4. RESULTS

The present study was carried out to investigate the extent of genetic damage in PBLs and buccal epithelial cells of cancer patients. MN assay and comet assay were used as the biomarkers for the assessment of the genetic damage in cancer patients as well as normal control subjects.

4.1 GENERAL PROFILE OF THE SUBJECTS UNDER STUDY

The general characteristics (average age, sex, smoking, drinking, and dietary habits and duration of disease) of both cancer patients and matched normal subjects under the study are given in Table 1. Out of the 200 subjects studied, 120 were cancer patients and 80 were normal subjects. A majority of the subjects under study were illiterate (64%) with only 36% subjects having completed matriculation. Slightly less than half of the subjects were employed while the rest were unemployed.

4.1.1 Cancer Patients

A total of 120 cancer patients were randomly selected over a period of three years. The average age of these persons was 51.18 years with a range of 25-80 years. Out of these, 72 (60%) were women while 48 (40%) were men (Table 1). The dietary habits of the cancer patients divided them into 99 (83%) vegetarians and 21 (17%) omnivores. Of all the cancer patients, 40 (33%) were smokers and 80 (67%) were non-smokers. Regarding alcohol drinking habits, 25 (21%) were alcoholics and 95 (79%) were abstainers. The average duration of disease in the study group was 15.61 months.

The details of prevalence of different types of cancer in the present study group are given in Table 2. The classification of types of cancer was done in accordance with ICD-10 (International Classification of Diseases and Related Health Problems, 10th edition) guidelines. With 33 (27.5%) cases, breast cancer was the most prevalent type of cancer in the study group followed by 24 cases (20%) of oral cancer and 12 cases (10%) of oesophageal cancer. Digestive system was the most affected body system with 66 cases (55%), followed by the reproductive system with 47 cases (39%) while there was only one patient with the cancer of circulatory system.
4.1.2 Normal Subjects

A total of 80 normal subjects matching the cancer patients, with respect to their age, sex, smoking, drinking, and dietary habits, were randomly selected. The average age of the group was 45.09 years with a range of 23-84 years. Out of these, 35 (44%) were women while 45 (56%) were men (Table 1). The dietary habits of the normal subjects divided them into 57 (71%) vegetarians and 23 (29%) omnivores. Of all the normal subjects, 28 (35%) were smokers and 52 (65%) were non-smokers. Regarding alcohol drinking habits, 23 (29%) were alcoholics and 57 (71%) were abstainers.

4.2 MN ASSAY

4.2.1 Comparison of Normal Subjects and Cancer Patients

The comparison of the mean frequencies of nuclear anomalies MNC, TMN, BE, BN, KH and KL in both cancer patients and normal subjects is given in Table 3. The difference in the mean frequencies of MNC and TMN in cancer patients (4.50±0.375 and 5.50±0.469, respectively) and normal subjects (0.75±0.141 and 0.78±0.146) was found to be highly significant (P<0.001), indicating an association between cancer and the increased occurrence of MNi. Similarly, there was a more than four-fold increase in the mean frequencies of BE and KH from normal subjects (1.74±0.236, 0.99±0.332, respectively) to cancer patients (7.09±0.490 and 4.11±0.371, respectively). A significant increase was observed in the mean frequencies of BN and KL from normal subjects (3.64±0.344 and 39.09±2.920, respectively) to cancer patients (6.53±0.506 and 61.15±3.428, respectively). The difference in the mean frequencies of all the nuclear anomalies was highly significant (P<0.001) between both the groups (Figure 12).

