CHAPTER 5: DISCUSSION

Chronic obstructive pulmonary disease (COPD) has been a major health problem and will remain a challenge in 21st century for clinicians. Worldwide, COPD is highlighted due to its high prevalence, morbidity and mortality, which creates alarming challenges for health-care systems (Lopez-Campos et al., 2016). The development and progression of COPD have been related to the increased oxidative stress or reduced antioxidant resources (Boots et al., 2003).

Oxygen breathing leads to the formation of reactive oxygen species (ROS) in the body which play important roles in the cellular signaling processes. The generation of small amounts of reactive oxygen species and free radicals is a normal side effect of aerobic metabolism and it is necessary for normal functioning of the human body (Wachtel-Galor and Benzie, 2011).

In smoker subjects the oxidant burden in lungs have been further enhanced by the release of ROS from the alveolar macrophages and sequestered neutrophils in the lungs (Schaberg et al., 1992; Rahman et al., 1996; Morrison, et al., 1999). Increased burden of ROS in pulmonary microvasculature i.e. plasma, red cells, and leukocytes due to exposure of cigarette smoking occurs either directly by diffusion in the blood (Fishman, 1985; MacNee et al., 1989) or indirectly from the reactive oxygen species generated from activated inflammatory cells in the lungs or peripheral leukocytes (Ludwig and Hoidal, 1982; Postma et al., 1988; Schaberg et al., 1992; Rahman et al., 1996; Pinamonti, 1996; Morrison et al., 1999).

Oxidants produced in the biological fluids are systematically scavenged by the antioxidants. Under normal conditions, the lung and blood are abundantly protected by various antioxidant components (Toth et al., 1984; Van Asbeck et al., 1985; Agar et al., 1986; Heffner and Repine, 1991).

Various investigations have revealed the role of the reactive oxygen species in the generation of the inflammatory response occurring in both central and peripheral airways of the COPD patients (MacNee, 2001 and Saetta et al., 2001). In respiratory system, ROS may be generated either exogenously i.e. inhaled gaseous or particulate agents such as cigarette smoke,
air pollutants, ambient high-altitude hypoxia, and some occupational dusts, or endogenously generated in the context of defence mechanisms of body against such infectious pathogens as viruses, bacteria, or fungi. ROS may also damage body tissues depending on the amount and duration of exposure (Domej et al., 2014).

Oxidative damage to DNA causes alterations in bases of DNA. If left unrepaired, the modifications of DNA bases in turn lead to genetic defects (Tsuboi et al., 1998). Recent studies have reported that the lung tissue of COPD patients displays oxidative DNA damage (Igishi et al., 2003; Caramori et al., 2011).

5.1 Genetic Damage and COPD

5.1.1 Nuclear anomalies

In the present investigation cytological damage was assessed by evaluating the mean frequencies of MN, BN, BE, KL and KH. COPD patients had higher frequencies of all the nuclear anomalies viz. MN, BE, KL, KH as compared to the control subjects. About two fold increased BN frequency was observed among COPD patients as compared to the control subjects. A negative and non-significant correlation was reported between various nuclear anomalies in control subjects. Pearson correlation coefficient between various nuclear anomalies was found to be positive in COPD patients. Previously, Maluf et al. (2007) have also reported that COPD patients had comparatively increased frequencies of BN and MN as compared to the matched controls.

Various studies have also reported the elevated frequency of MN in patients as compared to controls: cancer patients (Devi et al., 2011), head and neck cancer (Khilfi et al., 2013), lung cancer (Lou and Dean, 2007), type-2 diabetes (Martinez-Perez et al., 2007) and asthmatic patients (Herrstroma et al., 1998).

In the present investigation significant (P<0.001) difference was observed in the frequencies of MN and KL among severe COPD patients as compared to the moderate COPD patients, which implied that with severity of COPD there was increased level of DNA damage.
In male COPD patients significantly (P<0.001) higher frequencies of MN, BN, BE and KL were encountered in buccal epithelia cells as compared to control male subjects. The mean value of KH showed significant difference at P<0.01. In case of COPD females the mean values of all the nuclear anomalies showed a significant difference at (P<0.001) and the mean value of KH showed a non-significant (P>0.05) difference when compared with control female subjects. Earlier, Cheng (1996) found significant higher MN frequency in females than males. Bajpayee et al. (2002) studied the healthy Indian male and female population and reported the significantly higher level of basal DNA damage in males as compared to the females. In the present investigation significantly elevated frequencies of all the nuclear anomalies were reported in male COPD patients as compared to females. In contrast, Ganguly (1993) observed a higher MN frequency in females as compared to the male subjects. The Human micronucleus project depicted a gender difference after analysis of a large number of subjects and laboratories, with women demonstrating a higher incidence of MN as compared to the men (Bonassi et al., 2001).

