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Analysis of Cytogenetic Effects of Radiation in Dental Personnel Exposed to Diagnostic X-rays

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ABSTRACT The incidence of chromosomal aberrations were evaluated in the lymphocytes of peripheral blood of 40 persons working in different dental colleges and clinics in and around Bangalore occupationally exposed to X-rays. The age range of the study group was 25-65yrs and duration of exposure of dental personnel ranged from 5-35yrs. For comparison blood samples were also collected from 20 subjects (controls) who were not exposed to any diagnostic radiations. The radiographers showed a significant increase of chromosomal aberrations (mean of 1.00) when compared to controls (mean of 0.50). Though dental personnel showed increase in frequency of chromosomal aberrations but the results were not statistically significant. Frequency of chromosomal aberrations was also compared on bases of age, gender and duration of exposure, results were not statistically significant.

INTRODUCTION

Diagnostic radiology uses ionizing radiations which, as distinct from non ionizing radiation, have sufficient energy to ionize atoms or molecules in biological and other systems. X-rays used in diagnostic radiology are a potent mutagenic agent, capable of inducing both gene mutations and chromosomal aberrations. They act directly on the DNA molecule or indirectly through the formation of reactive compounds that react with this molecule. Chromosomal aberrations especially the double stranded break in DNA is regarded as being the most sensitive biological indicator of radiation induced genetic alteration. The use of ionizing radiation in dentistry, like in general medicine has rapidly being increasing. Today a complete radiographic survey of the mouth is considered an essential adjunct to diagnosis. Dental professionals are the only practitioners who perform radiographical examination of their patients themselves. Also there is increase in number of dental radiographers taking dental radiographs. However, an integral part of radiography is exposure of patients and, potentially, clinical staff to X-rays. Radiation in doses required for dentistry may not present any major risks, however these small doses are not necessarily risk free. No exposure to X-rays can be considered completely free of risk, so the use of radiation by dentists is accompanied by a responsibility to ensure appropriate protection.

Numerous studies have been conducted on the cytogenetic effects of radiation on the occupationally exposed workers in medical field. But studies conducted on exposed workers in a dental set up are few. Stuart (1967) in his study on effect of dental X ray radiation in everted cheek pouch of Chinese hamsters which were exposed to 0.25R, 2.9R and 5.4R radiation dose found significant amounts of chromosomal damage for all doses of radiation. Rozgaj et al. (1999), Maddileti et al. (2002), Abolfazl et al. (2007) in their studies in radiation exposed groups working in various medical fields compared to controls found significant increase in frequency of chromosomal aberrations. Cintia and Ilc (2002) in their cytogenetic study on Brazilian dentists occupationally exposed to low dose of X radiation found no significant difference between the dentists and the unexposed controls.

Objectives

The main objective of the study was to examine the various alterations in chromosomes caused by radiation in dental personnel and an attempt was also made to correlate the variation in the frequency of chromosomal aberrations based on gender, age and duration of exposure.

MATERIAL AND METHODS

Study consisted of 60 subjects divided into three groups of 20 each, group 1 of dental radiographers, group 2 of general dental practi-

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tioners and group 3 of controls. Subjects were selected randomly from various dental colleges and dental clinics in and around Bangalore; controls were selected randomly from the Department of Oral Medicine, Diagnosis and Radiology Dayananda Sagar College of Dental Sciences, Bangalore. The total cumulative dose was obtained from the previous three years record of the TLD badges worn by the dental radiographers. The group 2 of general dental practitioners were not wearing any TLD badges or following the safety measures.

Inclusion criteria for group 1 was personnel exposed to dental diagnostic radiations – IOPA radiographs, panoramic and skull radiographs, following standard ICRP safety measures and exposed to radiations for more than 3yrs. Criteria for group 2 was subjects who have been taking dental radiographs regularly for more than 3yrs. Subjects in group 3 included were those who have not been exposed to occupational or diagnostic radiation. Age range of the study was 25- 65yrs. A questionnaire was made in which details of any accidental exposure, duration of exposure, safety measures taken, etc was obtained as well as the cumulative dose was obtained from the past 3yrs report of TLD badges (chest) worn by dental radiographers.