The Pearson correlation coefficients of different nuclear anomalies observed in normal subjects are given in Table 4. The value of Pearson correlation coefficient was found to be statistically significant (P<0.01) only between MNC and TMN. Non-significant correlations were observed between the rest of the nuclear anomalies. In cancer patients, the Pearson correlation coefficients between different nuclear anomalies were found to be highly significant (P<0.01) between following pairs; MNC, TMN; MNC, BE; MNC, BN; TMN, BE; TMN, BN; BE, BN. A negative but statistically insignificant correlation was observed between BN and KL (Table 5).
4.2.2 Genetic Damage in Cancer Patients with respect to the Disease and Treatment Taken

The mean frequencies of nuclear anomalies in cancer patients according to the organ system affected are given in Table 6. The mean frequencies of MNC and TMN were the highest in patients with cancer of circulatory system (11.00±0.000 and 13.00±0.000, respectively) and the lowest in patients with cancer of respiratory system (0.75±0.750 each). The mean frequencies of BN and KH too were found to be the highest in patients with cancer of circulatory system (11.00±0.000 and 8.00±0.000, respectively), while the lowest values were observed in the patients with cancer of excretory system (1.00±1.000 and 0.50±0.500, respectively). Surprisingly, in case of L, the highest mean frequency was observed in patients with cancer of excretory system (80.00±15.000) which was more than twice the mean frequencies found in patients with cancer of circulatory system (35.00±0.000) (Figure 13).

While the mean frequencies of MNC and TMN were found to be the lowest in case of patients with lung cancer (0.75±0.750, each), the highest values were observed in patients with colon cancer (6.00±1.327 and 8.14±1.805, respectively). The mean frequency of BE varied from the lowest in patients with lung cancer (2.50±0.866) to the highest in patients with cancers of oesophagus (8.50±1.062) and liver (8.50±3.500). In case of KH, the mean frequency was the highest in patients with colon cancer (7.00±3.185) and the lowest in patients with the cancers of gastro-intestine (2.00±2.000) and gall bladder (2.00±1.000). The difference in the mean frequencies of MNC, TMN, BE and KH with respect to the organ affected was found to be statistically non-significant (Table 7). The difference in mean frequencies of BN in patients with gall bladder cancer (11.00±4.041) and patients with gastro-intestinal cancer (1.33±0.882) was found to be statistically significant (P<0.01). Also, the difference in the mean frequencies of KL was statistically significant (P<0.01) in case of patients with gall bladder cancer (28.00±13.796) and those with ovarian cancer (88.33±19.335) (Figure 14).

The cancer patients were categorized into 3 groups, based on the duration of the disease, i.e., 1-12 months, 13-24 months and >24 months. The analysis of mean frequencies of nuclear anomalies of cancer patients with respect to the duration of disease is given in Table 8. The mean frequencies of MNC and TMN increased from 1-12 months (3.15±0.299 and 3.74±0.357, respectively) to 13-24 months (6.23±0.826 and 8.15±1.126, respectively) and >24 months (10.75±1.199 and 13.38±1.488,
respectively), with the difference between the groups being highly significant (P<0.01). The mean frequencies of BE and BN were not significantly different in the first two groups but significantly higher mean frequencies were observed in the group with >24 months of disease exposure. The differences in the mean frequencies of KH and KL were homogeneous for all the groups (Figure 15).

Pearson correlation coefficients were positive between duration of disease and all the nuclear anomalies observed while being statistically significant (P<0.01) with MNC, TMN, BE and BN (Table 9).

For each patient, the stage of cancer was noted from their medical record. The patients were then grouped into 3 categories; viz. Stage II (early locally advanced), Stage III (late locally advanced) and Stage IV (metastasized) on the basis of the stage of cancer (Table 10). No patient with Stage I (localized) cancer was encountered in the present investigation. The mean frequency of MNC increased continuously with the stage of cancer and the difference was highly significant (P<0.01) between patients with Stage II cancer (2.08±0.720) and those with Stage IV cancer (5.31±0.498). The mean frequency of MNC in patients with Stage III cancer (3.74±0.661) did not differ significantly from either of the group. Similarly, the mean frequencies of TMN and BE were significantly different (P<0.05) between patients with Stage II cancer (2.69±1.015 and 4.31±1.052, respectively) and patients with Stage IV cancer (6.47±0.614 and 8.25±0.667, respectively). The mean frequencies of BN, KH, and KL increased gradually from patients with Stage II cancer to patients with Stage IV cancer, though not significantly (Figure 16).