In the present study elevated frequencies of MN, BN, BE, KL and KH were observed in smoker COPD patients as compared to the control smoker subjects. During the present investigation the micronuclei frequency in buccal epithelial cells was found to be markedly higher in smokers (consuming >6 cigarettes/day) than non-smokers. Mean frequency of BE were significantly (P<0.05) different in smokers consuming 1-6 cigarettes/day and >6 cigarettes/day as compared to non-smokers. Pearson correlation coefficient was positive between all the nuclear anomalies except KH with respect to the cigarette smoked daily in COPD patients and was highly statistically significant (P<0.01). Recently, Singh and Yadav (2016a) have reported significantly increased frequencies of MN, BN, BE, KL and KH in COPD smokers subjects as compared to the control smoker subjects. They have also reported that mean frequencies of MN, BN were significantly (p<0.05) higher in COPD subjects consuming >6 cigarettes/day as compared to the non-smokers and those consuming 1-6 cigarettes/day. The frequencies of BE and KL were significantly higher (P<0.05) in smokers consuming >6 cigarettes/day as compared to the non-smokers. Earlier, Yadav and Saini
(2015) have reported elevated frequencies of MN, BN and KL in smoker subjects as compared to the non-smoker control subjects. They have also reported significantly (P<0.05) elevated frequency of MNC (micronucleated cells) and TMN (Total micronuclei) in subjects with increase in the dose of the cigarettes. Wu et al. (2004) reported the positive relation between the micronuclei frequency and smoking intensity with increasing number of MNi in heavy smokers.

Previously, Au et al. (1991) analysed the factors which caused genetic damage in leukocytes of 67 cigarette smokers and 59 matched non-smoking control subjects. They observed slightly higher mean MN frequency in smokers than non-smokers. Bonassi et al. (2003) observed that micronucleus frequency was increased in heavy smokers, those smoked more than 30 cigarettes per day as compared to the moderate smokers. Bharti et al. (2015) found higher micronuclei number in buccal mucosa cells of smokers of more than 10 years duration than smokers of less than 10 years duration. They have also reported that diabetic patients have higher number of micronuclei than smokers. Similar results were found for cancer patients with smoking habits. Cheng et al. (1996) studied the micronucleus frequency in patients of lung cancer with smoking habits. They observed significantly higher MN frequency in lung cancer patients as compared to the controls (P<0.01). They further analysed that cancer patients showed significantly higher MN frequency in current and former smokers than controls. McHugh et al. (2013) studied the genetic damage in 500 lung cancer cases and 500 controls by using the CBMN assay to evaluate instability and risk of lung cancer associated with exposure to smoking. A difference was found in lung cancer between heavy smoker men and women when compared to non-smoker although it was not statistically significant. This data suggested that heavy smoking may have an effect on DNA repair capacity.

Previous studies have reported that various forms of tobacco consumption are associated with increase in the frequency of micronuclei in buccal epithelial cells (Stich et al., 1982a; Stich and Rosin, 1984; Majer et al., 2001; Nersesyan et al., 2006; Kamboj and Mahajan, 2007; Joshi et al., 2011). The present findings are in agreement with these studies.
Comparison of nuclear anomalies of non-alcoholics of both control subjects and COPD patients depicted significantly elevated mean frequencies of all the nuclear anomalies in non-alcoholic COPD patients than non-alcoholic controls. The mean frequencies of MN, BN, BE and KL were significantly (P<0.001) higher in alcohol consuming COPD patients than in alcohol consuming control subjects. Mean MN frequency was found to be significantly (P<0.05) increased in control subjects, consuming alcohol >4 times/week as compared to <4 times/week consumers and once a while and non-consumers. Previously, many authors have proposed a possible aneugenic effect of ethanol and relationship between alcohol consumption and increased MN frequency (Stich and Rosin, 1983; Crebelli et al., 1989; Maffei et al., 2000; Burim et al., 2004). Stich and Rosin (1983) observed an increased MN frequency related to the mutagenic effects of alcohol in buccal mucosal cells of alcoholic smokers.

