CHAPTER -5

DISCUSSION

COPD is a leading cause of chronic morbidity and mortality and should be a major public health concern. Reliable COPD prevalence data are lacking for many parts of the world, despite the frequency of major risk factors for COPD, such as cigarette smoking, use of biomass fuels, and air pollution. Approximately 15% of smokers would acquire COPD (Fletcher *et al.*, 1977; Tashkin *et al.*, 1983). The World Health Organization (WHO) estimates that there are approximately 1.1 billion smokers in the world, or approximately one third of the global population >15 years old. (Tobacco alert, WHO 1996).The use of biomass fuel, such as wood for cooking, increases the risk of COPD by three to four times, (Malik *et al.*, 1985; Dennis *et al.*, 1996) and may be an important contributor to COPD prevalence for some parts of the world, particularly in developing countries and rural areas (Chen *et al.*, 1990; De Koning *et al.*, 1985). Air pollution increases the prevalence of COPD by an estimated 2% for each 10µg increase in particulate matter—10/m^3 (Kunzli *et al.*, 1997). The WHO has published data placing the world-wide prevalence of COPD at 0.8% (Murray *et al.*, 1996). Other reports place the prevalence of COPD substantially higher, at approximately 4 to 6% (National Heart, Lung and blood Institute, 2000; Gulsvik *et al.*, 1999).

Our study in the coal mine area reported significantly greater prevalence (69.4%) of COPD than the Non-Coal mine area (33%) [$Z = 9.06$, $p<0.01$, (at 95% CI)]. A total of 225 males and 61 females were reported to have COPD in the Coal mine area; 58 males and 48 females suffered from COPD in the non-Coal mine area. In both the areas, we found a male predominance in COPD cases. There were around 486 people living within the radius of 1-1.5km of the mine area. Our statistical population was that these people who have been living there for 11 to 60 years within this 1.5km of the mine area are effected. However, the duration of residing of different families was different and thus the exposure to the pollutants was also different.

Among the 486 people, we drew 412 people randomly as the sample for our study irrespective of age, sex, and livelihood. Prior to the study it was not possible to detect people having COPD and Non-COPD categories; hence the fractions of the sample were not of equal size. Moreover, our sample also depended on the willingness of the people for demographic data, spirometry and blood samples for analyses. Later on, we categorized the sample into different fractions of male-female, age, smoking status etc. The same criteria was also followed while surveying the non-coal mine area. Moreover, majority of the male population were either smokers or daily wage workers in the coal mine.

The conflicts among published COPD prevalence rates may be due to many factors, including true differences in disease occurrence, differences in defining obstructive lung disease, cultural biases, and whether spirometry was used to confirm the diagnosis .The resulting confusion may result in under recognition of the burden of COPD, with significant implications. These potentially include the failure of physicians to consider the diagnosis of COPD in their practices and the failure of governments and other payers to allocate appropriate resources for COPD health care (Halbert *et al.*, 2003).In order to assess possible reasons for conflicting COPD prevalence estimates, Halbert *et al.* performed a critical evaluation of the published COPD epidemiology literature; and found that overall COPD prevalence rates ranged from 1 to 18%, and tended to vary by the method used to estimate prevalence. In general, rates tended to be higher for male than female subjects which are similar to our findings. A summary of field studies from some states of India on prevalence of COPD (published in last three decades) shows prevalence in the range of 5-12.5% in males and 3.2-4.5% in females (Jindal , 2006) .Hardly, any information on the disease is available from the rest of India.

The COPD prevalence data presented by (Halbert *et al.*, 2003) demonstrates the paucity of well-designed epidemiologic studies from most regions in the world. In many areas, available information gives an impression of very low prevalence rates. Consequently, policymakers in these areas—thinking that COPD is relatively uncommon—often assign a low priority to COPD when allocating health expenditures. Physicians in these areas may be less likely to suspect COPD, and less likely to correctly diagnose it, reinforcing the impression that COPD is rare. However, comparable regions with similar population compositions and risk factor exposures have often shown significantly higher COPD rates when rigorous measurement has been done. The well-documented relationships between these risk factors and the occurrence of COPD suggests that much of the prevalence data used in these areas is derived from methods that may not accurately represent the true burden of disease. The most striking example of the potential mismatch between risk factor exposure and COPD prevalence concerns the primary risk factor for COPD, as cigarette smoking. Many countries or regions with high rates of smoking have a low reported COPD prevalence. In some cases, local theories involving genetic differences have been developed to explain differences in the expected rates of COPD (Tatsumi *et al.*, 2001; Sevama *et al.*, 2001).