Pearson correlation coefficients of the stage of cancer with MNC, TMN and BE were highly significant (P<0.01) while that of stage of cancer with BN and KH were significant at P<0.05 (Table 11).

Comparison was also made between the mean frequencies of nuclear anomalies in cancer patients with respect to the treatment taken by them. The treatment histories of the cancer patients were taken from their medical records. Based on the information received the patients were divided into eight groups, viz. no treatment; surgery; chemotherapy; radiation therapy; surgery & chemotherapy; radiation therapy & chemotherapy; surgery & radiation therapy and surgery, chemotherapy & radiation therapy (Table 12). The mean frequencies of MNC (2.96±0.465) and TMN (3.35±0.541) were the lowest in patients who had received no treatment so far and the highest in patients who received a combination of surgery and
radiation therapy (8.00±0.707 and 10.75±0.250, respectively). Among the treatment groups, the lowest mean frequencies of MNC, TMN, BE and BN were observed in patients who had undergone surgery alone, while the highest mean frequencies were observed in treatment groups with radiation therapy as an integral part of the treatment. This may be due to the genotoxic (clastogenic) effects of the radiations used in radiation therapy (Figure 17).

### 4.2.3 Comparison with respect to Inherent Factors

The comparison of mean frequencies of different nuclear anomalies between men and women under study is given in Table 13. In both normal subjects and cancer patients, the mean frequencies of MNC were higher in women (0.77±0.201 and 4.92±0.527, respectively) than men (0.73±0.197 and 3.88±0.499, respectively). While in normal subjects, the mean frequency of TMN in women (0.78±0.208) was slightly lower than in men (0.77±0.201), in case of cancer patients, the mean frequency of TMN was higher in women (5.85±0.650) as compared to men (4.98±0.653). Women showed higher mean frequencies for BE and BN as well. Surprisingly, men showed higher mean frequencies of KH and KL in both the groups. The differences were not statistically significant (P<0.05) in any case.

On the other hand, when the mean frequencies of all the nuclear anomalies in men in the normal subjects group were compared to men in the cancer patients group the differences were significantly high (P<0.001) Similar highly significant (P<0.001) differences were observed on comparing the nuclear anomalies in women in the normal subjects group with the women in the cancer patients group. The differences were slightly less significant (P<0.01) in case of BN and KL in men and BN in women (Table 14; Figure 18).

The mean frequencies of nuclear anomalies of cancer patients and normal subjects having different blood groups have been compared in Table 15. While the mean frequencies of all the nuclear anomalies except KL (in blood group O) were significantly higher in cancer patients with blood groups A, B and O as compared to normal subjects with the same blood groups, none of the nuclear anomalies showed a significant difference between cancer patients and normal subjects having blood group type AB (Figure 19).

Among cancer patients, those having a family history of cancer had mean frequencies of MNC (7.55±1.065) and KH (7.73±2.224) were statistically higher
than those who didn’t have a family history of cancer (4.19±0.388 and 3.74±0.328, respectively) (Table 16). Difference in the mean frequency of TMN in patients with a family history of cancer (8.91±1.378) and patients without a family history of cancer (5.16±0.487) was statistically significant at P<0.05. The mean frequencies of all other nuclear anomalies under observation were higher in patients with a family history of cancer although the differences were not statistically significant (Figure 20).

4.2.4 Comparison with respect to Lifestyle Factors

The nuclear anomalies of cancer patients and normal subjects with respect to their dietary habits have been compared in Table 17. The mean frequencies of all the nuclear anomalies in normal subjects group were found to be lower in vegetarians as compared to omnivores. While the mean frequencies of KH and KL in vegetarians and omnivores in cancer patients showed a similar trend the mean frequencies of MNC, TMN, BE and BN were higher in vegetarians than in omnivores. The mean frequencies of all the nuclear anomalies of vegetarians and omnivores in both cancer patients and normal subjects were not significantly different, except the mean frequency of BN which was significantly higher (P<0.01) in vegetarians (6.89±0.587) than omnivores (4.86±0.744) in cancer patients group (Figure 21). Mean frequencies of nuclear anomalies of both vegetarians and omnivores in cancer patients group were significantly higher than their counterparts in the normal subjects group expect for the mean frequency of BN in which the difference between the mean frequency between normal subjects and cancer patients was not statistically significant (Table 18).