Several previous studies have also reported the increased frequency of micronuclei in alcohol consumers. Reis et al. (2002) reported the increased frequency of micronuclei in buccal mucosa cells in the group of alcoholic individuals as compared to the control group, although that difference was not statistically significant. Sellappa et al. (2010) found the increased MN frequency due to smoking and alcohol consumption in control as well as in welder subjects exposed to hexavalent chromium during welding. Smoker and alcoholic welders showed more MN frequency than control groups. Chadha and Prabha (2013) have also reported the increased frequency of micronucleated (MN) and binucleated (BN) in alcoholics as compared to non-alcoholics. Recently, Singh et al. (2015) have studied the effect of alcohol consumption on the micronucleus frequency in buccal epithelial cells and reported a significant difference in the mean value of MN among alcohol consumers as compared to the controls. The results of the present study are in substantial agreement with these studies. Contrarily, Santovito et al. (2015) observed no significant difference in the MNi frequency among alcoholics and non-alcoholics and no association between MNi and duration of alcohol intake.

Analysis of results in relation to dietary habits in COPD patients showed significantly (P<0.001) increased frequencies of MN, BN, BE among
vegetarian COPD patients as compared to vegetarian control subjects in the present study. Amongst non-vegetarian group, COPD patients had elevated mean frequency of KH than control subjects. Various studies have proposed that this may be due to intake of vegetarian food containing antioxidants, which reduce the genetic damage (Duthie et al., 1996; Pool-Zobel et al., 1997). Several previous studies (Giovannelli et al., 2002; Staruchova et al., 2006) have reported a small effect of vegetables and fruit on oxidative DNA damage. Bonassi et al. (2011) studied that the subjects with a daily consumption of green vegetables and fruits had lower frequencies of MN in their exfoliated buccal cells as compared to those who were not consuming all of these foods.

In the present study, in control subjects the mean frequency of BE was significantly increased in the higher age group (>60 years) as compared to the lower age group (≤60 years) while in COPD patients the mean frequency of BN was significantly higher in age group of >60 years as compared to the age group of ≤60 years. Previous studies have also reported age related alterations in micronucleus frequency. Tice and Setlow (1985) proposed that age has significantly affected the frequency of spontaneous micronucleus formation in lymphocyte of male and female subjects. Fenech and Morley (1985) observed a four-fold increase in micronuclei in 80 year old persons as compared to younger ones, they have also found a significant (P<0.001) positive correlation of micronucleus expression with increasing age. Ganguly (1993) observed that the aged male and females showed larger number of aberrations and the correlation of micronucleus formation with donor’s age is highly significant in peripheral lymphocytes of 127 healthy individuals. Bakou et al. (2002) evaluated non-disjunction, spontaneous and aneuploidogen-induced micronucleus frequencies in chromosomes of cultured binucleated lymphocytes of women with two age groups by using fluorescence in situ hybridization. They reported that micronucleus frequency increases with age and chromosome containing micronuclei predominated in older female. Acentric chromosome fragments in micronuclei were also enhanced with advancing age. Wojda et al. (2007) have also observed the increase in MN frequency in the higher age group.
In a recent study, Saini et al. (2014) have also studied the effect of ageing on micronucleus frequency in different age groups of healthy subjects. They have reported that the mean frequencies of MN, BN, BE, KL and KH were significantly different (P<0.05) in all the age groups. They have also observed a positive correlation between age and nuclear anomalies. In the present study also the mean frequencies of BE and KH also showed a significant positive correlation with respect to the age (years) among control subjects.

During the present investigations comparison of COPD patients were also done on the basis of duration (years) of COPD. The frequencies of MN and BN were significantly elevated with increase in the duration of COPD. The significant differences was observed for MN (P<0.001) and BN (P<0.05). Positive Pearson correlation coefficients were observed for MN (P<0.01) and BN (P<0.05) with duration of COPD. Thus, indicating that the COPD triggers the factors that increase the level of genetic damage in patients.