5ml of venous blood samples were drawn from each subject after obtaining written consent. Blood samples were collected in heparinized vacutainers and processed at Triesta Sciences India Pvt, Bangalore using method of Moorhead et al. (1960). Blood culture was set using 10ml of RPMI1640 medium (Hi Media, Mumbai) supplemented with 20% fetal bovine serum (European grade, Biological Industries Israel), 1% of penicillin and streptomycin (Penstep, Mediatech Inc, Hemdon, USA). To this 200ml of PHA (phytohemagglutinin, Gibco, USA) was added. Tubes were incubated at 37ºC in CO_2 incubator for 68hrs. At 68th hr 50µl of colcemide (Karyomax Gibco) was added and incubated further for 4hrs. At 72hrs the culture was terminated and tubes were centrifuged for 5mins at 2000rpm, supernatant was removed leaving behind 0.5ml liquid. It was resuspended in 10ml of 0.075M KCL and incubated at 37º for 20 mins. At this stage 1ml of freshly prepared cold Carnoys fixative was added slowly until 8ml of Carnoys fixative was added. The tubes were refrigerated for 1 day. Tubes were removed and centrifuged at 2000rpm for 5mins and the supernatant was removed. Cells were rewashed with the fixative until a clear white pellet was obtained. Slides were prepared using cold slides stored in methanol. Drop of pellet was dropped from a height of 12-15 inches for evenly spread. The slides was stained with Giemsa stain and examined under Nikon 80 light microscope. 50 well spread metaphases were analyzed for each subjects. Chromosomal aberrations such as gaps, breaks, fragments, dicentrics and acentrics were analyzed. The frequency of the aberrations was documented. The results were tabulated and subjected to statistical analysis using Kruskal – Wallis test, Mann Whitney test and Spearman's Rank test.

RESULTS

A total of 60 subjects were involved in the study divided into three groups of 20 subjects in each group selected randomly. Age range in the group 1 was 25-64yrs, group 2 was 27-45yrs and group 3 was 25-39yrs. Equal number of females and males that is 10 each were taken in group 2 and 3 but in group 1 no female dental radiographers were available hence the group 1 consisted of only males. The duration of exposure in group 1 and 2 ranged from 6-35yrs and 5-20yrs respectively. A total of 3000 metaphases that is 50 metaphases for each subject were analyzed for various chromosomal aberrations (some shown in Figs. 1,2,3) and tabulated (Table 1).

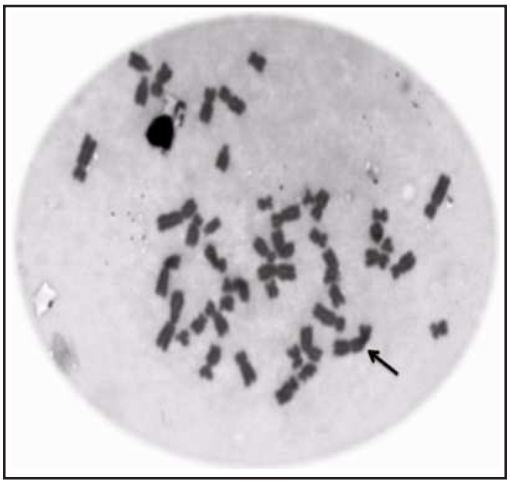

Fig. 1. A metaphase spread from a human lymphocyte exhibiting chromosomal aberrations-dicentric

