ABSTRACT

Chronic obstructive pulmonary disease (COPD), a heterogenous disorder is characterized by air-flow limitation that is not fully reversible, in which there gradually occurs declining lung function causing chronic bronchitis and emphysema. Smoking and occupational exposures are important exogenous risk factors with a causal relationship and synergistic effect resulting in greater severity of the disease with genetic predisposition increasing the likelihood of COPD. In the present study, therefore, lung function test identified COPD cases and genetic insult was assessed in peripheral blood leukocytes using the alkaline Single Cell Gel Electrophoresis and enzymatically-modified comet assays), and chromosomal damage using the buccal buccal micronucleus cytome and the urothelial MN assays. COPD cases (n=200) among migrant workers from Bihar, engaged in stone-crushing and adults from same area without any occupational exposures comprised age-and sex- matched control group (n=200). Xenobiotic metabolic genotypes of Glutathione S transferase (T1, M1, P1 (313A>G) and of the candidate gene Serpin peptidase inhibitor, clade A (alpha-1 antiprotinase, antitrypsin) member1 (SERPINA1 rs 6647 (710T>C), rs 709932 (374G>A), rs 17580 (863 A>T), rs 28929474 (1096G>A) were analyzed for disease-risk and for modulating genetic damage. The study was carried out after clearance from the Institutional Ethics Committee and after voluntary written informed consent from the study participants.

Cases were identified in situ by spirometry from among the workers at 22 stone-crushing units in the Pathankot and Gurdaspur districts of Punjab. Male COPD cases (41.69±0.47y) and controls (41.58± 0.58y) were matched for dietary habits and smoking duration but differed for smoking frequency, BMI,WC,WHR and physiometric analysis. Cases had been working for 5-21y (12.87±0.24y) with daily work shifts of 11-13h (9.83±0.14h) and performed drilling (35.50%), dressing (34.00%) and loading (30.50%) activities. Cases had significant (p=0.000) decrease in lung function and were categorized as the cases as having moderate (34.00%), severe (41.50%) and very severe (24.50%) COPD cases; respiratory distress in cases included shortness of breath, persistent cough, blocked nose and dry/sore throat. Abortions, miscarriages and still births were more among cases but pedigrees did not reveal COPD-family history.

DNA damage and oxidative DNA damage in peripheral blood leukocytes, chromosomal damage, cell-proliferation and cell death markers in buccal cells and also frequency of micronucleated cells, apoptotic and necrotic events in urothelial cells were
significantly (p=0.000) increased in cases as were total oxidant status (TOS), oxidative stress index (OSI) and malondialdehyde (MDA) levels. Total antioxidant capacity (TAC) and alpha-1-antitrypsin (AAT) activity were however decreased. The buccal micronucleus assay had maximum specificity and sensitivity followed by the SCGE assay and the urothelial MN assays on ROC analysis.

Genetic Damage, oxidative stress and biochemical marker levels differed significantly by age, study sites, workplace exposure, smoking history, physiometric characteristics and anthropometric variables. The Energy Dispersive X-ray Spectroscopy with Scanning Electron Microscope (EDX-SEM) analysis revealed presence of particulate matter (PM 0.23-0.53μm) and 11 elements carbon, oxygen, silica, aluminium, calcium, magnesium, sodium, copper, silver, potassium and iron) in the top-soil layers of the stone-crushing units. Predictors for genetic damage comprised diet, smoking history, duration of work, per day schedule of work, lung dysfunction, silica, aluminium, silver, sodium, magnesium, potassium and iron.

The allelic and genotypic distribution of GSTP1 (rs1695, 313A>G), SERPINA1 M1 (rs6647, 710 T>C) and M2 (rs709932, 374G>A) gene variants was in accordance with Hardy-Weinberg equilibrium (HWE) but not of SERPINA1 S (rs17580, 863A>T) and Z (rs289294, 1096G>A) as the genotypes were lacking in cases and controls. GSTM1 null genotypes were significantly higher in cases and SERPINA1 M1 (TC/CC) and SERPINA1 M2 (AA) significantly conferred risk for severe COPD. Multidimensionality factor Reduction (MDR) analysis revealed the gene-gene interaction of GSTT1, SERPINA1 M1 (710 T>C), SERPINA1M2 (374G>A) and the gene-environment interaction of GSTT1, GSTM1 and the workplace activities as increased disease-risk.

The present study as a first of its kind has identified in situ COPD cases from occupational exposure at stone-crushing units in an ethnic specific group (scheduled caste) from Bihar who were assessed for genetic damage in three biological samples with oxidative stress, lipid levels and AAT activity determined in blood serum samples. Highly significant increased DNA damage and chromosomal damage, cytokinetic defects, apoptotic and necrotic events were observed in cases. The increased oxidative stress in cases provides support for the causation of the observed genetic damage, which if unrepaired may lead to age-related diseases and malignancy. Genetic-damage modulation by GST and SERPINA1 as disease-genotypes have increased the significance of the study. The present study has generated a database on genotypic and allelic variants and gene-gene and gene-environment influences on disease-causation in an occupationally exposed population sub-group.