Despite the documented high rates of cigarette smoking in many countries in Asia, Latin America, and Africa, (Halbert *et al.* 2003) found no nationally representative measurements of COPD for these areas. We observed that 22.3% smokers in the coal mine and 24% in the non-coal mine site were having COPD which is almost near to the estimates of Hogg (2004) and Piqueras (2001).

A systematic review of available data, however, suggests that prevalence underestimates rather than novel biological mechanisms may be at work. These prevalence estimates depend on many factors. COPD varies with age and smoking status, occurring rarely in individuals >40 years old, and less frequently in nonsmokers. Thus, it is essential to note the base population for reported prevalence. Some published rates included children and younger adults in the denominator, (Brabin *et al.*, 1994) whereas others reported prevalence among adults or a subset of adults only. When reported by gender, the prevalence of COPD was generally higher for male than for female subjects. Higgins *et al.* (1977) reported that 14% of adult men and 8% of adult women had chronic bronchitis, obstructive airways disease, or both. Generalizability of study findings to the wider population depends on the degree to which the study subjects were drawn from a representative subset of the population.

The most possible reason for the increased occurrence of COPD amongst males (irrespective of smoking status) in the coal mine site could be that more number of males were found to be working at the coal mine as daily wage labourers and were residing with their families near to the coal mine. The male predominance was also observed in the non-coal mine area.

Many studies used spirometry, either in conjunction with a clinical examination or used alone, to reach a prevalence estimate. Three estimates from two northern European countries were based on a combination of clinical examination and spirometry (Isoaho *et al.*, 1994; von Hertzen *et al.*, 2000; Gulsvik *et al.*, 1979) and a fourth used a combination of symptoms and spirometry (Bakke *et al.*, 1991). Nine estimates were based on spirometry alone, even though some of these studies collected other data (symptoms, smoking status, etc) concurrently. There was considerable variation in the spirometric criteria for defining COPD. Only three studies clearly stated that reversibility testing was done to exclude persons with completely reversible obstruction (*ie*, asthma). Two of these were performed in Spain and represented a broad range of ages,(Marco *et*

al. , 1998; Pen *et al.*, 2000) while the third was limited to ages 60 to 75 years (Dickinson *et al.* ,1999).Spirometry-based studies produced rates ranging from 2 to 22.1%, but the majority of estimates were between 4% and 10%.Two studies provided separate estimates based on clinical examination plus spirometry and spirometry alone in the same population, allowing a comparison between these two methods (von Hertzen *et al.*, 2000;Bakke *et al.,* 1991).

We diagnosed COPD on the basis of observation of respiratory symptoms such as breathing difficulty, chest pain, H/O coal dust exposure, chronic cough and phlegm, chronic bronchitis, pre-bronchodilator and post bronchodilator spirometry. Lack of agreement on the definition of COPD is another source of variation in prevalence estimates. Terminology varies by country, by physician, and by time period. Some studies define COPD narrowly as chronic bronchitis or emphysema. Others use the terms *chronic obstructive lung disease* or *chronic obstructive airways disease*. Reactive airways disease or asthma may be included in the definition of COPD, and in other studies it may be excluded. These differences in terminology can influence the reported prevalence rates for COPD. What is considered COPD has also been confounded by revisions in coding terminology over time (Thom *et al.*, 1989). Specifically, during the time that the eighth revision of the International Classification of Diseases (ICD) codes (ICD-8 from 1968 to 1978) was in use, the United States and Canada introduced a code (519.3) for "chronic obstructive lung disease without mention of asthma, bronchitis, or emphysema." All countries using the ninth revision of the ICD-9 in 1979 adopted the new code 496 for "chronic airways obstruction not elsewhere classified." Over the past decade, there has been a growing consensus that COPD includes airway obstruction that is not completely reversible, and spirometry is becoming the "gold standard" for definitive diagnosis. The use of spirometry in epidemiologic studies, however, is influenced by the variation in the lung function parameters that "define" COPD according to various guidelines. For example, the American Thoracic Society (ATS) defines an FEV1/FVC ratio "below the lower limit of normal" as COPD (ATS, 1995b). The European Respiratory Society (ERS) guidelines for COPD list FEV1/slow vital capacity (VC) <0.88 predicted in male subjects or <0.89 in female subjects as the criteria (Sikafas *et al.*, 1995).Applying different COPD definitions, they found rates of obstruction ranging from 11 to 57% of their study population. They found a disparity based on a large prevalence of mild obstructive abnormalities when ATS criteria were applied, as compared to ERS and typical