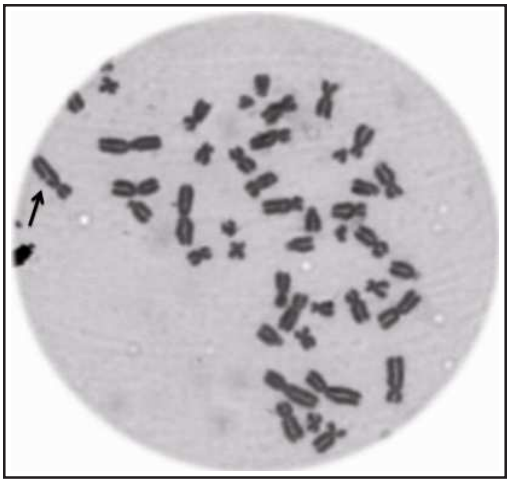

Fig. 2. A metaphase spread from a human lymphocyte exhibiting chromosomal aberrations- chromatid type of Gaps

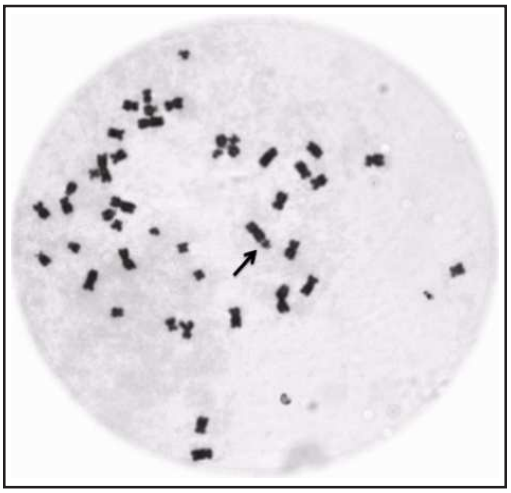

Fig. 3. A metaphase spread from a human lymphocyte exhibiting chromosomal aberrations-chromosome type of Gap

Correlation in number of chromosomal aberrations detected between the three groups was done (Table 2). Mean score was found to be higher in general dental practitioners (1.00), followed by dental radiographers (0.60) and controls (0.50) (Fig. 4). But the difference in scores between the groups was not found to be statistically significant (P>0.05). Correlation in the number of chromosomal aberrations between the different groups that is between radiographers and controls, clinicians and controls and radiographers and clinicians was done separately. The difference of score between the groups was not found to be statistically significant. But the key marker for the radiation exposure the dicentrics was noted in dental personnel.

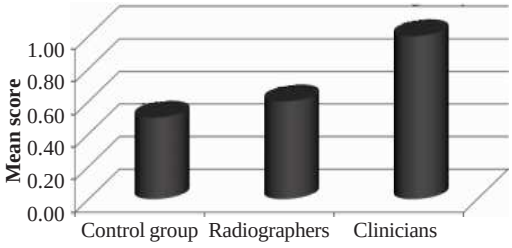

Fig. 4 Mean score of chromosomal aberrations recorded across the group

Correlation was also done taking into consideration the gender, age and duration of exposure. Correlation of frequency of chromosomal aberration between the genders was done in group 2 and 3 as group 1 consisted of only males using Mann Whitney test. A higher mean score was observed in males when compared to females in group 2 (Table 3) and group 3 (Table 4). But the difference in the scores was not statistically significant.

Age was distributed into four groups of age range of 10yrs from 25-65yrs. Frequency was recorded in each group and tabulated. Correlation of frequency of chromosomal aberration between the four age groups could not be done as we were not able to get equal number of subjects in each group. Hence an overall correlation was done between the number of chromo-

Table 1: Frequency of CA across the groups

Subjects	*No of samples*	*No of metaphases scored*	*Chromosome type aberrations*		*Chromatid type of aberrations*		*Dicentrics*	*Acentrics*	*Total*
			Gaps	*Breaks*	*Gaps*	*Breaks*			
Radiographers	20	1000	3	5	1	1	2	0	12
Controls	20	1000	1	3	3	3	0	0	10
General dental practitioners	20	1000	6	5	3	4	2	0	20

