SUMMARY

Despite the tremendous advancements in clinical research and medical facilities, diseases like cardiac vascular diseases, cancer, diabetes, AIDS and malaria, etc. are the most dreaded enemies of the human beings, contributing a significant number to the world wide mortality and a still higher percentage of handicapped survivors. With a rapid increase in new incidences and mortality rate of about eight million people a year, cancer has emerged as a chief cause of death worldwide.

Cancer is one of the major diseases all over the world. As per WHO report, in 2008, an estimated 12.7 million new cases of cancer were diagnosed worldwide with lung, female breast, colorectal and stomach cancers accounting for about 40% of all the cancers diagnosed worldwide. In 2008, cancer caused 7.6 million deaths (~13% of all deaths) worldwide with a prevalence of approximately, 29 million..

Increasing life span and progressive control of communicable diseases have led to the rise of cancer and other non-communicable diseases as a major health issue in India and other developing countries. There were 948,900 new cancer cases in 2008 of which 430,100 were men and 518,800 were women. Cancers of cervix, breast, oral cavity, and lung were the most common types of cancer diagnosed in the country. While the age adjusted incidence rate of oesophageal cancer (8.3 per 100,000) in women of Bangalore and gall bladder cancer (10.6 per 100,000) in women of Delhi are amongst the highest in the world, the highest incidence rate (8.8 per 100,000) of cancer of tongue in males in the world is found to be in Bhopal. In India, cancer is the fourth major cause of death after cardiovascular diseases, respiratory diseases, and trauma. It accounted for an estimated 555,000 deaths in 2010, which was roughly 6% of all the deaths occurring that year. In the end of 2008, the prevalence of cancer in India was about 1.71 million people (adult population).

In India, cancer death rates are high as more than 80% of the cancer patients report to cancer care facilities in advanced stages when the cancer has metastasized to such an extent that very little can be done to save the patient from their impending death. Treatment is more effective when cancer is detected while it is still in a localized (pre-metastasis) state. Therefore, early detection and treatment is the key to
cancer control and can decrease more than one-third of the global cancer burden.

Valid biomarkers may be used for the timely identification of increased cancer risk, and can be used in the prevention or control of disease. The assumption that the observed relationship between disease exposure and the marker will translate into a similar relationship between exposure and disease supports the use of a biomarker as a surrogate of disease.

Keeping in view the above, the aim of the present study was planned to investigate the levels of genetic damage in the peripheral blood lymphocytes (PBLs) and buccal epithelial cells of cancer patients with following objectives:

1. To make an epidemiological survey of the proposed human subjects.
2. To look for DNA damage, if any, in the proposed subjects along with healthy matched controls.
3. To compare the frequency of micronuclei in buccal epithelial cells of the diseased persons with controls.
4. To provide a baseline data for further studies in this field.

The present study was planned and conducted in accordance with the declaration of Helsinki (WMA, 2008). The samples were collected from Department of Surgical Oncology, Pt.B.D.Sharma, P.G.I.M.S., Rohtak. Therefore, ethical clearance for conducting this study was obtained from the Institutional Ethics Committee, Kurukshetra University, Kurukshetra, vide Letter No. IEC/10/229 dated 15-05-2010 as well as from Institutional Ethics Committee, Pt.B.D.Sharma, P.G.I.M.S., Rohtak, vide Letter No. Surg-V/IEC/12/468 dated 03-11-2012. An informed consent was taken from each individual, who took part in the study, prior to obtaining the samples. A total of 200 individuals were studied, of which, 120 were randomly selected cancer patients and 80 were normal healthy subjects, matched with the cancer patients with respect to age, sex, smoking, and alcohol drinking habits, drug intake, if any, and social class.