European clinical practice criteria. Application of these different criteria for obstructive lung diseases to data available from the third US National Health and Nutrition Examination Survey yielded COPD prevalence estimate variations of> 100% (Celli *et al.*, 2001). Thus measuring COPD prevalence is complicated by several factors, which relate to the disease state itself, the variability of diagnostic criteria, the difficulty of measuring COPD in a population, and potential diagnostic bias.

Age wise, we did not find any significant difference amongst COPDs and Non-COPDs in both the sites. The mean age was above 30 in all the categories. The NHBLI reports 12.1 million adults 25 and older were diagnosed with COPD 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 People over the age of 50 are more likely to be considered disabled, however, the damage started years before [COPD International (Some Alarming Statistics, United States 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.

Airflow limitation, measured by reduced FEV1, progresses very slowly over several decades, so that most patients with symptomatic COPD are in late middle age or are elderly. Thus, the prevalence of COPD is age dependent, (Kazuhiro *et al.*, 2009).The link between aging and the pathogenesis of COPD is strongly supported by numerous studies (Tuder *et al.*, 2006; Buchman *et al.*, 2008; Karrasch *et al.*, 2008; Vogelmier *et al.*, 2007; Kojima *et al.*, 2007).Senescence is a complex outcome of both intrinsic and environmental factors, especially oxidative stress, and therefore the role of cigarette smoke/noxious gas is a key factor linking aging lung to COPD. The close relationship between COPD and smoking has been reported by several authors (USDHHS 1984b; USDHHS, 1990; USDHHS, 1986; Higgenbottam *et al.*, 1980; Fletcher *et al.*, 1976b; Lange *et al.*, 1989; Dossman *et al.*, 1981).

Approximately 15 to 20% of smokers develop COPD (US Department of health and Human services, 1984; Lange *et al.*, 1989; Dossman *et al.*, 1981). A significant inverse relationship

exists between the number of cigarettes smoked per day and the cumulative cigarette consumption measured in pack-years and FEV1 values (Fletcher *et al.*, 1976b, Lange *et al.*, 1989; Dossman *et al.*, 1981).

We found a significant ($p < 0.05$) prevalence of 24.3% amongst male and female COPD smokers in the non-coal mine area. We also found a significant inverse relationship of lung function versus smoking pack years amongst symptomatic and asymptomatic people in the Non-coal mine area [$r = -0.816$, $R^2 = 0.665$, $p = 0.00$].

The pack years of smoking was significantly different amongst COPD smokers and Non-COPD smokers [$t = 29.22$, $df = 148$, $p = 0.002$].For every unit change in smoking pack years, there was 0.889 times significant decline in lung function [Beta = -0.889, $t = -17.15$, $p = 0.00$].