Table 2: Correlation of frequency of CA among the groups

Group	*N*	*Mean*	*Std dev*	*Min*	*Max*	*Kruskal-Wallis Chi-square*	*P-Value*
Control group	20	0.50	0.97	0	3	2.046	0.360
Radiographers	20	0.60	1.07	0	3		
Clinicians	20	1.00	1.05	0	3		

Table 3: Gender comparison in clinicians group

Gender	*Mean*	*Std dev*	*Mean difference*	*Z*	*P-Value*
Male	1.40	1.34	0.800	-0.986	0.324
Female	0.60	0.55			

Table 4: Gender comparison in control group

Gender	*Mean*	*Std dev*	*Mean difference*	*Z*	*P-Value*
Male	1.00	1.22	1.000	-1.936	0.053
Female	0.00	0.00			

some aberration and age using Spearman's Rank test (Table 5). We observed a weak correlation (r=0.377) in controls, positive but weak (0.321) in radiographers and positive but very weak (0.142) in clinicians. But this correlation was not found to be statistically significant.

Table 5: Overall correlation of age and frequency of CA

Group	*R*	*P-Value*
Control group	-0.377	0.283
Radiographer's group	0.321	0.366
Clinician's group	0.142	0.696

We also attempted to do grouping of duration of exposure, it was grouped under 3 groups of 10yrs duration from 5-35yrs. But we were not able to obtain equal number of sample size in each group. Hence a overall correlation was done in group 1 and 2 (Table 6). We observed positive but weak (r=0.370) in group 1 and positive but very weak (r=0.168) in group 2. But this correlation was not found to be statistically significant.

Table 6: Correlation between the duration of exposure and frequency of CA

Group	*R*	*P-Value*
Radiographer's group	0.370	0.292
Clinician's group	0.168	0.643

DISCUSSION

The importance of cytogenetic study of peripheral lymphocytes in persons exposed to ionizing radiation has been reported for more than 30 years. According to Upton (1982), the effects of exposure to low radiation doses accumulate in the body and may damage health after several years of exposure. Human lymphocytes when exposed to 1.5Gy X rays in vitro shows significant increase in the frequency of chromosomal aberrations when compared to normal cells (Mosesso et al. 2001). There are numerous studies on the induction of the chromosomal aberration by radiation in exposed workers of various fields, however to our knowledge; assessments of the cytogenetic impact of chronic exposure to low radiation doses in dental field are scarce.

In the study the cytogenetic effects of radiation in dental personnel was evaluated by analyzing the chromosomal aberrations and an attempt to correlate the frequency of chromosomal aberrations between the exposed group (group 1 and group 2) and controls (group 3), also taking into consideration the age, sex and duration of exposure was made.

The first objective was to detect the chromosomal aberrations. An increase in frequency of chromosomal aberration in group 1 and group 2 when compared to the controls was found but the difference was not statistically significant. It is consistent with Cintia and Ilc (2002) study of cytogenetic biomonitoring of Brazilian dentists occupationally exposed to low doses of X radiation

The results in the study though not statistically significant, but still there was increase in frequency of chromosome aberrations observed in group 1 and group 2 compared to group 3. And also dicentrics in group 1 and group 2 were detected. As known dicentrics are considered as excellent indicator of radiation exposure and have been widely used as a key marker in the radiation dosimetry. Also the frequency of chromosome type of aberrations i.e. double strand breaks were more in exposed personnel compared to controls. Chromosome type of aberrations are said to be more of radiation induced.

Many cytogenetic studies have shown statistically significant results in relation to frequency

of chromosomal aberrations between exposed group and controls in other fields of medicine. The reason for not obtaining a statistically significant result could be the small sample size when compared to the other studies were the number radiographers taken ranged from 50 - 1200 and also the amount of dose involved in dental radiography which is less compared to the dosage used in other fields of medicine like medical diagnostic and therapeutic radiation, nuclear medicine etc which were included in the studies. Studying the effects of radiation on survivors of the Hiroshima and Nagasaki A-bombs, Awa (1990) observed a significant increase in the frequency of dicentric chromosomes and rings when the number of cells analyzed was increased to 500 per individual. In this study, the average number of analyzed cells from the groups was 50 cells per subject perhaps a increase in the number of analyzed cells as well as the sample number would lead to different results.