In the present study, MN assay and comet assay were used as the biomarkers of choice for the evaluation of genetic damage in the cells under observation. In MN assay, the exfoliated buccal epithelial cells were collected from the inner cheek for
both cancer patients and normal subjects. The mean frequencies of micronucleated cells (MNC), broken egg (BE), bi-nucleated cells (BN), karyorrhexis (KH) and karyolysis (KL) in the buccal epithelial cells were evaluated to monitor the genetic damage. For the comet assay, blood samples from both cancer patients and normal subjects were collected intravenously into a vacuum tube containing EDTA, using sterile disposable needles. The samples were subjected to alkaline comet assay. Comet parameters, viz. % DNA in tail, integral intensity, tail length, tail moment, Olive tail moment, and tail area were evaluated to assess the extent of genetic damage by using Lucia Comet Assay v6.22 software.

During the present study, 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. The value of Pearson correlation coefficients between various nuclear anomalies was found to be positive in both normal subjects and cancer patients. This positive correlation with MN and BN suggests the possibility of a similar mechanism of formation of these nuclear anomalies.

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 showed that 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). A highly significant (P<0.01) positive correlation was observed in the different comet parameters in cancer patients as well as normal subjects.

In the current investigation, a significantly high level of genetic damage was observed in cancer patients as compared to normal subjects. The positive correlation observed between duration/stage of cancer and the genetic damage was highly significant too, suggesting that the progress of cancer results in great levels of oxidative stress, which causes genetic damage in the whole body. Interestingly, there was an increase in genetic damage from untreated patients to the ones taking treatment, with highest damages being observed in the patients receiving radiation therapy. This means that non-cancerous tissues may also be damaged during the radiation therapy process which may lead to the development of secondary tumours. A correlation was also observed between MN, BE and BN, suggesting a similar mechanism of their formation.

Higher levels of genetic damage were also observed in women as compared to men. This may be due to the preferential loss of the inactive X chromosome in women. Among different blood groups, cancer patients with the blood group type AB showed the lowest genetic damage which was not significantly different from the normal subjects with the same blood group. This indicates that probably the AB blood type may be more resistant towards genetic damage. Significantly higher genetic damage was also observed in cancer patients with a family history of cancer as compared to those without it. No significant different in genetic damage was observed with respect to dietary or alcoholic habits of the subjects, while in smokers, only heavy smokers (>20 cigarettes/ day) showed an increased genetic damage.

India is expected to overtake China to become the world’s most populated country and cancer is one of the major health issues it faces. The main problem encountered by any cancer control program is its late detection, when it is difficult to cure. Treatment is more effective when cancer is detected while it is still in a localized (pre-metastasis) state. Therefore, early detection and treatment is the solution to this problem with a potential to decrease more than one-third of the global cancer burden.
Both the tests employed in the current investigation are least invasive and low cost. Thus, these methods are not only readily accepted by the patient but can also be used for repeated examinations. Since only basic infrastructure is needed for these tests and the technicians can easily be trained, the screening facility can be provided readily throughout the country. About 10% of the clinically healthy donors, enrolled in the present study, had high levels of genetic damage. Mass screening of the whole population for such individuals will not just reduce the overall cost of the cancer control program but also help in early diagnosis of cancer and preventive therapy.

Cancer is spreading at an alarming rate and this has become the cause of concern for everyone. Therefore, further studies should be conducted with emphasis on the following:

i. To develop an understanding about the various underlying factors (genetic and environmental), which may affect an individual’s susceptibility to cancer, and on the basis of which individuals at high risk could be screened and monitored for disease development.

ii. To validate rapid, reliable, and cost effective mass screening tools for the screening of the whole population for high risk groups.

iii. To develop tests for the demonstration of mutated tumour genes in body secretions and excretions for the early diagnosis of tumours.

iv. To measure the genotoxic effect of different treatments (radiation therapy, chemotherapy, etc.) on the non-cancerous tissue and its correlation with the chances of development of secondary tumours.

v. To analyze individual treatment response for the development of person specific treatment.

vi. To develop a better understanding of the various protective factors (diet, antioxidants, etc.), which may enhance an individual’s chances of developing cancer.

vii. To develop a molecular therapy approach using substances those counteract the molecular processes which in turn promote the clonal development of tumours by activating/inactivating different pathways in the tumour cells.