ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a major cause of health problems in many countries including India characterized by slow, progressive, irreversible airflow obstruction, which is due to the loss of lung elasticity resulting from parenchymal destruction and peripheral obstruction that is not reversible. There is likely to be a complex interplay between genetic and environmental factors in the pathogenesis of the COPD. Cigarette smoking is currently major environmental factors in more than 90% of patients. However, only 10–20% of chronic heavy cigarette smokers develop symptomatic COPD, suggesting that genetic factors are likely to be important determinant. COPD is a complex disease which is still poorly understood at a molecular level and plausible genetic susceptibility is suggested one of the major factors. Major promising approach for the case control study is to select genes that are likely to be involved in the pathogenesis and then to study polymorphisms in these candidate genes and relate these to disease severity in terms of clinical and biochemical changes. This involves an understanding of the disease process at a cellular and molecular level. There are many possible genes that could contribute to the occurrence, severity, and progression of COPD and also the response to the treatment, such as antiproteolysis, metabolism of xenobiotics, airway hyperresponsiveness, inflammatory response and oxidative stress. In present thesis, genes involved in detoxification of xenobiotics, oxidative stress, inflammation and response to the treatment were investigated for the possible association with the disease. The genes included Cytochrome-P450 enzymes 2E1 (CYP2E1), Cytochrome-P450 enzymes 2D6 (CYP2D6), N-Acetyltransferase-2 (NAT2), Mn Super oxide dismutase (Mn-SOD), Myeloperoxidase (MPO), Endothelial Nitric Oxide (eNOS), Cyclooxygenase 2 (COX2), P53 and β2-adrenergic receptor (ADBR2). Biochemical markers GSH, GPx, CAT, GST, Nitrite and MDA were estimated for the possible correlation with the genetic variants, where as SNPs were also correlated with the clinical parameters, mainly FEV1, FEV1/FVC and BMI for any possible genotype-phenotype correlation. Pathophysiological complication due to acute cigarette exposure and preventive role antioxidant N-Acetylcysteine were investigated in murine model. The review of literature very pertinent to the objectives of the present thesis is presented in Chapter 1.
The objectives of the present thesis have been worked out in the following chapters. In Chapter 2, we attempted to understand the possible roles of genetic variants -1053C/T, -1293G/C, 7632T/A and 9893C/G of CYP2E1, 1934G/A of CYP2D6, 481C/T, 803A/G, 857G/A and 590G/A of NAT2 from detoxification pathway, whereas, the 1183C/T of Mn-SOD and -463G/A of MPO from oxidative stress pathway. Biochemical levels of GSH, GPx, CAT, GST and MDA were estimated. Single locus genetic variants as well as multilocus variants were correlated with these biochemical parameters as well as clinical parameter for their possible role in the disease susceptibility. The study showed altered oxidative stress markers in circulating system, whereas, genetic variants of xenobiotics metabolizing enzymes, particularly -1053T and -1293C alleles of CYP2E1 and NAT2*6 and NAT2*/7 alleles of NAT2, to the susceptibility of COPD. Further the minor allele associated haplotypes of CYP2E1 such as -1293T:7632T, 7632C:9893C, 7632C:9893G and -1293C:7632T:9893C associated with the disease. The study has provided important information on the oxidant-antioxidant status of the circulating system and genetic predisposition due to altered variants in detoxification genes.

The objective of Chapter 3 was to investigate the possible association of -786T/C, -922A/G, 4B/4A and 894G/T eNOS variants and their contribution to nitrite and MDA levels. Moreover, we look for an interaction between the investigated genetic, clinical and biochemical parameters. NO is a multifunctional molecule involved in the endothelial dysfunction and oxidative stress, the major pathophysiological consequences of COPD. In the present study, over-representation of -786C, -922G and 4A alleles was observed in patient, which was correlated with decreased nitrite levels. Interestingly, it was observed that overall nitrite levels were higher in patients as compared to the controls, which could be attributed to other factors. It is envisaged that the eNOS containing -786C, -922G and 4A alleles becomes faulty resulting in reduced NO generation, however, as a compensatory mechanism, the decrement of NO in endothelium may stimulate the inducible NOS (iNOS), which can also get activated as explained in earlier findings. Under such pathophysiological conditions more of the NO combines with \( \text{O}_2^{* -} \) to produce peroxinitrite radical, which contributes to increased oxidative stress and hence increased MDA levels was.
observed in patients. Therefore this finding provides the novel mechanism of increased oxidative stress in COPD due to the altered genetic variants of eNOS gene.