The frequency of chromosomal aberrations was found to be more in clinicians than the radiographers and control but not statistically significant. As known in India dentistry is only profession wherein the dentist take the radiographs by themselves in their clinical set ups, hence occupationally exposed to low levels of ionizing radiation, and there is no regular effective inspection of their work environment – the prevention of exposure to X-rays generally depends only on the common sense of the professional. Sitra et al. (2008) in a survey of radiation protection protocol observed among the dentist population in the union territory of Puducherry observed that though many of the dentists adapted the exposure time to faster film speed classes, the decreased use of rectangular collimators, shielding wall and other protective measures were disappointing. The clinicians included in the study were not following any standard safety measures while taking radiographs. This could be the reason why an increase in the frequency of chromosomal aberrations were detected in clinicians than the radiographers who were taking more radiographs compared to clinicians including extra oral and panoramic radiographs but following the standard safety measures.

An attempt was made to correlate the variation in the frequency of chromosomal aberrations based on age, gender and duration of exposure. There has been positive correlation of age and chromosomal aberrations as demonstrated by various studies. But certain studies have also reported negative correlation. Chung et al. (1996) reported negative results for the effect of age on chromosome damage in cytogenetic study of nuclear plant workers. Ruzica et al. (1998) in their study on 1260 occupationally exposed radiographers found that age, sex and duration of exposure were not significant predictor of analyzed chromosomal aberrations. Cintia and Ilc (2002) in their cytogenetic study of dentists occupationally exposed to low doses of X radiation found no association between the confounding factors age and sex and the frequency of chromosomal aberrations. Ruzica et al. (1998) and Abolfazl et al. (2007) in their studies on radiotherapy workers found that neither age, sex or duration of exposure were significant predictors of chromosomal aberrations. The reason for obtaining not so statistically significant results could be that other factors such as smoking which can cause chromosomal aberrations were not included in our study

Various studies have shown a positive relation between the duration and frequency of chromosomal aberrations. Most of these studies carried out had large sample size and the subjects included were exposed to more dosage of radiation than the dental personnel. Oesch et al. (1987) showed that the same individual has a different repair capacity at different times, which is a consequence of differences in endogenous physiological status or changing exposure to exogenous compounds. It is also known that human lymphocytes consist of cell sub populations with different sensitivity. There is evidence to suggest differences in sensitivity as a function of cell cycle position. Olive and Banath (1993) showed that damage repair also depend on cell cycle position.

CONCLUSION

Last few years we have witnessed an increase in use of diagnostic radiation in dentistry. Though the dose involved in dentistry is low it is not risk free. As chromosomal aberrations are result of accumulated dose of X ray radiation it indicates that long term exposure to the low dose of X ray radiation is potentially a risk. The data presented in the study indicated that the frequency of chromosomal aberrations among the

dental personnel and controls who were not exposed was not statistically significant but the slight increase in the frequency of chromosomal aberrations was noted and also presence of dicentrics in dental personnel exposed to the X ray radiation though in small number do indicate the importance of this study. Dicentrics are proved to be excellent markers of radiation exposure and have been widely used as a key marker in the radiation dosimetry. The slight difference in the frequency of chromosomal aberration between the dental radiographers and the general dental practitioners indicate the importance of safety measures to be followed during the radiographic procedures. Magnitude of the change appears to be relatively small in the study, so further research including large sample size and increasing the number of sampled cells to be analyzed is necessary to give a clearer view of what is actually happening, but it is utmost important that all prescribed safety measures be followed whether in a hospital or clinical set up and awareness of such risk taken up more seriously.

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