As expected, in the normal subjects group, the mean frequencies of MNC, TMN and BE were lower in non-smokers than in smokers. An opposite trend was observed among cancer patients, where non-smokers had higher mean frequencies of MNC, TMN, BE and BN. These differences were non-significant except for MNC and TMN in cancer patients where the mean frequencies were significantly higher (P<0.01) in non-smokers (5.18±0.497 and 6.36±0.624, respectively) as compared to smokers (3.15±0.468 and 3.78±0.567, respectively) (Table 19). This may be due to the exposure to passive smoking in case of non-smokers.

When the mean frequencies of nuclear anomalies in non-smokers in cancer patients group were compared with those of their counterparts in the normal subjects group highly significant (P<0.001) differences were observed. Similarly, significantly
higher mean (P<0.001) frequencies were observed in smokers of cancer patients group as compared to their counterparts in normal subjects group, except for BN and KH where slightly less significant (P<0.05 and P<0.01, respectively) differences were noted (Table 20; Figure 22).

On the basis of the number of cigarettes smoked daily, subjects in both normal subject and cancer patient groups were classified into four categories, viz. 0 cigarette/day; 1-7 cigarettes/day; 8-20 cigarettes/day and >20 cigarettes/day. The difference in the frequencies of all nuclear anomalies was found to be non-significant in all the categories in normal subjects group (Table 21). Similar non-significant differences were observed in the mean frequencies of all nuclear anomalies in cancer patients group, except for KH in which the mean frequency was significantly (P<0.05) higher in patients consuming >20 cigarettes/day (6.06±1.518) when compared to patients consuming 0 cigarette/day (3.20±0.408) (Table 22; Figure 23).

In normal subjects, Pearson correlation coefficients of number of cigarettes smoked with respect to nuclear anomalies under observation were positive for MNC, TMN and BE, while they were negative for the rest of nuclear anomalies, none of them being significant (Table 23). Pearson correlation coefficients of number of cigarettes smoked with respect to nuclear anomalies present in cancer patients were negative for MNC, TMN and BN whereas they were positive for BE, KH and KL. Of these, only the coefficient for KH was significant at P<0.05 (Table 24).

As for the alcohol drinking habit, all the nuclear anomalies showed higher mean frequencies for abstainers when compared to alcoholics in the normal subjects group. Similar trend was observed in case of MNC, BN and KL in the cancer patients group, while in case of TMN, BE and KH alcoholics had higher mean frequencies than abstainers. But the differences were non-significant in both cancer patients group and normal subjects group (Table 25; Figure 24). When the abstainers of the normal subjects group were compared to their counterparts in the cancer patients group, highly significant (P<0.001) differences were observed for all the nuclear anomalies. Similarly in case of the mean frequencies of MNC, TMN and BE of alcoholics in both normal subjects and cancer patients highly significant (P<0.001) differences were there. Slightly less significant differences were obtained for the mean frequencies of KH (P<0.01) and BN and KL (P<0.05) (Table 26).

The subjects of both study groups were categorized into three categories, viz. 0 litres/month; 1-8 litres/month and >8 litres/month, according to the amount of
alcohol consumed per month (Figure 25). While in normal subjects the mean frequencies of MNC, TMN, BE and KL first decreased from the abstainers (0 litre/month) to the light drinkers (1-8 litres/month) and then again increased for the heavy drinkers (>8 litres/month) an opposite trend was observed in case of MNC, TMN, BE and KL in cancer patients where the mean frequencies were the highest for light drinkers (1-8 litres/month). No significant difference was observed in the mean frequencies of nuclear anomalies in either of the groups (Table 27, 28).