In cross-sectional surveys, smoking history is strongly associated with chronic respiratory symptoms, diagnosed chronic bronchitis and emphysema, and obstructive pulmonary function abnormalities. Cohort studies have revealed that adult smokers experience faster longitudinal pulmonary function decline than non-smokers (Anonymous, 1984)and that this accelerated decline returns to the normal rate of aging-related decline following smoking cessation even if the cessation is intermittent (Fletcher *et al.*,1976b; Kaufmann *et al.*,1979). In addition, smoking has an adverse impact on lung function and growth and on the peak pulmonary function attained among those who take up the habit early. Smoking teenagers experience a reduced rate of lung function growth, and young adult smokers experience an earlier onset of pulmonary function decline from the plateau of maximal function achieved in the third decade of life (Tager *et al.*, 1988).Substantial evidence suggests the accelerated decline of lung function in cigarette smokers results from smoke induced inflammatory processes. 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). 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. A longitudinal study by Tager *et al.* (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).

In the coal mine site, the association of smoking (22.3%; $p > 0.05$) towards the development of COPD was not significant and 47.08% of the study population suffering from COPD were non-smokers; thus indicating the influence of other independent variables causing the disease; in the non-coal mine area only 8.75% of the population comprising of non-smokers were suffering from COPD.

Cohort studies suggest that the rate of longitudinal lung function decline in adults is related to ambient air pollution levels (Tashkin *et al.*, 1994).

The quantitative impact of particular pollutants on pulmonary function and dose-response relationships have been demonstrated in several studies further suggesting an impact of air pollution on long-term lung function (Cullinan *et al.*, 1997; Xu *et al.*, 1998).

Less is known about the long term effects of air pollution on pulmonary function. Some cross sectional studies have revealed lower mean pulmonary function levels and a higher prevalence of airflow obstruction among adults living in more polluted communities (Tzonou *et al.*, 1992; Schindler *et al.*, 1998; Xu *et al.*, 1998).

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.* 1996;Moolgavkar *et al.*, 1997; Morgan *et al.*, 1998;Schwartz *et al.* 1994;Spix *et al.* , 1998) may cause increased respiratory symptoms and hospitalizations in persons with COPD. 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 NO_2, 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).

The importance of dust exposure specifically, mineral dust in underground miners for the development of respiratory symptoms, airflow obstruction, and COPD is well established (Becklake *et al.*, 1989b; Oxman *et al.*, 1993). Over recent decades it has increasingly been recognized that occupations with biological or inorganic dust exposure have an increased prevalence of respiratory symptoms and chronic bronchitis.

Deficits of lung function, associated with increasing cumulative dust exposure, have previously been reported in underground coalminers in the United Kingdom (Soutar *et al.*, 1986; Love *et al.*, 1982). Reports from United states suggest that such deficits have also been found in drillers from open-cast mines (Amandus *et al.*, 1987).

We performed a detailed seasonal analysis of concentration of air components viz., RSPM, SO_2 and NO_2 in the coal mine area and non-coal mine area for two consecutive years (2009 to 2010); and found very high levels of RSPM , SO_2 and NO_2 in the coal mine site during both the years. There was significant seasonal variation in these parameters. The highest values of RSPM and NO_2 in the coal mine area and non-coal mine area were observed during the period Dec-March and the lowest was observed during the period Apr-July. The most possible reason for this could be that the period Dec-Mar which is relatively dry and there are records of less rainfall (Table 7b, 7c) during this period in both the sites and comparatively higher rainfall during the period Apr-Jul. which could cause the air components to settle. The annual averages were very high for all these parameters.

The overall annual data reveals that our study site is considerably polluted and the population in the area is exposed to recurrent episodes of acute air pollution.As our study was specifically focussed on the residential areas very near to the coal mine areas of Ledo, India, undoubtedly, respirable mixed coal dust was a significant contributor to the suspended particulate matter. Moreover, we also found that the symptomatic COPDs were exposed for a significantly longer duration than the Non-COPDs ($t = 7.03$, $df = 132$, $p = 0.009$). Several longitudinal, epidemiological and associative studies have established that acute episodes of atmospheric pollution causes increased risk of adverse pulmonary events (Dockery *et al.*, 1993; Laden *et al.*, 2006 ; Brook *et al..*, 2004; Pope *et al.*, 2006) .

