CHAPTER 4: RESULTS

Results pertaining to micronucleus (MN) assay are given in Tables 1-26 and Figures 12-21. Figures 1 to 6 showed different types of nuclear anomalies observed in buccal epithelial cells of subjects. Figures 7 to 11 depict different types of comets observed in the lymphocytes of subjects. Results regarding comet assay are depicted in Tables 27 to 51 and Figures 22-30. FRAP assay values are presented in Tables 52-62 and Figures 31-34.

4.1 General characteristics of controls and COPD patients

The general characteristics of controls and COPD patients (average age, sex, smoking, biomass smoke exposure, drinking and dietary habits) are given in Table 1. A total of 200 subjects were analysed out of which 110 were COPD patients and 90 were control subjects.

4.1.2 COPD patients

A total of 110 COPD patients were randomly selected. The average age of the COPD patients was 56.736±1.210 years. Out of 110 subjects, 77 (70%) were males and 33 (30%) were females, 49 (54.44%) smokers and 61 (55.45%) non-smokers, 48 (43.64%) were biomass smoke exposed and 64 (58.18%) were non-exposed. In relation to drinking habits, 21 (19.09%) were alcoholics and 89 (80.90%) non-alcoholics. Regarding dietary habits, 68 (61.82%) were vegetarians and 42 (38.18%) were non-vegetarians (Table 1).

4.1.3 Control subjects

A total of 90 subjects matched with respect to their age, sex, smoking, socio-economic status, drinking and dietary habits with 110 COPD patients were taken as controls in the present study. The average age of the control subjects was 53.667 ±1.613 years. Out of 90 subjects, 63 (70%) were males and 27 (30%) females, 59 (65.55%) non-smokers and 31 (34.44%) smokers, 29 (32.22%) were biomass smoke exposed and 61 were biomass smoke non-exposed. In case of
drinking habits subjects were divided into 71 (78.89%) non-alcoholics and 19 (21.11%) alcoholics. The dietary habits included 57 (63.33%) vegetarians and 33 (36.67%) non-vegetarians (Table 1).

4.2 MN assay analysis

4.2.1 Control subjects and COPD patients

The mean frequencies of nuclear anomalies viz. MN, BN, BE, KL and KH in both control subjects and COPD patients are given in Table 2. The mean frequency of MN in COPD patients (3.618±0.238) was significantly (P<0.001) higher with respect to control subjects (0.867±0.115). Similarly, there was about two fold significantly (P<0.001) increased BN frequency in COPD patients (14.354±0.404) as compared to control subjects (7.122±0.355). A significant (P<0.001) difference was observed in mean frequencies of BE (15.327±0.425) and KL (16.264±0.634) in COPD patients as compared to control subjects (9.956±0.350, 1.372±0.187). Whereas the mean frequency of KH in COPD patients (1.372±0.187) showed a significant difference at P<0.01 level with respect to controls (0.811±0.120). Mean frequencies of nuclear anomalies viz. MN, BN, BE and KL were observed to be significantly different (P<0.001) in COPD patients as compared to controls. In case of KH the significant difference was observed at P<0.01 (Figure 12).

The Pearson correlation coefficients of all the nuclear anomalies in control subjects are given in Table 3. Negative and non-significant correlation was observed between BE and MN; KL and MN; KH and MN; KH and BE; KH and KL. Non-significant (P>0.05) correlation was observed between rest of the nuclear anomalies.

In COPD patients a significant (P<0.001) and positive correlation was observed between following nuclear anomalies: KL and MN; BE and BN; KL and BN; KL and BE. Negative and non-significant (P>0.05) correlation was observed between KH and MN; KH and BN; KH and BE (Table 4).
4.2.2 Severity of COPD

COPD patients were categorized into two groups i.e. moderate and severe according to the severity of disease (GOLD, 2006). The significant (P<0.001) difference was observed in the mean frequency of MN and KL in severe COPD patients as compared to the moderate COPD patients (Table 5, Figure 13).

