SUMMARY

Chronic Obstructive Pulmonary Disease (COPD) is characterized by chronic airway inflammation, lung parenchymal inflammation and destruction resulting obstruction in the expiratory airflow. COPD includes chronic bronchitis and pulmonary emphysema. Chronic bronchitis is a chronic inflammatory condition in the lungs that causes the respiratory passages to be swollen and irritated, and there is an increase in the mucus and cough production in respiratory passage. Increased cough and sputum production arise from an innate immune response to inhaled toxic particles and gases of smoke. In case of emphysema destruction of the airway walls results in the enlargement of the distal airspace due to that there is less surface area for gas exchange and the person feels breathless.

It is estimated that in 2020, out of 68 million deaths worldwide, 11.9 million will be caused by lung diseases: 4.7 by COPD, 2.5 by pneumonia, 2.4 by TB and 2.3 million by lung cancer. At current time, COPD is the fourth most common cause of death in the United States of America. The world is estimated to be inhabited by a record of 7.3 billion people with COPD in 2015. According to WHO, India contributes a growing percentage of COPD cases which is estimated to be amongst the highest in the world i.e. more than 64.7 estimated age standardized death rate per 100,000 amongst both sexes. This report showed approximately 556,000 cases in India (>20%) out of a world total of 2,748,000 every year. COPD kills half a million people in India every year, more than those who die due to tuberculosis, malaria or HIV-AIDS.

The most important risk factor for progression of COPD is tobacco smoking. Other risk factors include α 1- antitrypsin deficiency, air pollution, socio-economic status and lower birth weight. Skeletal muscles in individuals with COPD generate free radicals at rest, and production increases during muscle contractile activity. Overproduction of free radicals may result in oxidant–antioxidant imbalance in favour of oxidants.
The formation of reactive oxygen species (ROS) by the cigarette smoke and inflammatory cells which were generated in the pulmonary epithelial tissues of lungs has been associated with slow growing and irreversible decrease in forced expiratory volume in one second (FEV1), loss of muscle mass and muscle dysfunction.

Different compounds in cigarette smoke can react directly with cellular components to form radicals, and other procarcinogen substances may get activated to produce single and double strand breaks into DNA.

The present study was undertaken to analyse the genetic damage in COPD patients using MN assay and comet assay and oxidative stress by using FRAP assay with the following objectives:

1. To conduct an epidemiological survey of the COPD human subjects and healthy matched controls.
2. To compare the frequency of micronuclei in buccal epithelial cells of the COPD patients with controls.
3. To detect genetic damage, if any, using comet assay in the COPD human subjects along with healthy matched controls.
4. To estimate the total antioxidant activity in the COPD human subjects along with healthy matched controls.
5. To provide a baseline data based on which further studies may be planned.

The present investigation included epidemiological survey of COPD patients and healthy individuals for the study of genetic damage, if any. The epidemiological data of 110 COPD patients and 90 control individuals matched with respect to age, sex, smoking and alcohol drinking habits, biomass smoke exposure and socio-economic class along with their blood samples and buccal smears were collected.

The data was recorded on a standard proforma. Before obtaining the samples, an informed consent was taken from each subject and a questionnaire was filled which determined their lifestyle pattern (smoking, alcohol drinking, diet, daily activity, disease etc.). The samples were collected with the permission and help of the registered medical practitioner. Care was taken to avoid any type
of infection/contamination during sampling. COPD was diagnosed with the help of GOLD (2006) guidelines, using clinical history, physical examination, and confirmation by airflow obstruction which is defined as a ratio of forced expiratory volume in one second (FEV1) to forced vital capacity (FVC) below 70% of predicted value. Ethical approval was obtained from Institutional Ethics Committee Kurukshetra University Kurukshetra vide letter no. IEC/14/371. Dated 1/10/14.

In the present study evaluation of nuclear anomalies, genetic damage and oxidative stress in COPD patients and matched control subjects was carried out using Micronucleus (MN) assay, SCGE assay/comet assay and FRAP assay. In MN assay exfoliated buccal epithelial cells were obtained from the inner chick of both COPD patients and healthy matched controls. The mean frequency of MN (micronucleus), BN (binucleate), BE (broken egg), KL (karyolysis) and KH (karyorrhexis) were evaluated to analyse the cytological damage. For the present study, 5 ml venous blood samples were drawn from each COPD patients and matched control subjects and were collected in K2-EDTA vacutainers. Out of 5 ml blood 2 ml were centrifused for the extraction of plasma, which is used for FRAP assay analysis. For comet assay analysis blood samples of COPD patients and control subjects were subjected to comet assay. Comet parameters, viz. Tail DNA (%), Integral intensity, Tail length (µm), Tail moment, Olive moment, and Tail area (µm²) were analysed with the help of Lucia Comet Assay software to assess the extent of genetic damage.