Pearson correlation coefficients of the amount of alcohol consumed (per month) with the nuclear anomalies was negative but non-significant for all the nuclear anomalies in normal subjects, except KL where it was positive. A reverse trend in Pearson correlation coefficient was observed in cancer patients where all the nuclear anomalies had a positive correlation with the amount of alcohol consumed (per month) except for KL. None of the correlations were found to be significant (Table 29, 30).

4.3 COMET ASSAY

4.3.1 Comparison of Normal Subjects and Cancer Patients

The comparison of the mean values of different comet parameters, viz. % tail DNA, integral intensity, tail length, tail moment, Olive tail moment and tail area between cancer patients and normal subjects is given in Table 31. There was at least a two-fold increase in the mean values of % tail DNA, tail length and tail area in cancer patients (35.81±1.222, 33.70±1.274 and 765.50±36.452, respectively) with respect to that of normal subjects (18.37±1.504, 16.25±1.544 and 366.05±44.141, respectively). The mean values of tail moment and Olive tail moment showed a 2.3 fold increase in cancer patients (17.94±0.853 and 12.40±0.567, respectively) from that of normal subjects (7.34±0.996, respectively). The differences in the mean values of all the comet parameters were highly significant (P<0.001) (Figure 26).

The Pearson correlation coefficients for different comet parameters observed in normal subjects are given in Table 32. The correlation was found to be highly significant (P<0.01) in all the parameters. Similarly, a highly significant (P<0.01) positive correlation was observed in different comet parameters in cancer patients as well (Table 33).
4.3.2 Genetic Damage in Cancer Patients with respect to the Disease and Treatment Taken

The mean values of comet parameters in cancer patients with respect to the organ system affected are given in Table 34. Mean values of tail length, tail moment and Olive tail moment were observed to be highest in circulatory system (67.02±0.000, 28.59±0.000 and 17.93±0.000, respectively) while mean values of % tail DNA and tail area were highest in respiratory system (42.22±5.347, 940.81±146.890, respectively). Digestive system showed the lowest mean values in % tail DNA (33.30±1.713), tail length (30.89±1.684), tail moment (16.17±1.150) and Olive tail moment (11.26±0.769) (Figure 27).

Mean values of comet parameters in cancer patients with respect to the organs affected are compared in Table 35. Liver cancer patients had the highest mean value of % tail DNA (46.15±10.700) while the cancer patients with neck cancer had the lowest mean value (27.48±5.836). Gall bladder cancer patients had the highest mean value of tail moment and Olive tail moment (24.59±4.677 and 16.37±2.557, respectively) in the group while neck cancer patients had the lowest mean values of these parameters (12.08±3.683 and 8.44±2.447, respectively). The mean values of integral intensity and tail length were again the highest in cancer patients with gall bladder cancer (217.91±35.647 and 48.15±9.187, respectively) and the lowest in cancer patients with gastro-intestinal cancer (86.41±14.201 and 24.48±2.122, respectively). The difference in the mean values of all the comet parameters was statistically non-significant except for the mean value of tail area for gall bladder (138.74±338.607) which differed significantly from rest of the affected organs (Figure 28).

The mean values of different comet parameters in cancer patients with respect to the duration (in months) of disease are given in Table 36. Strangely, the mean values of % tail DNA, tail length, tail moment, Olive tail moment and tail area decrease from cancer patients with 1-12 months to cancer patients with 13-24 months of disease, but the differences were non-significant. The mean values of comet parameters then rose significantly (P<0.05) in the group with disease duration of >24 months as compared to 13-24 months category. A reverse phenomenon was encountered in integral intensity which had lower mean value in >24 months category as compared to 1-12 months category (Figure 29).
Pearson correlation coefficients of duration of disease were positive but non-significant for all comet parameters except integral intensity where the Pearson correlation coefficient was negative (Table 37).

The comparison of mean values of comet parameters in cancer patients with respect to the stage of cancer is shown in Table 38. The mean values of % tail DNA, tail moment and Olive tail moment showed a significant increase from Stage II (15.71±4.335, 6.35±2.132 and 4.43±1.419, respectively) to Stage IV (33.31±1.800, 15.55±1.229 and 10.75±0.806, respectively). Rest of the comet parameters too increased significantly across the stages, except for integral intensity, where the difference was non-significant (Figure 30).