When the COPD patients were compared with respect to the exposure of biomass smoke a significant difference was observed in the mean frequencies of MN, BN, BE and KL in biomass smoke exposed COPD patients than the biomass smoke exposed control subjects. Among control subjects the significant difference was reported in the mean frequencies of MN, BN and KL with the increased duration of the biomass smoke exposure. In COPD patients MN and BN showed elevated frequencies with the increased duration of biomass smoke exposure. Previously, Sisenando et al. (2012) studied the effect of exposure to biomass burning through micronucleus assay in exfoliated buccal cells of school children in the Brazilian Amazon region and reported that those children exposed to biomass burning showed a significant difference between micronucleus frequencies as compared to non-exposed children. In a study, women using biomass fuels for cooking purposes compared with those using LPG, showed that women exposed to biomass fuel had higher levels of cytogenetic abnormalities such as chromosomal aberrations and formation of micronucleus in peripheral lymphocytes (Musthapa et al., 2004). The present results also correlate with these studies which suggest that exposure of biomass can lead to higher level of cytogenetic changes i.e. nuclear anomalies.
5.1.2 DNA damage

In human biomonitoring studies lymphocytes are the cell types most frequently tested with the comet (Angerer et al. 2007; Faust et al. 2004; Moller, 2006b). The percentage of DNA present in the tail reveals quantitatively the frequency the DNA break frequency (Collins et al., 1995). Therefore, increase or decrease in DNA damage will lead to the increase or decrease in Head DNA (%), Tail DNA (%), Integral intensity, Head radius, Tail length, Tail moment, Olive moment, Head area and Tail area. Thus all these parameters can be used to detect the level of DNA damage. Some workers (Collins, 2004; Villela et al., 2007) used comet types for studying the genotoxic effects. During the present investigation, comet parameters viz. Tail DNA (%), Integral intensity, Tail length, Tail moment, Olive moment and Tail area were used to evaluate the DNA damage in COPD patients and matched controls.

In the present investigation, DNA damage was observed to be markedly increased among COPD patients as compared to the control subjects. All the comet parameters viz. Tail DNA (%), Integral intensity, Tail length, Tail moment, Olive moment and Tail area were significantly (P<0.001) different in COPD patients than control subjects. Pearson correlation coefficients between various comet parameters were observed to be significant in both COPD patients and control subjects. The present results detecting increased DNA damage as a measure of various comet parameters in COPD patients are in agreement with the previous studies (Ceylan et al., 2006; Maluf et al., 2007; da Silva et al., 2013) that have also reported increased DNA damage among COPD patients measured with the help of comet assay.

Comet assay was also used for the evaluation of DNA damage in other respiratory disease such as asthma and lung cancer. Zeyrek et al. (2009) observed higher level of lymphocyte DNA damage level in children with bronchial asthma and proposed that the elevated DNA damage level may be related to the increase in the level of oxidative stress. Lou et al. (2007) used comet assay and MN assay to the blood samples of 36 untreated lung cancer patients and 30 controls and observed a significantly (P < 0.05) higher mean value of tail moment in lung cancer patients as compared to the controls.
During the present investigation the mean value of Tail DNA (%) was observed to be significantly elevated in severe COPD patients as compared to the moderate patients. This depicted that due to severity of disease there was increase in the DNA damage in COPD patients. Previously, da Silva et al. (2013) also studied the level of DNA damage after 3-h MMS (methylmethane sulfonate) alkylating agent treatment and reported that the level of DNA damage was significantly higher in patients with moderate, severe and very severe COPD as compared to the control subjects, while Maluf et al. (2007) found that the values of DNA damage for patients with moderate, severe and very severe COPD did not differ significantly.

During the present study a significant difference was encountered (P<0.001) in the mean values of all the comet parameters among male COPD patients as compared to their counterpart control subjects. In case of COPD female patients all the parameters of comet showed significant difference except integral intensity when compared with control female subjects. Wojewódzka et al. (1998) have also reported higher levels of DNA damage in men compared to the women. Lam et al. (2002) also reported increased DNA damage in elevator manufacturing male workers in China. Recently, Alija et al. (2016) studied environmentally polluted areas in Kosovo and noted the Differences in basal DNA damage in blood cells from men and women with the help of comet assay and reported a statistical significant (p<0.001) DNA damage level in the females from cement factory polluted area as compared to the females of non-polluted area.