In the present investigation, the mean frequencies of all the nuclear anomalies (MN, BN, BE, KL and KH) were significantly higher (3.618±0.238, 14.354±0.404, 15.327±0.425, 16.264±0.634 and 1.372±0.187, respectively) in COPD patients as compared to control subjects (0.867±0.115, 7.122±0.355, 9.956±0.350, 1.372±0.187 and 0.811±0.120, respectively). Pearson correlation coefficient between various nuclear anomalies was significant and positive in COPD patients. The mean frequencies of MN and KL in severe COPD patients were found to be higher as compared to the moderate COPD patients.
The comparison of mean values of different nuclear anomalies (MN, BN, BE and KH) between smoker COPD patients and control smoker subjects depict highly significant (P<0.001) difference. The mean frequencies of MN and BN were significantly (P<0.05) higher in COPD patients smoking >6 cigarettes/day as compared to the 0 cigarette/day consumers and those smoking 1-6 cigarettes/day and the frequency of BE and KL were significantly (P<0.05) higher in smokers smoking > 6 cigarettes/day as compared to the non-smokers.

The frequency of BN and BE were observed to be significantly increasing with increased dose of alcohol (>4 times/week) among COPD patients. Significantly higher MN, BN and BE frequencies were observed in the vegetarian and non-vegetarian COPD patients than controls. The significant difference was observed in the mean frequencies of BN in COPD patients with >60 years age (15.595±0.585) as compared to COPD patients having ≤60 years age (13.588±0.527). Significant differences were observed in the mean values of MN and BN with the duration of the disease. The significant differences were reported in the mean frequencies of MN, BN, BE and KL among biomass smoke exposed COPD patients as compared to the control biomass smoke exposed subjects.

The mean values of different comet parameters viz. Tail DNA (%), Integral intensity, Tail length, Tail moment, Olive moment and Tail area were significantly (P<0.001) higher in COPD patients with respect to the control subjects. Mean value of Tail DNA (%) showed a significant difference in severe COPD patients when compared with moderate COPD patients. In male subjects, all the comet parameters were significantly (P<0.001) different in COPD patients than control subjects. In case of females the mean values of Tail DNA (%), Tail length, Tail moment, Olive moment and Tail area were significantly (P<0.001) different among COPD patients as compared to controls. Markedly significant (P<0.001) difference was found for all comet parameters in non-smoker and smoker COPD patients as compared to control subjects. Tail DNA (%) in the control subjects those smoked 1-6 cigarettes/day and >6 cigarettes/day (16.301±0.898, 16.541±1.436, respectively) showed a significant difference as compared to the non-smokers (11.696±0.466). Among COPD patients Tail DNA
 (%) (39.110±1.749) and Tail area (4320.600±223.110) in smokers smoking >6 cigarettes/day were significantly different from the 0 cigarette/day smokers (35.227±0.685 and 3343.000±200.900, respectively). In case of Tail length the mean values were significantly (P<0.05) higher in patients smoking 1-6 cigarettes/day and >6 cigarettes/day as compared to the 0 cigarette/day smokers. Markedly significant (P<0.01) and positive correlation was observed for Tail length and Tail area with number of cigarettes smoked daily among COPD patients. Tail DNA (%) and Olive moment showed correlation at P<0.05.

The mean values of all the comet parameters in non-alcohol consuming COPD patients were significantly (P<0.01) higher as compared to the non-alcohol consuming control subjects. Significant changes were observed in the comet parameters with increased dose of alcohol among COPD patients and control subjects. Highly significant (P<0.001) differences were observed in mean values of all the comet parameters of vegetarians and non-vegetarian COPD patients when compared with control subjects. No significant changes were observed in all the comet parameters among COPD patients with age. Among COPD patients those exposed to biomass smoke, the mean value of Tail DNA (%), Tail length, Tail moment, Olive moment and Tail area were significantly higher as compared to the control biomass smoke exposed subjects. Significant positive correlation was observed for Tail DNA (%) in both COPD patients and control subjects when correlated with biomass smoke exposure.

In the present study FRAP values of control subjects (966.51±7.347) were significantly (P<0.001) higher (765.580±7.571) as compared to COPD patients (392.280±06.602). Severe COPD patients have lower FRAP values than moderate. The FRAP values were significantly lower in male COPD patients (402.570±8.078) as compared to control males (787.290±8.388). Similarly, female COPD patients have lower value of plasma than control females. Vegetarian COPD patients had lower FRAP values than non-vegetarian patients. FRAP values were found to be greatly reduced in smokers COPD patients as compared to control smokers. Biomass smoked exposed COPD patients have also lower values of FRAP as compared to control biomass smoke exposed subjects.
In the present investigation both micronucleus assay and comet assay results showed significant differences between COPD patients and controls. This suggested that there was increased level of genetic damage in COPD patients. Decreased FRAP values of plasma in COPD patients as compared to controls indicated that there was reduced antioxidant level of plasma in COPD patients.

The present study can be helpful in spreading awareness among people about adverse effects of cigarette smoking and biomass smoke exposure which cause oxidative stress in the body and inflammation in respiratory tract which can lead to COPD or other respiratory disease and consequently genetic damage.

The future studies may be carried out with the following objectives:

1. To trace the extent of interaction of various factors i.e. environmental and genetic which can may lead to progression of the COPD.
2. To devise easy, efficient and economical methods for early diagnosis in occupationally exposed workers.