A cross-sectional study in Merseyside conducted by Brabin *et al.* (1994) in school children aged 5-11 years exposed to coal dust and air pollution; established significant [Odds Ratio 1.55 (1.17—2.06) at 95% CI] increased prevalence of lung function decline irrespective of their parents being smokers or non-smokers. Standard dust deposit gauges on the schools confirmed significantly higher dust burden in the exposed zone. A Swiss study looked at the respiratory health of primary school children and noted that respiratory symptoms were highest in the area with the highest level of suspended particulates in the air and that they were independent of levels of nitrogen and sulphur oxides (Braun *et al.*,1992) .The Monkton coking works study from the North of England and the Munich/Leipzig study from Germany found similar results (Brunkreef *et al.*, 1992). Such form of inhalational injury causes a low grade inflammatory exudation of fluid and cells into both large and small bronchi, bronchioles with minimal effects on lung function; but in susceptible individuals, this normal inflammatory response is amplified (Hogg *et al.*, 2009).

Undoubtedly, the coal dust at the open cast mining site at Ledo constituted most of the respirable suspended particulate matter and we recorded the cumulative exposure to coal dust for each individual staying near the coal mine in terms of years of exposure depending on their stay in the coal mine site. The coal mine has been there since 1870 and most of them were either permanent residents or emigrants since 30 to 40 years ; and depended on the coal mine for their livelihood. People belonging to COPD group were found to be exposed for a longer duration to coal dust than the Non COPDs ($p<0.05$).There was significantly higher prevalence of COPD in the coal mine site than the non-coal mine site [$Z = 9.06$, $p<0.01$, (at 95%CI)].

The relative prevalence and severity of mining related occupational lung diseases are a function of the commodities mined, airborne hazard exposure levels, and co-existing illnesses or environmental conditions and lifestyle. The contamination of atmosphere from anthropogenic sources such as coal mining, industrial sources as well as local conditions generated either in home or workplace make a significant contribution as environmental factors, to development of chronic airflow obstruction. The coal-based industries in India are considered to be one of the chief industrial emitters in India. North East part of India has coals with different physicochemical properties compared to other Indian coals with higher sulphur content (Khare *et al.*,2010).

The air analysis of the non-coal mine area showed normal levels of all the air components thus attributing the cause of COPD to other independent variables.

Cross sectional analyses of respiratory diseases in coal miners have provided strong evidence for an exposure-relationship between cumulative exposure to dust and decrements in pulmonary function and increased prevalence of symptoms of chronic bronchitis (Rogan *et al.*, 1973; Morgan *et al.*,1974; Hankinson *et al.*, 1977;Marine *et al.*,1988).

Studies of Rogan *et al.*, (1973) and Kibelstis *et al.*, (1973) have reported either obstructive lung disease occurring as a chronic process requiring the accumulated insult of dust exposure over many years or restrictive lung disease among subjects who evidence large opacities on chest x-ray films.

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.

Besides environmental factors, exposure to other factors such as oxidative injury also plays an important role in the pathogenesis of COPD (Behera *et al.*, 2010). Such injury, resulting from an imbalance between free radicals and protective mechanisms can alter the conformation of protease inhibitors and reparative enzymes, injure cell membranes, and result in mutagenesis. Free radicals appear in the lung by inhalation from the environment or by release from inflammatory cells. Genetically controlled antioxidant defense systems may also play an important role in determining susceptibility, both to free radicals released by inflammatory cells and to oxidants inhaled from the environment. The lungs possess several enzymatic scavengers including glutathione which are under genetic control. The observations that the enzymatic antioxidants are under genetic control and that allelic variation 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 glutathione system is the major antioxidant mechanism in the airways. Several common variants of GSTs have been well characterized and are associated with certain respiratory diseases (Hayes *et al.*, 2000).

Chronic Obstructive Pulmonary Disease (COPD) is thought to be the result of environmental triggers in genetically susceptible individuals .Although cigarette smoking is the main

environmental risk factor ,only about 15% of smokers develop clinically significant disease (ATS,1996), suggesting other influences on disease expression. This is supported by family studies showing ancestral aggregation of spirometric abnormalities both in general population (Lewitter *et al.*, 1984) and in the relatives of patients with COPD (Kueppers *et al.*,1977). Moreover, differences in the rate of decline of lung function between smokers (Fletcher *et al.*,1977) suggests a gene-environment interaction.

