CHAPTER 6

SUMMARY & CONCLUSION

Most of the information available on COPD prevalence, morbidity, and mortality comes from developed countries. Even in these countries, accurate epidemiologic data on COPD are difficult and expensive to collect. Prevalence and morbidity data greatly underestimate the total burden of COPD because the disease is usually not diagnosed until it is clinically apparent and moderately advanced. The imprecise and variable definitions of COPD have made it hard to quantify the morbidity and mortality of this disease in developed (Pride *et al.*, 1989) and developing countries. Mortality data also underestimate COPD as a cause of death because the disease is more likely to be cited as a contributory than as an underlying cause of death, or may not be cited at all (Mannino *et al.*, 1989).

Risk factors of COPD include both host factors and environmental exposures and the disease usually arises from an interaction between these two types of factors. The host factor that is best documented is a rare hereditary deficiency of α1- Antitrypsin. The major environmental factors are tobacco smoke, heavy exposure to occupational dust and chemicals and indoor and outdoor pollution. The role of sex as a risk factor for COPD remains unclear. In the past, most studies showed that COPD prevalence and mortality were greater in men than women (Buist *et al.*, 1994; Thom 1989; Xu *et al.*, 1994; Feinleib *et al.*, 1989).

It is believed that many genetic factors increase or decrease the risk of developing COPD. The genetic risk factors that is best documented is a rare hereditary deficiency of α1-Antitrypsin(Laurell *et al.*, 1963; Hubbard *et al.*, 1991;McElvaney *et al.*, 1997).

We had studied some of the environmental and molecular risk factors amongst the people staying very near to the open-cast coal mines of Ledo, Assam. The non-coal mine site (Jorhat, Assam) was taken as control. We found that about 69% individuals were diseased at the coal mine site and 33% individuals at the non-coal mine site. In the coal mine site, amongst COPD non-smokers (n=194) and Non-COPD non-smokers (n=84), significantly, more number of symptomatic males [$\chi^2 = 7.08$, p = 0.007, OR = 0.39 (0.18 – 0.83)] and symptomatic females [$\chi^2 = 7.08$, p = 0.007, OR = 0.39 (0.18 – 0.83)] was recorded.

In the Non-Coal mine area, significantly less numbers of symptomatic COPDs were recorded in male COPD smokers versus Non-COPD smokers [$\chi^2 = 4.56$, p = 0.03, OR = 0.48 (0.23 – 1.00)] ; in female COPD Smokers and Non-COPD smokers [$\chi^2 = 4.56$, p = 0.03, OR = 2.08 (1.00 –

4.33)] ; and in male COPD non- smokers versus Non-COPD non- smokers [$\chi^2 = 4.21$, p = 0.04, OR = 0.17 (0.42 – 1.06)] ; in female COPD non-smokers and Non-COPD non-smokers [$\chi^2 = 4.21$, p = 0.04, OR = 0.17 (0.42 – 1.06).In the coal mine area, no significant difference was observed in smoking pack years between COPDs and Non-COPDs (t = 0.21, df = 132, p = 0.8).Regression analysis between lung function and smoking pack years showed that for every unit change in smoking pack years, there was no significant decline in lung function. [Beta = 0.251, t = 0.785, p = 0.434].There was no significant correlation in lung function versus smoking pack years amongst symptomatic and asymptomatic people in the Coal mine area. [r = 0.068, R^2 = 0.005, p = 0.434].In the Non-Coal mine area, the pack years of smoking was significantly different amongst COPD smokers and Non-COPD smokers [t = 29.22, df = 148, p = 0.002].Regression analysis of lung function versus smoking pack years showed that for every unit change in smoking pack years, there is 0.889 times significant decline in lung function [Beta = -0.889, t = -17.15, p = 0.00]. A significant negative correlation in lung function versus smoking pack years was observed amongst symptomatic and asymptomatic people in the Non-coal mine area [r = -0.816, R^2 = 0.665, p = 0.00]. In the coal mine area, the coal dust exposure years also significantly differed amongst COPD smokers versus Non-COPD smokers [t = 7.03, df = 132, p = 0.009]; and COPD Non-Smokers versus Non-COPD non smokers [t = 9.71, df = 276, p = 0.02].In the coal mine area, regression analysis of lung function versus years of exposure to coal dust showed that for every unit change in exposure years, there is 1.02 times significant decline in lung function [Beta = -1.020, t = -11.31, p = 0.00].A significant negative correlation was observed in lung function versus coal dust exposure years amongst the COPDs at coal mine site. [r = -0.488, R^2 = 0.238, p = 0.00].