Bajpayee et al. (2002) used comet assay to measure the basal level of DNA damage in a normal, healthy population (124 Indian male and 106 female) belonging to a comparable socio-economic background and aged between 20 and 30 years. They were also matched for their dietary habits and smoking and period of sample collection. They found a statistically significant higher level of DNA damage in males when compared to females. These results were evident by an increase in the Olive Tail moment, Tail DNA and Tail length in males as compared to female. While Garaj-Vrhovac and Kopjar (2003) found similar levels of primary DNA damage in both men and women subjects. Earlier, genotoxic differences by sex in blood leucocytes and nasal epithelium in subjects residing in highly polluted places were observed by
Fortoul et al. (2004). In males, DNA damage was high in leucocytes and nasal cells as compared to females and healthy subjects. Moreover, nasal epithelium showed higher percentage of squamous metaplastic changes in males than females and controls.

On comparing the smoking habits of subjects, the mean value of all the comet parameters of non-smokers and smokers COPD patients were significantly different as compared to their counterpart control subjects. Control subjects were compared according to the number of cigarette smoked daily. Mean value of Tail DNA (%) were significantly elevated with the increased consumption of cigarette smoke daily. In case of COPD patients the mean value of Tail DNA (%), Tail length and Tail area increased with increase in the number of cigarette smoked daily. Ceylan et al. (2006) also observed the DNA damage through the alkaline comet assay and reported markedly increased levels of oxidative stress and DNA damage in peripheral blood cells of COPD patients using biomass fuels and had smoking habit. They also found that increased level of DNA damage was correlated with the use of biomass fuel and smoking status. Previously, Zhu et al. (1999) studied 148 workers from a cigarette manufacturing factory, 107 occupationally exposed to tobacco dust from the production department and 41 unexposed controls to investigate whether there were separate or combined effects of occupational exposure to tobacco dust and smoking on genetic damage in lymphocyte. Both groups had similar mean age, mean duration of work and smoking prevalence. The Tail moment was used to analyse DNA damage. They found significant higher Tail moment in exposed workers than controls. Smokers had significantly larger Tail moment than non-smokers. They also observed that occupational exposure and smoking had a significant and independent effect on Tail moment. Tail moment value was not affected by age and gender. This study suggested that smoking and tobacco dust exposure can induce DNA damage in lymphocyte and there was a synergy between tobacco dust exposure and smoking. Likewise, Rojas et al. (1996) used comet assay for the evaluation of DNA strand breaks and observed a significantly higher level of DNA damage in smokers as compared to the non-smokers. Various previous studies reported increased level of DNA damage in smokers and heavy smokers as compared to the control subjects (Betti et al., 1994, 1995; Betti and Nigro 1996;
Piperakis et al., 2000; Dhawan et al., 2001; Lam et al., 2002; Hininger et al., 2004). In contrast, De Marini (2004) and Cessela et al. (2006) found no difference between smokers and non-smokers induced DNA damage.

In the present study, a positive and significant (P<0.001) correlation was reported for Tail DNA (%) with respect to number of cigarettes smoked daily in control subjects, while Integral intensity was negatively correlated with number of cigarettes smoked daily. Tail DNA (%), Tail length, Olive moment and Tail area were significantly (P<0.05) correlated with number of cigarette smoked in COPD patients which suggested that there is increased level of DNA damage in COPD patients with increase in the consumption of cigarettes.

In the present study, the mean values of all the comet parameters in both non-alcohol and alcohol consumers COPD patients were significantly (P<0.01) higher as compared to the non-alcohol consumers and alcohol consumers control subjects. Significant changes were observed in mean value of Tail DNA (%) with increased dose of alcohol in controls and COPD patients. Previously, Sellappa et al. (2010) studied the effect of alcohol consumption on the DNA damage in building construction worker in South India with comet assay and reported a significant difference in the Tail length as compared to alcohol users and non-users of the control subjects. Similarly, Mutlu-Turkoglu et al. (2000) have reported increased H₂O₂ induced DNA damage in the lymphocytes of patients with chronic alcoholism. Contrarily, Van Zeeland et al. (1999) studied the level of 8-OHdG (8-hydroxy-2'-deoxyguanosine) in DNA from peripheral leucocytes among healthy adults of North Itlay and observed no association between DNA damage and alcohol consumption.