Oxidative stress plays an important role in the pathogenesis of interstitial lung diseases. This is especially relevant for the lung which is exposed to oxidant pollutants, and endogenous oxidants produced by inflammatory cells (Yucesoy *et al.*,2005).Many chemical and physical agents in the environment including mineral dusts are potent generators of reactive oxygen species (ROS).In response to these agents, various enzymatic and non-enzymatic defence system help to protect cells and tissues from oxidative damage, and it is possible that genetically acquired variations in these systems account for interindividual variations in the response to oxidative stress (Yucesoy *et al.*,2005).In this respect there is substantial that antioxidant genes such as glutathione S-transferases (GST) which are important components of lung defence in response to oxidative stress are highly polymorphic (Hayes *et al.*,2000).

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). There is an increasing evidence that oxidative stress is an important feature in COPD (Henricks,2001). The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract (Cantin *et al.*, 1990).

Tobacco smoke contains a heavy oxidative burden for the lungs both in the gas phase and in the tar components.(Anonymous, 1986) 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.

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).When we analysed the GST gene for the subjects from coal mine area, we found that the GSTM1 null type was significantly higher ($p < 0.05$) in the COPDs compared to Non-COPDs in the non-smoker group. The difference was not significant in the smokers. The frequency of GSTT1null type was also significant ($p<0.05$) in the non-smoker group amongst COPDs and Non-COPDs and not the smoker group.

In the non-coal mine area, GSTT1 null type was significantly higher ($p <0.05$) in both the non-smoker and smoker group amongst COPDs versus Non-COPDs. In the smoker group, the difference was not significant. GSTM1 null type was significantly high ($p<0.05$) in the non-smoker amongst COPDs and Non-COPDs. The difference was not significant in the smoker group.

Studies have been conducted on the role of polymorphism of genes regulating the GST enzyme, including GSTT1, GSTM1 with respect to COPD with controversial results (Cheng *et al.*, 2004;Lu *et al.*, 2002).Such studies have been conducted in US, Slovak, Taiwan, Chinese, Turkish, African, Korean population. Studies conducted by Cheng *et al.*, (2004), have established that GSTM1 null genotype in combination with other genes is a significant predictor in increased susceptibility to COPD.

As such we can interpret that the nonsmoker COPDs and Non-COPDs in the coal mine site are genetically predisposed due to higher frequency of GSTT1 and GSTM1 null type. The smoker COPDs and Non- COPDs were not genetically predisposed to the disease. It also implies that in the nonsmoker group, the factors influencing disease development are GSTT1, GSTM1 null type and cumulative exposure to coal dust. It is also interesting to note that there seems to be no genetic predisposition to the disease in the smoker group .We also previously reported that smoking itself did not significantly influence the development of COPD in the people of the coal

mine site as we did not find any significant association of smoking (χ^2 = 0.14, p > 0.05).Thus amongst the attributable variables, coal dust is the most potential factor for causing COPD amongst the smokers. The effect of coal dust on lung function cannot be ruled out in the non-smoker group because we also established that for every unit change in exposure years, there was 1.02 times decline in lung function and a significant negative correlation of lung function versus exposure years was also reported by us. We found no significant correlation of smoking and lung function in this population.

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).

In the non-coal mine site, most of the COPDs were at higher risk due to GSTT1 null type irrespective of smoking status. The nonsmoker COPD group seemed to be at an additional risk due to higher frequency of GSTM1 null type. We also reported a significant association (χ^2 = 4.56, p <0.05) of smoking with the development of COPD in this population. The factors causing COPD are both genetic predisposition by high GSTT1 and GSTM1 null type in non-smoker group, and GSTM1 null type in the non-smoker group. The non-smoker group is obviously at higher risk due to deficiency of both the genes in majority of the cases. It is an established fact that COPD is polygenic and the interplay of gene – environment interaction has a role to play in disease development. 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 (Ross *et al.*, 2004; Mapp *et al.*, 2005).