Air quality was assessed simultaneously during each survey and sampling of air was done with Respirable Dust Sampler (Envirotech Model APM 460 BL).Data were calculated for Respirable suspended particulate matter (RSPM), SO_2 and NO_2 .All the air components were found to be very high in the coal mine site whereas in the non-coal mine site, they were within standard limits.

Genomic DNA from whole blood was extracted using standard phenol chloroform method. Genomic DNA was amplified by multiplex PCR method (Arand *et al.*, 1996).

The occurrence of GSTM1 deficiency was concluded from the absence of the specific 215bp fragment and GSTT1 deficiency from the absence of 480 bp fragment. Albumin gene fragment used as internal positive control resulted in constant 350bp band in all the samples. For both GST genes, individually, subjects were categorized as having null-type (0/0), non-null type (+/0) or (+/+).In the coal mine area,the frequency of GSTM1 null significantly differed amongst COPD Non-Smokers versus Non-COPD Non-smokers [χ^2 = 13.91, p = 0.00, OR = 2.73 (1.55-4.84)].In the smoker group the difference was not significant [χ^2 = 2.77, p = 0.09, OR = 0.49(0.19- 1.23)].

The frequency of GSTT1 null type was not significantly different in COPD smokers versus Non-COPD smokers [χ^2 = 2.84, p = 0.09, OR = 0.53 (0.24-1.18)] but a significant difference was observed in COPD Non-Smokers versus Non-COPD Non-smokers [χ^2 = 3.85, p = 0.05, OR = 1.67(0.97- 2.90)].In the Non-Coal mine area, the frequency of GSTT1 null type was significantly high in COPD smokers versus Non-COPD smokers [χ^2 = 5.02, p = 0.02, OR = 2.11 (1.04- 4.29)] and in COPD Non-Smokers versus Non-COPD Non-smokers [χ^2 = 7.23, p = 0.007, OR = 3.66 (1.22-10.88)]. GSTM1 frequency was higher in COPD Non-smokers versus Non-COPD nonsmokers [χ^2 = 2.57, p = 0.02, OR = 3.66 (1.02-6.65)].In the smoker group (COPD versus Non-COPD), GSTM1 null type was not significant [χ^2 = 2.30, p = 0.13, OR = 0.92 (1.32- 1.89)].

PCR amplification for Alpha-1-antitrypsin gene was done by site directed mutagenesis PCR method as described by (Tazellar *et al.*, 1992).PCR amplification showed characteristic 179bp band indicating the presence of normal homozygous 'MM' type in all the samples. On restriction digestion, a band was observed at 157bp in all the samples. As such, there was no ZZ mutation in these subjects in their A1AT gene. Since all the samples were homozygous 'MM' type, our data did not fit the 'Hardy-Weinberg equation'. Sequencing of the α1AT gene also agreed to the findings of PCR and Restriction enzyme analysis. As such there was no single nucleotide polymorphism of α1AT (ZZ type) i.e., alpha -1 -antitrypsin deficient allele in either of the coal mine or non-coal mine population.

The sequences obtained were aligned with the mRNA of normal (MM type) Alpha-1-Antitrypsin gene. No difference was found in position Glu342 **G**AG to Lys **A**AG in COPD smoker, COPD Non-Smoker, Non-COPD Smoker and Non-COPD Non-smoker.

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 α1AT gene did not fit the logit model because all the results were normal MM type for all the subjects.

A cause and effect relation between dust exposure and respiratory health is well accepted (Soutar *et al.*, 1986). Against the background, a correlation of our results is that the dust related reductions in lung function were in fact a health effect of exposure to dust. The other covariables as associated risk factors were smoking and male gender.