In Chapter 4, our study focused on the role of the polymorphisms -765G/C and 8473T/C of COX2 and 72Pro/Arg of P53 for their possible association with the disease and their contribution to biochemical and clinical parameters. Both the genes are associated with common pathway of inflammation, oxidative stress and apoptosis, the major hallmarks of COPD. The genes play crucial role in respiratory diseases; moreover, interaction between these genes seems noteworthy, which made us to explore their combined genetic effect for the genetic susceptibility and their influence on the biochemical levels of MDA and clinical parameters such as BMI, FEV1 and FEV1/FVC.

Present study reveals that the alleles -765C and 72Pro and the third allele 8473C in the haplotype -765C:8473C were over-represented in patients. The genotypes-combinations of the two genes containing more of the risk-alleles -765C, 8473C and 72Pro associated with the disease. Combination of genotypes having ≥3 risk alleles also correlated with decreased BMI and increased MDA level in patients. Furthermore, gene-gene interaction between the two polymorphisms of P53 and COX2 were analyzed to find the interaction in disease susceptibility. The present study therefore highlighted the importance of these polymorphisms, in particular, in combinations toward pathogenesis of the disease.

In Chapter 5, after investigating candidates genes involved in the pathogenesis of the disease we looked for the ADRB2 gene, involved in response to the treatment. Beta2 (β2)-agonists are used widely in treatment of asthma and COPD and action of these drugs is mediated by the β2-adrenergic receptor (ADRB2), a transmembrane protein expressed by airway smooth muscle cells. We investigated the 46A/G, 79G/C, 491C/T, 252C/T and 523C/A polymorphisms for their possible association with the disease individually, in multilocus form such as haplotypes. Linkage disequilibrium and cross sectional haplotype analysis were performed to find the linked variants and to identify the risk as well as protective haplotypes. Present findings revealed that only 46G/A polymorphism is associated with the disease individually but multilocus haplotypes analysis reveals several risk and protective haplotypes. We have provided
the first evidence of the *ADRB2* haplotype association in north Indian subjects in context of COPD, which has potential role in alteration of the receptor functionality.

In Chapter 6, we looked for the effect of the acute cigarette smoke (CS) exposure in mice model to understand the complex mechanism of the disease pathophysiologies at biochemical and molecular levels; and to look the effect of N-Acetylcysteine (NAC) in CS exposed animals. In present study we looked for the histopathological changes and biochemical changes in the oxidative stress markers such as SOD, GSH, GPx, GR, CAT, GST and MDA. Expression analysis of the gene *Mn-SOD, GPx* and *CAT* were performed by RT-PCR. Lastly, we also looked for the DNA damage analysis using comet assay in blood cells. Present findings revealed that acute CS induces the inflammatory response observed in histopathological changes in lung tissue. It leads to the DNA damage in blood cells of the animals exposed to the CS and effect of the NAC is almost negligible to prevent in such a condition. There is increased oxidative stress due to increased levels of markers mainly SOD, CAT and GST in lung as well as in liver tissue. There are increments in the levels of GSH in lung. Increment in antioxidants may be a protective mechanism to protect the lungs as well as liver from the free radicals; however, increased levels of MDA indicated that increased antioxidants were not sufficient to combat the oxidative damage. NAC although significantly decrease the MDA levels but not sufficient to prevent the oxidative damage and histopathological changes.