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Original Article

Dentistry Section

Micronuclei Frequencies and Nuclear Abnormalities in Oral Exfoliated Cells of Nuclear Power Plant Workers

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ABSTRACT

Aim: Biomonitoring provides a useful tool to estimate the genetic risk from exposure to genotoxic agents. The aim of this study was to evaluate the frequencies of Micronuclei (MN) and other Nuclear abnormalities (NA) from exfoliated oral mucosal cells in Nuclear Power Station (NPS) workers.

Materials and Methods: Micronucleus frequencies in oral exfoliated cells were done from individuals not known to be exposed to either environmental or occupational carcinogens (Group I). Similarly samples were obtained from full-time Nuclear Power Station (NPS) workers with absence of Leukemia and any malignancy (Group II) and workers diagnosed as leukemic patients and undergoing treatment (Group III).

Results: There was statistically significant difference between Group I, Group II & Group III. MN and NA frequencies in Leukemic Patients were significantly higher than those in exposed workers &control groups ($p < 0.05$).

Conclusion: MN and other NA reflect genetic changes, events associated with malignancies. Therefore, there is a need to educate those who work in NPS about the potential hazard of occupational exposure and the importance of using protective measures.

Keywords: DNA damage, Exfoliative cytology, Genotoxicity, Micronucleus

INTRODUCTION

Radioactivematerial is used to produce electricity in presence of heavy water in Nuclear Power Stations (NPS), during this process the workers are exposed to toxic substances and radiations. These exposed toxic substances or radiations are absorbed into body of NPS workers either by inhalation or by direct contact. This leads to several health hazards which are mild, moderate and severe such as diabetes, thyroid, hypertension, leukemia etc. Epidemiological studies have shown increased risk of cancer to the people living in nuclear power corporation community due to radiation.

Cytogenetic endpoints such as sister chromatid exchange, chromosome exchange, chromosome aberration and micronucleated cell frequency have been proposed as sensitive parameters for assessing genotoxic effects of chemical or physical mutagens [1].

However, these methods are typically difficult and sometime time consuming or require highly qualified technicians to accurately read and interpret slides, for these reasons non-invasive investigation like application of the micronuclei test to exfoliated cells has been encouraged. It has also been adapted by numerous laboratories for the measurement in peripheral blood lymphocytes, epithelial cells, erythrocytes, and fibroblasts [2].

The buccal cell MN assay was first proposed in 1983 and continues to gain popularity as a biomarker for genetic damage [3]. The micronuclei (MN) assay in exfoliated buccal cells is potentially an excellent candidate to serve as such a biomarker. MN defines as microscopically visible, round or oval cytoplasmic chromatin masses next to the nucleus. They are the results of aberrant mitosis and consist of eccentric chromosomes, chromatid fragments, or aberrant chromosomes.

Assessment of the number of MN may be used as a strategy to identify the genotoxic damage in epithelial cells, which are exposed to carcinogens or mutagens. A rise in the numbers of MN in exfoliated cells indicates an increased risk for cancer [3].

Other then MN, comparison of frequencies of various types of metanucleated cells: binucleated, karyorrhexis (KR), karyolysis (KL) and broken egg (BE) with MN provide a more comprehensive assessment of genome damage then only MN [4,5]. Hence, the present study evaluated the frequencies of micronuclei and other metanucleated abnormalities from exfoliated oral mucosal cells in Nuclear Power Station (NPS) workers.

MATERIALS AND METHODS

Buccal smears were collected from control individuals not known to be exposed to either environmental or occupational carcinogens and were considered as Group I (n=15). Buccal smears obtained from Nuclear Power Station (NPS) workers were classified as Group II (n=15, healthy individuals of NPS) and similarly samples collected from known leukemia diagnosed patients undergoing treatment in NPS were classified as Group III (n=15,).

The workers and control subjects were informed of the objectives of the work, and gave expressed informed and written consent to participate in this study before the collection of buccal smears. The smears of buccal mucosa were coded and the indistinctness of the workers and control population was guaranteed. The ethical clearance for the study was obtained from the NPS.

Buccal smears were collected from consented volunteers at the end of the work shift in NPS campus according to the criteria established by Heddle & Tolbert et al., [6,7] i.e. after a thorough clinical oral examination, exfoliated oral mucosal cell are collected from buccal mucosa of the mouth from all the subjects. Exfoliated oral mucosal cells were collected using a cytobrush (Swan) moistened with water. Using moderate pressure, the brush was repeatedly rotated in one direction over the entire buccal mucosa many times until pin point bleeding was noted. The obtained material from the brush was then spread on the clean, dried microscopic glass slides. Smears were dried in the air and fixed in methanol: glacial acetic acid (3:1) solution for 15 min.