4.2.3 Gender

The mean frequencies of nuclear anomalies of males and females of both control subjects and COPD patients are given in Table 6. In male COPD patients, the mean frequencies of the nuclear anomalies viz. MN, BN, BE and KL showed a significant (P<0.001) difference as compared to the control males. In case of KH the significant difference was observed at P<0.01 (Table 6). The mean frequencies of BE and KL showed highly significant difference (P<0.001) in male COPD patients as compared to females. The mean values of MN (P<0.05), BN (P<0.01) and KH (P<0.5) also showed a significant difference among male COPD patients as compared to females.

In case of females all the nuclear anomalies showed a significant (P<0.001) difference except KH (Figure 14).

4.2.4 Smoking habits

Control subjects and COPD patients were divided into non-smoker and smoker groups (Table 7). Comparison of non-smoker group of control subjects and COPD patients showed a significant difference (P<0.001) in the mean frequency of BN, BE and KL. In case of smoker group, MN frequency (4.449±0.358) was significantly (p<0.001) increased as compared to the control subjects (1.193±0.209); similarly the mean frequencies of BN, BE and KH (16.612±0.666, 17.796±0.832 and 22.143±0.666, respectively) were also significantly (p<0.001) increased in COPD patients as compared to the control smoker subjects (8.419±0.514, 9.290±0.440 and 12.064±0.466, respectively). The mean frequency of KH showed significant difference at p<0.01 (Figure 15).
COPD patients and controls were further categorized according to the number of cigarettes smoked daily i.e. 0 cigarette/day, 1-6 cigarettes/day and >6 cigarettes/day. The mean frequencies of MN and BN in control subjects consuming >6 cigarettes/day were significantly (P<0.05) higher as compared to the subjects consuming 0 cigarette/day and 1-6 cigarettes/day. Whereas the mean frequencies of BE and KL in control subjects who have smoked >6 cigarettes/day were significantly (P<0.05) higher as compared to the non smokers (Table 8).

Similarly the mean frequencies of MN and BN were significantly (P<0.05) higher in COPD patients who consumed >6 cigarettes/day as compared to the 0 cigarette/day consumers and those consumed 1-6 cigarettes/day. The frequencies of BE and KL were significantly (p<0.05) higher in smokers smoking > 6 cigarettes/day as compared to the non smokers (Table 9).

A significant (P<0.01) and positive Pearson correlation coefficients were observed between MN, BN and KL with number of cigarettes smoked in control subjects. Negative and non-significant correlation was observed between number of cigarettes smoked and KH (Table 10). In COPD patients significant (P<0.01) and positive Pearson correlation coefficients were observed for all nuclear anomalies except KH with respect to the number of cigarettes smoked daily (Table 11).

4.2.5 Alcohol drinking habits

Mean frequencies of nuclear anomalies of non-alcoholics and alcoholics of both control subjects and COPD patients are given in Table 12. Significant differences (P<0.001) were observed in the mean frequencies of MN, BN, BE and KL in non-alcoholics COPD patients than non-alcoholics control subjects. Mean frequency of KH in COPD patients (1.157±0.183) was significantly different (P<0.01) with respect to control subjects (0.887±0.145) (Figure 16). Comparison of mean frequencies of nuclear anomalies among alcoholic COPD patients and control subjects showed significantly (P<0.001) increased frequencies of MN, BN, BE and KL in COPD patients as compared to the control subjects. In case of
KH, a significant difference was observed at P<0.05 in COPD patients when compared with control subjects.

COPD patients and control subjects were categorized into four categories according to their alcohol consumption habit i.e. never, once a while, <4 times/week and >4 times/week. MN frequency was found to be significantly (P<0.05) increased in control subjects consuming alcohol >4 times/week (2.500±0.428) as compared to <4 times/week (1.000±0.577) consumers, once a while (1.167±0.307) and non consumers (0.676±0.115) (Table 13). COPD patients consuming alcohol >4 times/week had significantly (P<0.05) elevated frequencies of BN and BE (19.2000±1.280, 19.800±2.417) when compared with non-alcoholics (14.056±0.439, 14.753±0.470) (Table 14). The mean frequency of KH was significantly different (P<0.05) in subjects consuming alcohol >4 times/week than other categories in COPD patients.

