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Comparing Pre And Post Exposure Genotoxic Effects Of Radiation On Buccal Epithelial Cells Using Exfoliative Cytology- An Observational Study

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Abstract
Ionizing radiations causes damage to DNA and DNA protein crosslinks and induces cellular death. Use of ionizing radiation is unavoidable in certain dental disorders thus leading to stochastic effects on exposed tissue. Present study is undertaken to evaluate association of routine diagnostics radiographic examination to increased risk of cancer due to cytotoxic effect of x-ray imaging and to minimize potential risk by prescribing radiographs where deemed necessary and using appropriate modality required.
Aim: To evaluate post exposure genotoxic effects of radiation on buccal epithelial cells using exfoliative cytology.
Objectives:To detect number of Micronuclei(Mn) in exfoliated buccal cells of adult male and female patients following radiographic examination using CBCT of single volume (SV), CBCT of single arch (SA) and a orthopantomogram (OPG).
Material and methods:Eighty seven healthy patients were recruited who fulfilled the inclusion and exclusion criteria. Patients were divided into three groupsExfoliated buccal cells were collected before CBCT and OPG exposure, thereafter 7-10 days post exposure and 18-20 days after exposure. Cytological smears were examined to detect changes in number of Mn.
Conclusion:Present study concludes that, radiation exposure from CBCT as well as OPG has inevitable health side effects that should be taken into consideration while prescribing diagnostic radiographs.

Keywords: Cytologic alterations, Spiral Cone Beam Computed Tomography, Pantomography, Mn, oral mucosal cells.
Key message:Considering genomic alterations caused by radiation as shown in this study we recommend judiciary use of 3D radiographs whenever necessary.

I. INTRODUCTION

Genetic and environmental agents can increase the risk of cancer.[1] 90% of human oral malignancies are epithelial in origin, making oral cancer sixth most frequent cancer in the world.[2] An average person is exposed to various radiation from different sources, such as global fall out, occupational exposure, treatment and diagnostic radiation procedures and other miscellaneous causes.[3] Most of these radiations appear to be harmless, while others could be potentially harmful. CBCT and OPG are established diagnostic aids which offers various benefits in diagnosing dental diseases.[4] But for many years, risks of ionizing radiation from various dental radiography procedures have been assessed, and a number of guidelines have been recommended to curb the harmful effects of radiation.

DNA protein cross-links, single and double strand breaks are both caused by ionizing radiation which can be detected by micronuclei formation within the epithelial cells. [5]Mn test is one of the most well-liked and exacting procedures to detect cellular aberrations in exfoliated buccal cells caused due to diagnostic radiations. [6, 7] with the increasing used of CBCT imaging to diagnose pathologies which cannot be discerned on 2d radiographs, there is a high risk of exposing patients to excessive radiation. Taking this into account we devised a study to evaluate the harmful effects of radiation on an individual by detecting frequency of micronuclei in the exfoliated buccal cells.

II. MATERIAL AND METHOD

Study design:
This is an observational prospective type ofstudy which was done in the department of Oral Medicine and Radiology.

Study size:
"The study required a sample size of twenty nine(number of pairs) to achieve a power of 80% and a level of significance of 5% (two sided), for detecting a mean ofdifferences of 0.06 between pairs, assuming the standard deviation of the differences to be 0.11". So, total number of eighty seven healthy patients were selected randomly, who fulfilled the inclusion and exclusion criteria.Informed consent was obtained from all the selected patients. The study was carried out in consonance with Helsinki's declaration and GCP guidelines.
The control group was the pre-exposure values of the participants exposed to various radiations.

Inclusion criteria:
Adult population aged 18-45 years, who were advised a radiograph; eithera OPG or CBCT for SA or SV for diagnostic purposes.

Exclusion criteria:
Subjects with systemic disorders, Restorations, H/O any previous radiation exposure within a month, Patients taking any medications, Alcohol or tobacco consumers, Red or white lesions.

Scanning protocol:
Subjects were exposed to CS 9600 using CS imaging version 8 software. Exposure units used for CBCT (SV) and CBCT (SA) were similar that is 120 kVp, current used is 6.3 mA; except for the time of exposure which was 19.0 seconds, for field of view (FOV) of 5 x 5 cm for the former and 18.8 seconds forFOV of 8 x 5 cm for the later. Exposure units used forOPGs taken were 73kVp and 8.0mA for 12.3 seconds.

Cell collection and slide preparation:
Patients were divided into three study groups. Buccal epithelial cells were collected using exfoliative cytology; before exposure and ten days and twenty days after the radiation exposure. Patient was asked to rinse the mouth to clear all the debris then the buccal mucosa was scraped with a moist wooden ice-cream stick to collect exfoliative cells.Smear was prepared from these cells andslides were stained using Papanicolaou(PAP)stainand were observed under the microscope.

Cytological analysis:
Two observers analysed the smears at different time interval. Observers were blinded toexposure history, and smears were detected in randomly selected microscopic field under x40 magnification.Hundredexfoliative epithelial cells were counted in each smear to detect changes in number of Mn. Two investigators performed the assessment on all the patients having an interclass correlation coefficient ranging from 0.89 to 0.92 and these values indicated that there is high agreement between the two observed measurements.