There are two major hypotheses in the pathogenesis of smoke-related COPD (Sethi *et al.* 2000; Rahman *et al.*, 1996). One is the protease–antiprotease hypothesis, which states that various proteases break down with the pathogenesis of cancer and emphysema (Spurdle *et al.*, 2001; Tan W *et al.*, 2000; Harrison *et al.*, 1997; Sandford *et al.*,2000). The other, nonmutually exclusive, hypothesis is the oxidant–antioxidant theory, which proposes that oxidant stress and reactive oxygen species (ROS), resulting from an oxidant/antioxidant imbalance, have important consequences for the pathogenesis of COPD.

Amongst many risk factors of COPD, the genetic deficiency of A1AT attributed to ZZ type is the best documented reasons (Carp, 1978).Phenotype M is the normal variant phenotypes S and Z are the two most frequent abnormal variants (Hutchinson, 1998). Calculated values of PiZZ prevalence are approximately: 1:1000—1:45,000 in Western and Northern Europe, 1:45,000 – 1:10,000 in Central Europe; and 1: 10,000 – 1:90,000 in Eastern Europe and in Southernmost and northern areas of the continent. In the White population of USA, Canada, New Zealand, PIZZ phenotype prevalence ranges from 1: 2000 –1:7000 individuals (Andolfatto, 2003). In our population subset, we found that all the subjects were having the normal MM type which was confirmed through site directed mutagenesis PCR and restriction digestion. Our investigation suggests that A1AT deficiency is not prevalent in our population subset.

Our investigation on Alpha 1 Antitrypsin (A1AT) gene for all the categories of subjects in both the sites revealed that all the samples were homozygous 'MM' type. Thus, our data did not fit the 'Hardy-Weinberg equation'. Sequencing of the A1AT gene also agreed to the findings of PCR and Restriction enzyme analysis. There was no change in position Glu342 GAG → Lys AAG of the sequences indicating normal MM type in all the individuals studied.

As such, we have attempted to study possible variables from both of the environmental and genetic factors influencing COPD. We have screened two genes namely GSTT1 and GSTM1 as per the antioxidant-oxidant theory; and one gene namely A1AT as per the antiprotease-protease theory. Besides, our study on environmental factors included tobacco smoking and coal dust exposure.

To understand the impact of coal dust on lung function in the subjects at the open-cast coal mine, we studied the non-coal mine area about 300kms far from the coal mine. In the non-coal mine area, we again studied the variables such as sex, smoking status, age, lung function, GSTT1,

GSTM1 and A1AT genes as discussed earlier. Finally, logistic regression analysis was done as a whole for the predictor variables at the coal and non-coal mine site; to find out the risk contribution of all the factors towards COPD. It was observed that the strongest variable that effected lung function was coal dust exposure. The people at the coal mine site were at 6.3 times greater risk than those at the Non-coal mine site [p = 0.00, OR = 6.34 (4.43 – 9.07)]. Males were found to be at 2.5 times greater risk than females [p = 0.00, OR = 2.54 (1.71 – 3.78)].People who smoked were 1.95 folds higher risk than the non-smokers [p = 0.00, OR = 1.95 (1.37 – 2.78); and age greater than 35 years was significantly higher in the COPDs than the Non-COPDs [p = 0.01, OR = 0.653 (0.463 – 0.922)]. However, the risk association was not significant. Interestingly the genes GSTT1 and GSTM1 were not found to be as potent risk factors in COPD as per the logistic regression analysis. But, when we analysed each site individually, we found an association of either GSTT1 or GSTM1 in the development of COPD. The reason for this could be attributed to the confounding effect of coal dust exposure whose influence was much stronger than any other variables we studied. As such, in comparison to coal dust exposure, the significance of GSTT1 and GSTM1 genes in COPD as susceptible risk factors was reduced. Data on A1AT gene did not fit the logit model because all the results were normal MM type for all the subjects. Against the background of these findings, we interpret that the decline in lung function was in fact due to exposure to coal dust. The other covariables as associated risk factors are smoking and male gender.