4.2.6 Dietary habits

Comparison of nuclear anomalies of vegetarians and non-vegetarians of both control subjects and COPD patients is given in Table 15. In vegetarians and non vegetarians the significant difference (P<0.001) was observed in the mean frequencies of MN, BN, BE among COPD patients as compared to controls. Non vegetarian COPD patients showed elevated frequency of KH (1.857±0.130) than non-vegetarian control subjects (0.606±0.130) and significantly different at P<0.01 (Figure 17).

4.2.7 Age

Mean frequencies of nuclear anomalies in relation to age (years) in control subjects is given in Table 16. Control subjects were divided into two groups, on the basis of their age i.e. ≤60 years and >60 years. BE frequency is significantly (P<0.05) increased in subjects having >60 years of age (9.687±0.603) as compared to those having age ≤60 years (7.620±0.372). Rest of the nuclear anomalies showed no significant difference (P>0.05) (Figure 18).
The mean frequencies of BE and KH were showing significantly positive correlation with respect to the age (years) in control subjects. The mean values of MN, BN and KL showed negative and non-significant correlation with age (Table 17).

On the basis of age COPD patients were also divided into two groups i.e. ≤60 years and >60 years (Table 18). The significant difference (P<0.05) was observed in the mean frequency of BN in COPD patients with >60 years age (15.595±0.585) as compared to COPD patients group having ≤60 years age (13.588±0.527) (Figure 19). Positive and significant (P<0.05) Pearson correlation coefficient was observed between the mean frequency of BN with age. Negative non-significant correlation was observed in the mean frequencies of BE and KH with age (Table 19).

4.2.8 Duration of the COPD

COPD patients were divided into two groups on the basis of duration of COPD (years) i.e. ≤7 years and >7 years. The frequencies of MN and BN were significantly higher in >7 years group as compared to ≤7 years group. The significant differences were observed for MN (P<0.001) and for BN (P<0.05) (Table 20, Figure 20). Pearson correlation coefficients of all the nuclear anomalies observed with respect to the duration of COPD (years) are depicted in Table 21. MN and BN showed significant (P<0.01, P<0.05) and positive correlation with duration of COPD in the patient group.

4.2.9 Duration of biomass smoke exposure in the COPD

Mean frequencies of nuclear anomalies of biomass smoke exposed COPD patients and control subjects are given in Table 22 and Figure 21. The significant differences were observed 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. Control subjects were categorized into two groups on the basis of exposure of biomass smoke i.e. ≤8 years and >8 years group. The significant differences were observed in the mean frequencies of MN,
BN and KL in >8 years group than ≤8 years group. Significant difference was observed in case of MN (P<0.001) along with BN and KL (P<0.05) (Table 23). Positive correlations (P<0.01) were observed among MN and BN with duration of biomass smoke exposure (years). Mean frequency of KL showed negative but significant (P<0.05) correlation with duration of biomass smoke exposure (years) (Table 24).

COPD patients were also divided into two categories according to the exposure of biomass smoke i.e. ≤8 years and >8 years group. MN (P<0.001) and BN (P<0.05) showed elevated frequencies in >8 years group (4.768±0.518 and 16.059±1.059, respectively) as compared to ≤8 years group (1.903±0.264, 12.452±0.673, respectively) (Table 25). Significant (P<0.01) correlation was observed for MN and BN with respect to the duration of biomass smoke exposure (years). KL showed significant positive correlation at (P<0.05) (Table 26).

4.3 Comet assay analysis

4.3.1 Control subjects and COPD patients

The mean values of different comet parameters viz. Tail DNA (%), Integral intensity, Tail length, Tail moment, Olive moment and Tail area are given in Table 27. The significant (P<0.001) differences were observed in the mean values of all comet parameters in COPD patients and the control subjects.