MICRONUCLEI ASSAY

All the cytological smears were stained by papanicolaou technique using a commercially available staining kit RAPIDPAP (Biolab diagnostics, J-345, MDC, Maharashtra). Slides were examined at 40X magnification. Thousand cells on each case were observed for

	Age C	Age T	Age L
n=sample size	15	15	15
Mean	46.93	37.67	49.20
Std. Deviation	11.585	10.682	9.283
Minimum	28	25	30
Maximum	64	65	67
Sum	704	565	738
ANOVA	**F=5.019, df=2, p=0.011,S**		

[Table/Fig-1]: Age wise distribution of subjects

Groups	Gender			Age * Gender
	M	F	Total	
I	9	6	15	Pearson`s Chi-Square value - 0.008, df=11, p=0.299, NS
II	15	0	15	No statistics computed as gender is constant
III	15	0	15	
Chi-Square	Chi-Square value - 13.846, df=2, p=0.00099, S. Yate`s correction - 9.663, p= 0.00797455, S			

[Table/Fig-2]: Gender wise distribution of subjects

the presence of micronuclei and other metanucleated abnormalities by moving from one field to another systematically in a zigzag fashion avoiding the previous field. Micronuclei were scored only in cells with intact cellular and nuclear membranes. Over lapped cells, cell clumps were excluded. Criteria for identifying micronuclei as given by Heddle and Countryman[6] were followed. Other metanucleated abnormalities were also observed as given by Tolbert PE et al., [7]. Comparison between all three groups was done using ANOVA test. Pair wise comparison between the groups was done using Chi-Square test. Statistical significance was set at $p \leq 0.05$.

RESULTS

The average age and gender distribution of the study population are presented in [Table/Fig-1,2]. Mean age of subjects included was 35 to 50 y in all the groups. No statistics computed for gender in group II and III as it was constant and in Group I, 9 males and 6 females were included in the group.

The buccal smear of all the study groups were evaluated for micronuclei, Binucleated cells, Broken-Eggs, Karyolysis, Karyorrhexis, Condensed chromatin, and Pyknosis. Means and standard deviations were calculated for each Group and compared for significant differences using ANOVA. Group III (Leukemia individuals) showed highest number of micronuclei and other nuclear anomalies which was found to be statistically significant ($p<0.05$). There was significant difference between Group II (Toxic) and Group I(Control). [Table/Fig-3] represents Distribution and comparison of subjects with anomalies in different groups. Individuals of Group II and Group III showed an enhanced frequency of micronuclei and other nuclear anomalies in comparison to Group I. But there was no statistical difference for occurrence of micronuclei between Group II & Group III.

Since Condensed chromatin was not found in any of the study groups, statistical test could not be applied. Amongst the other nuclear anomalies tested, Group I had least occurrence of all abnormalities. Broken-Eggs, Karyorrhexis & Pyknosis were seen with significant difference between Group III & Group II. No statistical differences were seen for Binucleated cells, and Karyolysis between Group II & Group III.

The average number of micronuclei cells in Group I, Group II & Group III. There was significant difference between the mean percentages of micronucleated cells for these three groups (Group I= 0.20 ± 41, Group II= 2.93 ± 1.43 and Group III= 7.73±2.71).

Anomolies	Groups						Chi-Square
	Control		Toxic		Leukemia		
	n	%	n	%	n	%	
Micronuclei	3	20	14	93.3	15	100	Chi-Square value -8.313, df= 2, p =0.0157, S.
Binucleated cells	5	33.3	12	80	15	100	Chi-Square value - 4.938, df= 2, p = 0.085, NS.
Broken-Eggs	0	0	2	13.3	14	93.3	Chi-Square value - 21.5, df= 2, p = 0.00002, S.
Karyolysis	5	33.3	4	26.7	9	60	Chi-Square value - 2.333, df= 2, p = 0.31, NS.
Karyorrhexis	0	0	3	20	10	66.7	Chi-Square value - 12.154, df= 2, p = 0.002, S.
Condensed chromatin	0	0	0	0	0	0	Cannot be computed
Pyknosis	0	0	0	0	3	20	Chi-Square value - 6, df= 2, p = 0.049, S.
TOTAL	7	46.7	15	100	15	100	Chi-Square value - 3.459, df= 2, p = 0.177, NS.
Chi-Square	**Chi-Square value -22.199, df= 12, p =0.0353, S.**						

[Table/Fig-3]: Distribution and comparison of subjects with anomalies in different groups, *(S= significant, NS= Non-significant)*

In oral epithelial cells, broken eggs may be not associated with occurrences of chromosome damage, and thus reflect alterations relating to the normal tissue differentiation process.