All the comet parameters, except for integral intensity, showed a highly significant (P<0.01) Pearson correlation coefficient with the stage of cancer. With integral intensity the Pearson correlation coefficient was significant at P<0.05 (Table 39).

The comparison of different comet parameters in cancer patients with respect to the treatment taken by them is given in Table 40. The difference in the mean values of these parameters was non-significant in different treatment groups (Figure 31).

### 4.3.3 Comparison with respect to Inherent Factors

The mean values of different comet parameters of normal subjects and cancer patients with respect to their sex are given in Table 41. The mean values of % tail DNA in both normal subjects and cancer patients was higher in case of women (19.39±2.451 and 36.56±1.464, respectively) than in men (17.58±1.890 and 34.70±2.132, respectively). Similarly, all other comet parameters too, was slightly higher in women as compared to men in both normal subjects and cancer patients but the differences were not statistically significant (Figure 32).

When the mean values of different comet parameters in men and women in the cancer patients group were compared to their counterparts in the normal subjects group the differences were found to be highly significant (P<0.001) for all the comet parameters, except for integral intensity, where the difference in the mean values was significant at P<0.05 level (Table 42).

Table 43 shows the comparison of the mean values of comet parameters in normal subjects and cancer patients with respect to their blood groups. All parameters showed a statistically significant difference in the two for the blood group types A, B
and O except for integral intensity which showed a statistically significant difference only for the blood group type AB (Figure 33).

Among cancer patients, those who had a family history of cancer had higher mean values for all the comet parameters as compared to those who didn’t have a family history of cancer (Table 44). The differences in the mean values of % tail DNA, tail moment and Olive tail moment in cancer patients with a family history of cancer (44.81±3.295, 24.12±2.748 and 16.36±1.779, respectively) and cancer patients without a family history of cancer (34.91±1.275, 17.32±0.879 and 12.01±0.586, respectively) were found to be significant (P<0.05) (Figure 34).

4.3.4 Comparison with respect to Lifestyle Factors

The mean values of different comet parameters of normal subjects and cancer patients with respect to their dietary habits have been compared in Table 45. In the normal subjects group, the mean values of % tail DNA, tail moment and Olive tail moment in omnivores (19.06±2.866, 8.51±1.989 and 6.07±1.319, respectively) were found to be higher than vegetarians (18.09±1.780, 6.87±1.151 and 5.10±0.767, respectively). Similarly, the mean values of tail length and tail area were also higher in omnivores as compared to the vegetarians. In case of integral intensity the mean value in the omnivores (79.76±5.700) was slightly lower than that in the vegetarians. Surprisingly, in case of cancer patients, only the mean values of % tail DNA and integral intensity were higher in omnivores (36.03±2.721 and 118.27±9.008, respectively) in comparison with vegetarians (35.77±1.370 and 116.30±4.428, respectively), while the rest of the comet parameters showed increased mean values in vegetarians as opposed to omnivores. The difference in mean values of comet parameters in vegetarian and omnivore persons in both groups was non-significant (Figure 35).

The difference in the mean values of comet parameters of vegetarians in cancer patients and normal subjects were highly significant (P<0.001) except for that of integral intensity, where the difference was significant at P<0.05 level. In case of omnivores the mean values of % tail DNA, integral intensity and tail length were significantly (P<0.001) higher in cancer patients than that in normal subjects. The difference in the mean values of tail moment, Olive tail moment and tail area were also significant (P<0.01) (Table 46).
Comparison of mean values of different comet parameters of normal subjects and cancer patients with respect to their smoking habits is given in Table 47. Oddly, among normal subjects, the mean values of % tail DNA, integral intensity, tail length and tail area were higher in case of non-smokers (18.57±1.908, 95.93±9.212, 16.53±1.927 and 383.17±58.618, respectively) with respect to smokers (18.00±2.474, 82.24±4.623, 15.73±2.625 and 334.26±64.634, respectively) while the mean values of tail moment and Olive tail moment showed a reverse trend. In cancer patients, the mean values of % tail DNA, integral intensity and tail area were higher in smokers (35.86±2.397, 119.34±6.587 and 778.88±70.106, respectively) as compared to non-smokers (35.79±1.397, 115.29±4.972 and 758.81±42.279, respectively), while the mean values of tail length, tail moment and Olive tail moment were slightly lower in smokers. None of the differences in the mean values of comet parameters in both the groups were statistically significant (Figure 36).