Pearson correlation coefficients for different comet parameters analysed in control subjects are given in Table 28. The mean values of different comet parameters were observed to be significantly (P<0.01) different in control subjects except for Integral intensity and Tail DNA (%). Pearson correlation coefficients for different comet parameters observed in COPD patients are given in Table 29. Similar results were observed in COPD patients, all the comet parameters were significantly correlated at P<0.01 level whereas a non-significant (P>0.05) and negative correlation was observed between Integral intensity and Tail DNA (%).
4.3.2 Severity of COPD

Comparison of different comet parameter observed in moderate and severe COPD patients is given in Table 30. Mean value of Tail DNA (%) showed a significant difference in severe COPD patients when compared with moderate COPD patients. Rest of the comet parameters showed no significant (P>0.05) difference between moderate and severe COPD patients (Figure 23).

4.3.3 Gender

Mean values of different comet parameters of males and females of both control subjects and COPD patients are depicted in Table 31, Figure 24. 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 value of Tail DNA (%), Tail length, Tail moment, Olive moment and Tail area were significantly (P<0.001) different among COPD patients as compared to control subjects. The mean value of Integral intensity did not showed any significant difference.

4.3.4 Smoking habits

The mean values of different comet parameters in control subjects and COPD patients with respect to their smoking habits are given in Table 32. Markedly significant (P<0.001) differences were found for all comet parameters in non-smoker and smoker COPD patients as compared with control subjects with respect to the smoking habits (Figure 25).

The comparison of mean values of all the comet parameters observed with respect to number of cigarettes smoked daily in control subjects is given in Table 33. Control subjects were divided into three categories viz. 0 cigarette/day, 1-6 cigarettes/day and >6 cigarettes/day. Tail DNA (%) in the subjects those who smoked 1-6 cigarettes/day and >6 cigarettes/day (16.301±0.898 and 16.541±1.436, respectively) showed a significant difference (P<0.05) as compared to the non-smokers (11.696±0.466). Rest of the comet parameters showed no significant difference between different smoker groups. Tail moment (0.825±0.079) and Olive moment (1.587±0.104) were significantly (P<0.05)
different in non-smokers than other categories (1-6 cigarettes/day and >6 cigarettes/day). COPD patients were also divided into three groups according to number of cigarettes smoked daily i.e. 0 cigarette/day, 1-6 cigarettes/day and >6 cigarettes/day. Tail DNA (%) and Tail area in smokers smoking >6 cigarettes/day (39.110±1.749 and 4320.600±223.110, respectively) were significantly different from the 0 cigarette/day consumers (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 those consuming 0 cigarette/day consumers (Table 34).

Positive and significant Pearson correlation coefficient was observed for Tail DNA (%) with respect to number of cigarettes smoked daily in control subjects at (P<0.001) (Table 35). Integral intensity was observed to be negative but significantly (P<0.001) correlated with number of cigarettes smoked daily in control subjects. COPD patients were divided into three categories viz. 0 cigarette/day, 1-6 cigarettes/day and >6 cigarettes/day on the basis of number of cigarettes smoked daily. Markedly significant (P<0.01) and positive correlation was observed for Tail length and Tail area with number of cigarettes smoked daily. Tail DNA (%) and Olive moment showed significant correlation at P<0.05. While negative and non-significant correlation was observed in case of Integral intensity when correlated with number of cigarette smoked daily (Table 36).

4.3.5 Alcohol drinking habits

The mean values for different comet parameters among non-alcoholics and alcoholics of control subjects and COPD patients are presented in Table 37. The mean values of all the comet parameters in non-alcoholics COPD were significantly (P<0.01) higher as compared to the non-alcoholics control subjects. Similarly, alcoholics COPD patients also showed significant difference in the mean value of all the comet parameters when compared with the control alcoholic subjects (Figure 26).

Control subjects and COPD patients were categorized into four groups i.e. never, once a while, <4 times/week and >4 times/week according to the frequency
of alcohol consumption in control subjects (Table 38). Subjects who never consumed alcohol (11.958±0.423) had significantly (P<0.05) lower value of Tail DNA (%) as compared to the subjects who consumed alcohol once a while (20.037±0.836), <4 times/week (16.601±1.796) and >4 times/week (18.923±2.572). No significant difference was observed among rest of the comet parameters. Similar trend was observed in COPD alcohol consumers, the significant (P<0.05) difference was observed in the mean value of Tail DNA (%) who never consumed alcohol (35.121±0.636) when compared with subjects those consumed alcohol once a while (41.469±2.736), <4 times/week (43.150±2.804) and >4 times/week (45.369±2.082) (Table 39).

