimating thegenetic effects on quantitative traits (Khoury *et al*., 1986).

A variety of environmental factors have been associated with changes in lung function. Myriad exposures including workplace, atmospheric, domestic, dietary, and others, play putative roles in altering lung function. Tobacco smoking is one of the best documented environmental cause of chronic airflow obstruction and is implicated in more than 90% of COPD cases (Brandt *et al*., 1984).Neutrophils and macrophages produce respectively, human neutrophil elastase (HNE) and metalloelastases; these proteolytic enzymes have been implicated in the parenchymal destruction that results in emphysema.

Individuals with severe deficiency of API, the major inhibitor of HNE activity, experience premature development of emphysema (Snider *et al*., 1989).Elastase and elastase-API complexes (Gast *et al.*,1990) are found in higher concentration in the bronchoalveolar lavage fluid of smokers. The plasma of COPD patients contains elevated levels of elastin-derived peptides compared with that of non-smokers (Akers *et al.*,1992;Schriver *et al.*,1992).Intratracheal instillation of proteases with elastolytic activity provides an animal model for emphysema(Snider *et al.*, 1991).Protease and protease inhibitors may also play a role in the pathogenesis of small airway disease. In addition to the degradation of airway elastin, HNE and API have, respectively, pro and anti-inflammatory effects (Stockley *et al.*,1994;Doring *et al.*,1994).Increased elastin degradation has been observed in smokers with rapid decline in lung function, an effect seen equally in subjects with predominantly emphysema and those with predominantly airway disease(Gottlieb *et al*.,1996).Tobacco smoke contains a heavy oxidative burden for the lungs both in the gas phase and in the tar components (Anonymous, 1986a) and causes a transient decline in the antioxidant capacity 1hour after smoking a single cigarette (Rahman *et al.*,1997; Rahman *et al*.,1996).

Both current smoking and COPD exacerbations are associated with increased levels of markers with oxidative stress and decreased level of serum antioxidants (Rahman *et al.*,1997; Rahman *et al*.,1996).Depletion of the buffer against free radicals, either as a result of a decreased antioxidant capacity, may alter the protease/antiprotease balance by inactivation of antiproteases (Repine *et al.*,1997) thereby contributing to the pathogenesis of COPD.

Agent	Established	Suspected
Tobacco	Active Smoking	Environmental Tobacco Smoke
Biologic Dusts	Grain	Cotton Cork/wood/paper
Inorganic Dusts	Silica (gold and coal mining)	Chalk, talc
Fumes, gases		Metal ,Chlorine SO_2,H_2S,Styrene Polyvinyl chloride, Methyl methacrylate
Pollution		Particulate NO_2,SO_2, O^{3-},Biological Cooking Fuels
Dietary Factors		Antioxidant vitamins (A,C,E) (appear to be protective) Linoleic Acid,Fish Oils appear to be protective) Ethanol (effect mixed)

Table 4: Environmental factors influencing the risk of COPD (Walter *et al.*, 2000).

Passive exposure to environmental tobacco smoke (ETS) has been hypothesized to be a risk factor for the development of COPD. Dose response curve in smokers, measured in terms of pack years and reduction in FEV1, suggest that the estimated ETS exposure of non-smokers living and working with smokers may be sufficient to contribute to the development of airflow limitation (Anonymous 1986b).The composition of unfiltered sidestream tobacco smoke differs