Stich and Rosin (1983) observed an elevated frequency of MNi in buccal cells from the cheek and tongue only for subjects who both smoked and drank alcohol. Neither smoking alone nor alcohol drinking alone was associated with an elevated frequency of MNi, thus, indicating a synergistic effect of both smoking and alcohol drinking habits. Fenech (1993) showed that individuals with high cigarette usage (>30 cigarettes/day) had statistically greater MNi frequency as compared to non-smokers.
In the present study when the comparison was made with respect to the dietary habits, a significant (P<0.001) differences was observed in mean values of all the comet parameters of vegetarians and non-vegetarian among COPD patients when compared with their counterpart control subjects. Wojewódzka et al. (1998) analysed the level of DNA damage in the people exposed to low ionizing radiation and reported higher level of DNA damage in exposed subjects as compared to the control subjects; in control subjects they have reported higher level of DNA damage in men as compared to women. Bajpayee et al. (2002) have also reported higher levels of DNA damage in men as compared to women subjects. While Zhu et al. (1999) reported no difference in the effect of smoking on the DNA damage between men and women. Recently, Alija et al. (2016) studied the basal DNA damage in blood cells from men and women in a environmentally polluted areas of Kosovo in South Eastern Europe. They found that when the females from Peja (unpolluted area) were compared with the females of Hani Elezit area (cement factory polluted area) the statistically significant (P<0.001) difference was obtained.

During the present study the mean values of all the comet parameters in control subjects and COPD patients showed no significant differences with respect to age (years). In COPD patients Tail DNA (%), Tail length and Tail area showed positive and non-significant correlation with age. In contrast, Kruszewski et al. (1998) observed no correlation of DNA base damage with age while Moller (2006a) reported a positive correlation between age and DNA damage.

In the present investigation when the comparison was made between all the comet parameters of COPD patients with the duration (years) of COPD no significant difference was encountered. Tail length, Tail moment, Olive moment and Tail area showed non-significant correlation with duration (years) of COPD, while a significant correlation was observed in the mean value of Tail DNA (%) with respect to the duration (years) of COPD. This may be due to the accumulation of genetic damage with the increased duration of COPD.

During the current study, the mean values of Tail DNA (%), Tail length, Tail moment, Olive moment and Tail area were significantly elevated in biomass smoke exposed COPD patients as compared to control biomass
smoke exposed subjects. Similarly, Ceylan et al. (2006) reported the increased DNA damage in biomass smoke exposed COPD patients as compared to the non-exposed control subjects.

In the present investigation, correlation was significant and positive for Tail DNA (%) in both control as well as COPD patients with respect to the biomass smoke exposure. Previously, Mondal et al. (2010) studied the DNA damage in women using biomass fuel and LPG gas for cooking purposes. They reported 59% increase in mean comet Tail % DNA, 45% elevation in comet Tail and a remarkable 3-fold increase in the Olive Tail moment in biomass fuel using women as compared to LPG users. They suggested that the chronic exposure to biomass smoke causes chromosomal and DNA damage and up regulation of DNA repair mechanism.

5.2 Oxidative stress

Oxidative stress occurs in the body when free radicals and other reactive species overcome the availability of the antioxidants. Reactive oxygen species, reactive nitrogen species, and their counterpart antioxidants agent are important for the physiological signaling and host defence, as well as for the persistence of inflammation. When their normal steady state is disturbed then the imbalances between oxidants and antioxidants may generate pathological reactions which can cause a range of non-respiratory and respiratory diseases, especially COPD (Domej et al., 2014).

In the present investigation, FRAP values of plasma in COPD patients were reported significantly (P<0.001) lower as compared to control subjects. The lower value of FRAP suggested that oxidative power of plasma was reduced and hence there was increased oxidative damage in COPD patients. Previously, Nadeem et al. (2005) carried out a study to analyse the systemic oxidant-antioxidant status in COPD patients and related it to the severity of disease. They have reported that in plasma, FRAP and total protein sulfhydryls were lower and GSH-Px, lipid peroxides measured as MDA-TBA products, and protein carbonyls were higher in the COPD patients as compared to healthy non-smokers controls. Raut (2012) studied 60 patients with chronic bronchitis and 100 healthy non-smokers (controls). They have reported malondialdehyde (MDA), nitric oxide, alpha tocopherol and total antioxidant
capacity of plasma during study. They observed higher nitric oxide and malondialdehyde levels in the bronchitis patients than control subjects. Total antioxidant capacity and plasma alpha-tocopherol were lower in the patients as compared to control subjects. Similarly, Emin et al. (2010) analyzed the total antioxidant capacity of plasma in COPD patients with the help of FRAP assay and reported significantly lower value of plasma in COPD patients as compared to the control subjects. Ahmad et al. (2013) have also reported the lower value of FRAP in COPD patients than controls.