4.3.6 Dietary habits

The mean values of different comet parameters of vegetarians and non-vegetarians of both control subjects and COPD patients are given in Table 40. Highly significant (P<0.001) differences were observed in mean values of all the comet parameters of vegetarians and non-vegetarian in COPD patients when compared with control subjects (Figure 27).

4.3.7 Age

Comparison of different comet parameters with respect to age (years) in control subjects and COPD patients is given in Tables 41, 42. No significant difference was observed for the mean values of all the comet parameters in both control subjects and COPD patients having age >60 as compared to the subjects having ≤60 years age. Different comet parameters observed with respect to age are presented in Figure 28.

The mean values of different comet parameters in control subjects viz. Tail length, Tail moment, Olive moment and Tail area showed positive but non-significant correlation with age. A negative and non-significant correlation was observed for Tail DNA (%) with age (Table 43).
Pearson correlation coefficients for different comet parameters with respect to age are given in Table 44. Tail DNA (%), Tail length and Tail area showed positive non-significant correlation with age. Negative and non-significant correlation was observed for Integral intensity, Tail moment and olive moment with age in COPD patients.

4.3.8 Duration of COPD

Mean values of different comet parameters observed in COPD patients with respect to the duration of COPD (years) are given in Table 45 and Figure 29. No significant difference was analysed when comparison was made for all the comet parameters with respect to the duration (years) of COPD.

Positive Pearson correlation was observed in the mean value of Tail DNA (%) with respect to the duration (years) of COPD. The mean values of Tail length, Tail moment, Olive moment and Tail area showed a positive and non-significant correlation, while the Integral intensity showed the negative and non-significant correlation with duration (years) of COPD (Table 46).

4.3.9 Exposure to biomass smoke

Mean values of different comet parameters observed in control subjects and COPD patients according to the exposure (years) of biomass smoke are given in Table 47, Figure 30.

Markedly significant (P<0.001) difference was observed for Tail DNA (%), Tail length, Tail moment, Olive moment and Tail area between biomass smoke exposed COPD patients (38.789±1.002, 62.411±3.891, 35.638±2.47, 923.831±1.502 and 3430.200±250.392, respectively) as compared to the biomass smoke exposed control subjects (15.254±0.869, 25.933±2.578 10.463±1.320, 7.379±0.772 and 1226.000±136.084, respectively) (Figure 30).

Integral intensity showed a significant difference at P<0.05 level between biomass smoke exposed COPD patients and biomass smoke exposed control subjects. On the basis of duration of biomass smoke exposure, the control subjects and COPD patients were divided into two groups i.e. ≤8 years and >8 years
exposure group. Both control subjects and COPD patients showed no significant
difference between ≤8 years and >8 years group (Table 48, 49).

Pearson correlation coefficients for different comet parameters observed
with respect to duration of biomass smoke exposure (years) in control subjects are
given in Table 50. Significant (P<0.01) and positive correlation was observed for
Tail DNA (%) with respect to the biomass smoke exposure. Rest of the comet
parameters showed negative and non-significant correlation with biomass smoke
exposure.

Significant positive correlation was observed for Tail DNA (%) in COPD
patients when correlated with biomass smoke exposure (years). The mean values
of Integral intensity, Tail length, Tail moment, Olive moment and Tail area
showed positive and non-significant correlation with biomass smoke exposure
(years) (Table 51).

4.4 FRAP Analysis

4.4.1 Control subjects and COPD patients

FRAP value of plasma in both control subjects and COPD patients are
given in Table 52 and Figure 31. FRAP value of control subjects (966.51±7.347)
was significantly (P<0.001) higher (765.580±7.571) as compared to COPD
patients (392.280±06.602).