The mean values of comet parameters of non-smokers and smokers in cancer patients group are shown in Table 48. Both non-smokers and smokers showed significantly (P<0.001) higher mean values of comet parameters in cancer patients when compared to their counterparts in normal subjects group. Only in the case of integral intensity in non-smokers, the level of significance was P<0.05.

When the subjects under study were classified into four categories on the basis of the number of cigarettes smoked daily, no significant difference was observed between the mean values of different comet parameters in normal subjects group (Table 49). Similarly, non-significant difference was observed in different smoker categories in cancer patients group (Table 50; Figure 37).

Negative values of Pearson correlation coefficient of the number of cigarettes smoked (daily) were observed with respect to % tail DNA, integral intensity, tail length and tail area in normal subjects while the values were positive for tail moment and Olive tail moment. All the values were statistically non-significant (Table 51).

Similarly in cancer patients, the number of cigarettes smoked (daily) was negatively correlated with integral intensity, tail length and tail area while positive correlation was seen with % tail DNA, tail moment and Olive tail moment. Here also, the values were statistically non-significant (Table 52).

The mean values of comet parameters in abstainers and alcoholics among both cancer patients and normal subjects are given in Table 53. In normal subjects, the mean values of all the comet parameters viz. % tail DNA, integral intensity, tail length, tail moment, Olive tail moment and tail area were higher in abstainers
(18.96±1.854, 96.23±8.399, 16.99±1.918, 7.61±1.222, 5.57±0.811 and 394.02±56.524, respectively) as compared to alcoholics (16.91±2.535, 78.52±5.324, 14.42±2.523, 6.68±1.717, 4.91±1.151 and 296.73±62.252, respectively). Similar trend was observed in case of cancer patients. No significant difference was observed in the mean values of comet parameters in both the groups (Figure 38).

The comparison of the mean values of comet parameters of abstainers and alcoholics in normal subjects with their counterparts in cancer patients is given in Table 54. On comparing abstainers and alcoholics of normal subjects group with their counterparts in cancer patients group, highly significant (P<0.001) differences were observed for all comet parameters except for integral intensity where the level of significance was P<0.01 for both abstainers and alcoholics.

When categorized according to the amount of alcohol consumed (per month), the mean values of comet parameters in normal subjects decreased from abstainers to light drinkers and then increased again in heavy drinkers, although the difference in the mean values was not statistically significant (Table 55).

A trend similar to the one seen in normal subjects was observed in cancer patients when the mean values of comet parameters in different classes of alcoholics were compared, though the results were again not statistically significant (Table 56; Figure 39).

The Pearson correlation coefficients for the amount of alcohol consumed (per month) with respect to the comet parameters were found to be negative but statistically non-significant in both normal subjects (Table 57) and cancer patients (Table 58).

4.3 DISTRIBUTION OF ABO AND Rh (D) ALLELES

The phenotypes and gene frequencies of ABO blood group, observed in both normal subjects and cancer patients, are given in Table 59. In normal subjects, the highest frequency was that of allele O (0.533) followed by allele B (0.316) and allele A (0.151). A similar trend was observed in cancer patients, where the highest frequency was that of allele O (0.546) and lowest was again that of allele A (0.196). The $\chi^2$ values of the allele frequencies were non-significant (P<0.05) in both the cases.

The phenotypes and gene frequencies of Rh (D) blood group, observed in both normal subjects and cancer patients, are given in Table 60. The gene frequency for d was slightly higher in cancer patients (0.398) than normal subjects (0.354).