The present results contradicted the data of Hakhamaneshi et al. (2007) who found about two-fold increase in the values of FRAP in COPD patients as compared to the controls. Many studies have also reported reduced antioxidant ability in asthmatic subjects (Rahman et al., 1996; Comhair et al., 2000; Kanazawa et al., 2002; Nadeem et al., 2005; Ahmad et al., 2012; Yadav and Saini, 2016) and diabetic subjects (Gupta et al., 2007; Lodovici et al., 2008) as compared to the controls.

Recently, Singh and Yadav (2016b) have also reported significantly lower value of plasma in COPD patients as compared to the control subjects.

During the present study, when the comparison was made between FRAP values of plasma with respect to the severity of COPD, severe COPD patients had significantly lower values as compared to the moderate. Nadeem et al. (2005) and Ahmad et al. (2013) have also noticed lower values of FRAP in severe COPD patients as compared to the moderate COPD patients. Ahmad et al. (2012) proposed that antioxidant ability of plasma was decreased with the increase in the severity of disease in asthmatic patients.

In the present investigation mean FRAP values of COPD patients and control were also compared with respect to various correlates viz. sex, smoking, biomass smoke exposure, alcohol drinking and dietary habits.

When the comparison was made between the male control subjects with male COPD subjects the mean FRAP value of male control subjects was significantly elevated as compared to COPD patients. In control subjects highly elevated (P<0.001) FRAP values were observed in males as compared to females. COPD male patients also showed significantly increased FRAP values as compared to female COPD patients. Previous studies depicted a conflict in gender-based alteration in antioxidant ability of plasma. Choy et al.
(2000) used modified version of FRAP assay i.e. FRASC (Ferric reducing ascorbate assay) assay to study the total antioxidant activity and ascorbic acid concentration in the human tears. They have reported no significant difference in men and women in terms of total antioxidant ability of plasma in tears. In contrast, Benzie et al. (1998) had observed that plasma antioxidant level analysed as vitamin E and ascorbate plasma level was higher in females as compared to males. Katalinic et al. (2005) measured the antioxidant ability in heart, kidney and liver tissues of rats with the help of 2, 2'-azinobis (3-ethylben zothiazoline 6-sulfonate) and ferric reducing antioxidant power assays and they have reported the higher antioxidant ability of plasma in female than male rats.

In the current investigation, non-smoker and smoker COPD patients had significantly (P<0.001) increased FRAP values of plasma than their counterpart control subjects. When the comparison was made among control subjects, FRAP value was slightly elevated in non-smoker subjects as compared to smokers, but the difference was not significant. The plasma antioxidant values were also compared among COPD patients, significantly elevated FRAP values were reported in non-smoker COPD patients as compared to smokers. Recently, Emin et al. (2010) also reported that COPD patients who were current or ex-smokers have lower value of FRAP as compared to those who had never smoked. Earlier, Rahman et al. (1996) observed great reduction in plasma antioxidant capacity in smokers, asthmatic subjects and COPD patients in comparison to control. Dierckx et al. (2003) also found significantly lower total antioxidant capacity in smokers as compared to non-smokers. Recently, Jansen et al. (2014) studied the effect of smoking on the biomarkers of oxidative stress, antioxidant status and redox status in 48 healthy men. They have observed that the average concentration of ROM (reactive oxygen metabolites) and TOS (total oxidant status) was higher in smoker subjects as compared to the non-smoker subjects, and the average concentrations of BAP (biological antioxidant potential), FRAP and TAS (total antioxidant status) decreased with smoking.

Earlier, Goraca et al. (2015) studied the relationship between lipid peroxidation and total plasma antioxidant capacity in healthy smoking, non-smoking young and elderly subjects. They found significantly higher total
antioxidant capacity in plasma of healthy non-smoking young subjects than plasma antioxidant capacity of smoking elderly subjects. The concentration of thiobarbituric acid-reactive substances was found lower in young non-smoking volunteers than young smokers. TBARS concentration in elderly non-smoking subjects was less than elderly smokers. Concentrations of beta-carotene, alpha-tocopherol and ascorbic acid were significantly lower in plasma of elderly smokers than in elderly non-smokers of same age. They proposed that supplementation of antioxidants could be useful for the enhancement of plasma antioxidant status.