in component concentrations from that of smoke inhaled directly during active smoking , as it contains higher concentrations of some toxins .Studies of airflow obstruction and ETS in adults are complicated by the difficulties of assessing lifetime cumulative ETS exposure, accounting for other respiratory irritants, and ascertaining any active smoking in the past. Evidence definitively linking ETS exposure to chronic airflow obstruction in adults is lacking because of methodologic difficulties with existing studies (Anonymous, 1986a; Brunekreef *et al.*, 1985).There is a consensus, however, that passive exposure is associated with reduced pulmonary function in children. A longitudinal study by Tager *et al.*, 1983 demonstrated a 10.7% reduction in FEV1 among children with a parent that smoked compared to children of a non-smoker. It has been estimated that ETS exposure in childhood results in a 0.5% FEV1/year decrease in growth of FEV1(Anonymous, 1986b).The reduction of maximally attained function is hypothesized to predisposed to impairment of pulmonary function in later life (Samet *et al.*, 1996).Occupational exposure to biological dusts has been established as a risk factor for COPD (Buist *et al.*, 1988).Grain dust exposure has been established as a risk factor for COPD (Buist *et al.*,1988).Cotton dust exposure in the work place has been reported to be associated with accelerated longitudinal lung function decline (Christiani *et al.*,1999) but not all studies confirm these findings (Fox *et al.*,1973). Other workplace biologic dusts implicated in the development of chronic airflow obstruction include coke, (Alegre *et al.*, 1990) wood (Carosso *et al.*, 1987), sugar (Bohadana *et al.*, 1996), and paper dusts (Toren *et al.*, 1996). Exposure to certain inorganic dusts in mining and other occupations has also been shown to be a risk factor for the development of chronic airflow obstruction independent of smoking and pneumoconiosis (Coggon *et al.*, 1988; Meijers *et al.*, 1997; Becklake *et al.*, 1987). Occupational chalk dust (Bohadana *et al.*, 1996) and talc (Fine *et al.*, 1976) exposure have also been reported to be associated with chronic airflow obstruction. The degree of airflow limitation is directly related to the degree of exposure, and there is at least an additive effect of smoking (Hennberegr *et al.*, 1996; Sandford *et al.*, 1997). Outdoor air pollution is a hypothesized but unproven risk factor for the development of COPD (Sasaki *et al.*, 1998). Although the relative independent contribution of individual pollutants remains uncertain,(Anderson *et al.*,1997).Studies of the acute effects of air pollution, suggest that episodic increases in particulate matter (Schwartz *et al.*,1993; Schwartz *et al.*,1994; Schwartz *et al.* ,1996;Moolgavkar *et al.*, 1997) NO_2,SO_2 (Jorgensen *et al.*,1996; Sunyer *et al.*,1993) and ozone (Schwartz *et al.*,1993; Schwartz *et al.*,1994; Schwartz *et*

al. ,1996;Moolgavkar *et al.*, 1997) may cause increased respiratory symptoms and hospitalizations in persons with COPD. Less is known about the long term effects of air pollution and pulmonary function. Some crossectional studies have revealed lower mean functional levels and a higher prevalence of airflow obstruction among adults living in more polluted communities (Schindler *et al.*, 1998; Neri *et al.*, 1975; Xu *et al.*, 1998; Tzonou *et al.*, 1992; Tashkin *et al.*,1994).Studies of air pollution and lung function in children revealed lower levels of lung function (Schwartz *et al.*,1989) in children living in communities with higher levels of NO2, O3- , and particulate matter. Living in close proximity to a major point source of industrial emissions has been reported to be a risk factor for pulmonary function impairment among children (Hsuie *et al.*,1991) Cohort studies suggest that the rate of longitudinal lung function decline in adults is related to ambient air pollution levels (Taskin *et al.*,1994).The quantitative impact of particular pollutants on pulmonary function is not fully understood , but dose – response relationships have been demonstrated in several studies(Xu *et al.*,1998; Cullinan *et al.*,1997) further suggesting an impact of air pollution on long-term lung function.

The interaction among anthropogenic activities such as coal mining and human health is very complex and difficult to assess. The relative prevalence and severity of mining related occupational lung diseases are a function of commodities mined, airborne hazard exposure levels, the period of exposure and co-existing illnesses or environmental conditions and lifestyle. It is thus critical when evaluating the potential effects of air toxics on respiratory disorders or other health end points, to determine where the exposure occurs, the contribution of each pollutant, its anthropogenic origin as well as to define the target population. Understanding the environmental and genetic risk factors of accelerated in a population is a first step in prevention strategy against the worldwide increasing respiratory pathology of COPD.

It is believed that COPD is both environmental and genetic .However specific genetic factors in the development of COPD have not been clearly identified, except for protease inhibitor types. While the environmental determinants of pulmonary function have been studied extensively- eg. Smoking and ambient air pollution, the genetic determinants are receiving increased attention. Genetic epidemiological studies of pulmonary function are of potential importance in understanding normal pulmonary function and the etiology and prevention of COPD and other respiratory disorders. Differences in the prevalence of COPD in different ethnic groups are likely

to be accounted for by the differing frequency of genes relevant to pathogenesis, so that exploration of these differences at a molecular level may be informative .Oxidative stress is increased in COPD and may be an important determinant of disease severity and progression .Several enzymes regulate the formation of reactive oxygen species and the synthesis of endogenous antioxidants.

All of these enzymes could show genetic polymorphism resulting in alteration in oxidative stress responses. These polymorphisms may be associated with altered susceptibility to disease, to disease severity, and to the response to treatment.