4.4.2 Severity of disease

The mean FRAP value of plasma in moderate COPD patients was
significantly higher as compared to the severe COPD patients. The significant
difference was observed at P<0.001 level (Table 53, Figure 32).

4.4.3 Gender, smoking, biomass smoke exposure, alcohol drinking and
dietary habits

The mean values of FRAP in control subjects and COPD patients with
respect to various habits are given in Table 54 and Figure 33. The mean FRAP
values of control subjects viz. males, females, non-smokers, alcoholics, biomass
smoke non-exposed and exposed were observed to be significantly higher (787.290±8.388, 714.930±11.029, 772.290±9.919, 752.810±11.109, 783.820±8.420 and 727.210±12.976, respectively) when compared with COPD patients (402.570±8.078, 368.270±10.369, 410.950±9.089, 369.040±8.565, 415.810±8.275 and 361.900±9.056, respectively). Similarly, non-alcoholics, alcoholics, non-vegetarian and vegetarian of control subjects showed significant (P<0.001) increase in the mean values of FRAP when compared with COPD patients.

FRAP values of plasma in relation to various characteristics of control subjects are presented in Table 55. The significance difference (P<0.001) was observed in the mean FRAP value of males (787.290±8.388) as compared to the females (714.930±11.029). Biomass smoke non-exposed subjects showed lower value of FRAP (727.210±12.976) than biomass smoke exposed subjects (783.820±8.420). A small and non-significant (P>0.05) difference was observed in the mean FRAP value of non-smokers, non-alcoholics and vegetarian (772.290±9.918, 766.580±8.518 and 772.260±10.267, respectively) as compared to smokers, alcoholics and non-vegetarian subjects (752.810±11.109, 761.840±16.948 and 754.030±10.471, respectively).

In case of COPD patients, highly significant difference was observed in the mean FRAP values of non-smokers, biomass smoke non-exposed subjects and vegetarians (410.950±9.089, 415.810±8.275 and 409.810±8.472, respectively) than smokers, biomass exposed and non-vegetarian patients (369.040±8.565, 361.900±9.056 and 363.900±9.046, respectively). Mean FRAP values of males and non-alcoholics observed significance difference at P<0.05 level when compared with females and alcoholics, respectively (Table 56).

4.4.4 Age

Control subjects were categorized into two groups on the basis of age i.e. ≤60 years age and >60 years age group. The mean FRAP values showed no significant difference when comparison was made between ≤60 years and >60 years group. COPD were also divided into two groups on the basis of age i.e. ≤60
years age and >60 years age group. Slight increase in the value of FRAP was reported in >60 years group than ≤60 years group but the difference was non-significant (P>0.05) (Table 57).

Table 58 depicts the Pearson correlation coefficient for FRAP values of plasma with respect to age (years) in control subjects and COPD patients. Positive and non-significant (P>0.05) correlation was observed between FRAP values and age (years) in both control subjects and COPD patients.

4.4.5 Duration of the COPD
COPD patients were divided into two groups i.e. ≤7 years and >7 years group according to the duration (years) of COPD (years). The FRAP value was slightly decreased in >7 years age group (391.240±10.947) than ≤7 years age group (393.000±8.281) (Table 59).

Positive and non-significant Pearson correlation coefficient was observed between FRAP values and duration (years) of COPD in COPD patients (Table 60).

4.4.6 FRAP values of plasma in relation to biomass smoke exposure in control subjects and COPD patients
Table 61 represents the mean FRAP value of plasma in biomass smoke exposed control subjects and COPD patients. Control subjects and COPD patients were divided into two groups according to the exposure of biomass smoke i.e. ≤8 years and >8 years age group. A slight decrease in the mean value of FRAP was observed when comparison was made between >8 years group (718.500±21.288) and ≤8 (737.920±12.722) years group. No significant difference was observed in the FRAP values of >8 years group (366.060±15.504) as compared to the ≤8 years age group (359.610±11.321).

Negative and non-significant (P>0.05) correlation was observed in the mean value of FRAP with respect to the age (years) in controls. In case of COPD patients, positive but non-significant correlation was observed with age (years) (Table 62).