During the present investigation, control subjects who were exposed to biomass smoke were observed to have significantly higher FRAP value as compared to the exposed COPD patients. Non-exposed control subjects also showed elevated FRAP value when compared with COPD exposed patients. When the comparison was made among control subjects and COPD patients regarding the exposure to biomass smoke, significant differences were observed in non-exposed subjects than exposed subject. In COPD patients, when the comparison was made with respect to the duration of biomass smoke exposure, slightly increased FRAP values were observed with increase in the duration of biomass smoke. Earlier, Gani et al. (2000) studied the effect of biomass smoke exposure on Turkish women and they reported a decrease in the antioxidant enzyme activity.

Previously, Sezer et al. (2006) studied combined effect of both biomass and cigarette smoke exposure on rabbits and observed decreased antioxidant level with exposure to biomass smoke. Gurjar et al. (2010) also studied the effect of biomass smoke exposure and reported lower level of antioxidant in exposed subjects. Mondal et al. (2010) observed that total antioxidant status was reduced significantly among the subjects who were exposed to biomass smoke. The present results correlated well with the earlier studies.

During the present investigation both non-alcoholic and alcoholic COPD patients showed significantly lower values of FRAP as compared to the non-alcoholic and alcoholic control subjects. Slightly lower FRAP value was reported in alcoholic control subjects as compared to non-alcoholic control subjects but the difference was not significant, while in case of COPD
patients, significantly reduced FRAP values were observed in alcoholic COPD patients as compared to non-alcoholic COPD patients, this indicated that alcohol consumption leads to a decrease in the antioxidant ability of plasma.

When the FRAP values of plasma were compared for vegetarian and non-vegetarian between control subjects and COPD patients, significantly (P<0.001) lower FRAP values were observed in COPD patients than control subjects. Slightly increased FRAP values were observed in non-vegetarian controls as compared to vegetarian controls. Amongst COPD patients, significantly (P<0.01) increased FRAP values were found in vegetarians than non-vegetarians.

In the present study, no significant difference was observed in the FRAP value of plasma in control and COPD patients with increased age. However several studies have reported the age related increased oxidative stress (Nuttall et al., 1999; Mutlu-Turkoglu et al., 2003; Marzani et al., 2004).

On comparing FRAP values of plasma in relation to duration of COPD in patients, slightly elevated mean plasma FRAP value was observed in COPD patients with increased duration of COPD. A positive Pearson correlation was observed in the FRAP values of COPD patients with respect to the duration of the COPD. This depicted the increased level of oxidative stress among COPD patients with increased duration of COPD.

6. CONCLUSIONS

The present investigation results depicted that there was increased level of DNA damage caused by the oxidative stress. Nuclear anomalies and comet parameters showed significant differences between COPD patients and controls, suggesting that there was increased genetic damage in COPD patients.

Positive Pearson correlation coefficients observed for all nuclear anomalies except KH with respect to the number of cigarettes smoked daily in COPD patients depicted that the DNA damage increases in COPD patients with increase in the consumption of cigarettes.

Decreased FRAP values in COPD patients as compared to controls suggested that there was reduced antioxidant level of plasma. Oxidative stress increases in both patients with smoking and biomass-related COPD. The diet
forms the major source of antioxidants in the body. Optimising the antioxidant content of the diet might therefore be utilised to avert or treat metabolic stress in chronic obstructive pulmonary disease.

In rural areas women mostly use biomass fuel for cooking purposes. In the present investigation increased level of DNA damage was observed in women exposed to biomass smoke in control as well as COPD patients. The present study can be helpful in spreading awareness among people about adverse effects of cigarette smoking and biomass smoke exposure which causes oxidative stress in the body and inflammation in respiratory tract which can finally lead to COPD or other respiratory disease.

Further studies may be conducted with the following objectives:

1. To trace the extent of interaction of various factors i.e. environmental and genetic which may lead to progression of the COPD.
2. To device easy, efficient and economical methods for early diagnosis in occupational exposed workers.
3. To develop molecular and inflammatory markers for the early detection and treatment of COPD patients.
4. To reduce oxidative DNA damage in COPD patients through nutritional intervention.