DISCUSSION

Occupational exposure to carcinogenic forms of radioactive products occurs among workers in several professional groups particularly with high exposure among NPS (Nuclear Power Station) workers. Epithelial cells are highly proliferative and are the origin of more than 90% of all human cancers [1,3,8]. Buccal epithelial cells represent a recognized target site for early genotoxic events induced by carcinogenic substances. Therefore, the application of micronucleus test in buccal epithelium cells has been increasingly accepted in occupationally exposed groups as a sensitive tool for bio-monitoring the genetic damage in human population [8,9]. This method is reliable, simple, economic and can be easily applied on individuals who are exposed to carcinogenic substances. Radioactive waste comes from a number of sources. The majority of waste originates from the nuclear fuel cycle and nuclear weapons reprocessing [10,11]. Other sources include medical and industrial wastes, as well as naturally occurring radioactive materials that can be concentrated as a result of the processing or consumption of coal, oil and gas, and some minerals.

Waste from the front end of the nuclear fuel cycle is usually alpha-emitting waste from the extraction of uranium. It often contains radium and its decay products.

The back end of the nuclear fuel cycle, mostly spent fuel rods, contains fission products that emit beta and gamma radiation, and actinides that emit alpha particles, such as uranium-234, neptunium-237, plutonium-238 and americium-241, and even sometimes some neutron emitters such as californium (Cf). These isotopes are formed in nuclear reactors. Depleted uranium one of the waste products released from the nuclear industry. It predominately contains radionuclide 238U whose half-life is approximately 4470x10 y [10].

The difference of opinion in frequent usage of Uranium is that it possesses only 60 % of natural uranium radioactivity, having been “depleted” of much of its most highly radioactive 234U and 235U isotopes.

During decay, uranium isotopes emit alpha-particles, which possess high energy, but are poorly penetrating. When the shells containing Depleted Uranium (UD) hits a target, a fine aerosol is formed and particles are easily inhaled [11]. Depleted uranium is an internal health hazard predominantly affecting skeletal tissue [10]. It has also been connected with the increased number of leukemia's and other cancers.

Benova and co-workers found double the frequency of buccal MN in Chromium exposed workers when compared with control persons. Similarly our results showed statistically significant difference between full time NPS workers and unexposed healthy individuals.

In addition Benova et al., [12] showed the elevated prevalence of several other nuclear anomalies like binucleates, broken egg, karyorrhexis, karyolysis and pyknosis. Similarly our results also showed increased frequency of other nuclear anomalies in Group II and III patients. Our study results suggest that in oral epithelial cells, broken eggs may be not associated with occurrences of chromosome damage, and thus reflect alterations relating to the normal tissue differentiation process.

Therefore there are four recognised mechanisms of micronucleus formation: mitotic loss of acentric fragments, chromosome breaks and exchanges, mitotic loss of whole chromosomes, and apoptosis. There is abundant evidence that the micronucleus assay is sensitive to effects induced by ionizing radiation [2].

According to Gutierrez S et al., [13], increased frequency of micronuclei after radioactive iodine treatment has been found in buccal mucosa epithelial cells in hyperthyroidism and thyroid cancer patients. But there was no significant increase in the frequency of cells with micronuclei and total number of micronuclei in patients exposed to panoramic radiography [14].

This suggests that radioactive substances like Uranium, Tritium, and Iodine cause genotoxicity which is not the same with panoramic radiography. Further micronucleus frequency was also increased in buccal cells in Chromium Exposed Tannery Workers and silica-exposed workers [15,16].

As there was statistically significant difference between the study groups, it is evident that radioactive substances released in Nuclear Power Stations cause increase in micronuclei frequency and other nuclear abnormalities in buccal epithelial cells demonstrating chromosomal aberrations leading to high risk for malignancy.

CONCLUSION

From the results of the study MN and NA frequencies in Leukemic Patients were significantly higher than those in exposed workers & control groups ($p < 0.05$). Here by to conclude with the knowledge of the risk status, various interventions can be initiated and the above test can again be performed to assess the cellular improvements and prognosis after the completion of treatment / chemoprevention trials.

MN and other NA reflect genetic changes, events associated with malignancies. Therefore, there is a need to educate those who work in NPS about the potential hazard of occupational exposure and the importance of using protective measures. Further studies upon larger samples are required to assess genetic changes.

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