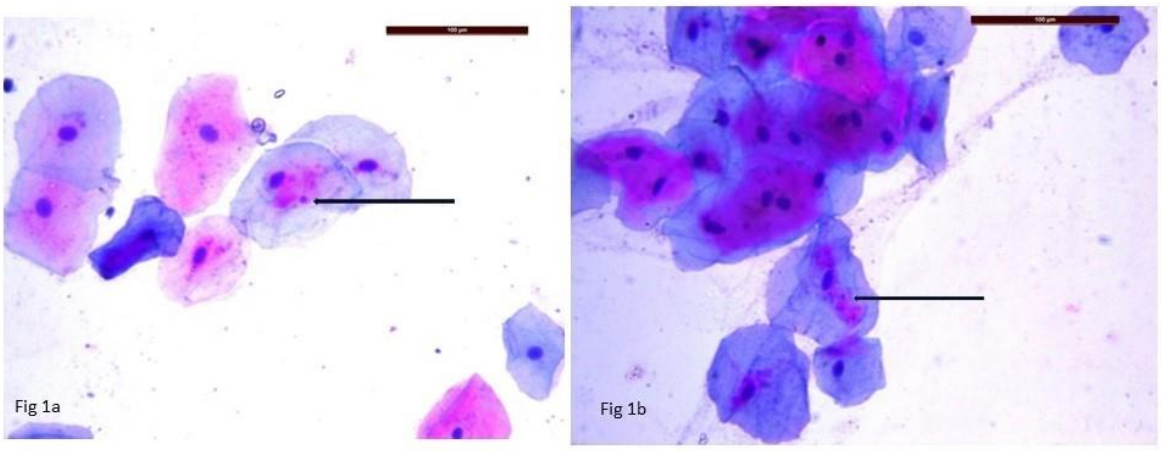

Fig 1a and 1b: Photomicrograph of buccal mucosal squamous cells showing Mn stained with PAP stain in a Cytological smear (PAP-40x)

Statistical methods:
"Statistical Package for Social Sciences (IBM SPSS Statisticfor window, version 21.0. Armonk, NY: IBM Corp.) was used for Statistical analysis". The quantitative data was expressed inmean + standard deviation (SD). The normality testing done usingShapiro – Wilk test

andKolmogorov-Smirnov test showed normally distributed data.

Repeated measures ANOVA and Tukey's post hoc was used to compare the intra group change in the mean count of Mn at pre-exposure, tenthday and twentiethday after the radiation exposure for OPG, CBCT(SA) and CBCT(SV) group. Kruskal Wallis ANOVAand Tukey's post hoc was used to compare the inter group change in the mean count ofMn at pre-exposure, tenth day and twentiethday after the radiation exposure for OPG, CBCT(SA) and CBCT (SV) group. For all tests p-value of 0.05 or less was considered for statisticalsignificance.

III. RESULTS:

This study was performed on eighty seven healthy patients, who were in the range of 18-45 years. Before and after the radiographic procedures, all examined cytological smears showed various nuclear changes in varying proportions and frequencies. Mn arerounded membranous extranuclear bodies which are smaller in size withsimilar stain intensity as that of the nucleus. Mean frequencies of Mn and other nuclear changes were compared per hundred cells,Prior to, after ten days, and twenty days of exposure to OPG and CBCT.

Repeated measure ANOVA along with Tukey's post hoc was done to compare the results between pre-exposure; tendaysand twentydays post exposure to radiation.

Frequency of Mn increased significantly in group A patients ontenthday. Whereas, the frequency was found to besignificantly reduced after the exfoliation of affected layer twenty days later. [Table 1] Similar results have been noticed in patients who were exposed to CBCT (SV) and CBCT (SA), wherevalues have increased significantly after ten days and have been reduced effectively afterexfoliation of the affected layer twenty days later. [Table 1] Kruskal Wallis ANOVA along with Tukey's post hoc was done to compare pre and post exposure values individually between group A, group B, group C. Difference between the frequency of Mn before exposure was not significant between groups A, B ∧ C. Considering the values on tenthday there was a significant difference between allthree groups. The highest number of Mn is observed in group C; that is the patients who were exposed to CBCT (SA), followed by patients who were exposed to CBCT (SV), and lastly the ones exposed to OPG [Table 3]. Similarly,values taken after twenty days also showed a significant difference between all three groups, however; it is still higher than the pre-exposure values [Table 3]. Values are mentioned in the tables below.[Table 1] [Table 2] [Table 3]

Table 1- Mean and standard deviation values for OPG, CBCT (SV), CBCT (SA) at Pre-exposure, 10th day and 20th day time interval

Technique	Time Interval	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
OPG	Pre-exposure	29	43.00	4.35	0.80	34	52
	10^{th} day	29	73.72	6.79	1.26	60	89
	20^{th} day	29	59.34	5.79	1.07	46	72
CBCT (SV)	Pre-exposure	29	44.03	5.51	1.02	29	52
	10^{th} day	29	87.55	3.80	0.70	81	95
	20^{th} day	29	73.07	3.56	0.66	65	80
CBCT (SA)	Pre-exposure	29	44.34	5.24	0.97	34	54
	10^{th} day	29	93.59	4.67	0.86	86	103
	20^{th} day	29	77.14	3.99	0.74	69	85

Table 2- Within the group comparison of exposure values for OPG, CBCT (SV), CBCT (SA) at Pre-exposure, 10th day and 20th day time interval using Kruskal Wallis ANOVA and Tukey's Post Hoc test

Dependent Variable			Mean Difference	Std. Error	Tukey HSD	Sum of Squares	Mean Square	ANOVA (F)	Significance
OPG	Pre-exposure	10^{th} day	-30.72	1.50	0.000*	13706.27	6853.13	208.39	0.000*
		20^{th} day	-16.34	1.50	0.000*				
	10^{th} day	Pre-exposure	30.72	1.50	0.000*				
		20^{th} day	14.37	1.50	0.000*				
	20^{th} day	Pre-exposure	16.34	1.50	0.000*				
		10^{th} day	-14.37	1.50	0.000*				

CBCT (SV)	Pre-exposure	10th day	-43.51	1.15	0.000*	28482.85	14241.42	742.10	0.000*
		20th day	-29.03	1.15	0.000*				
	10th day	Pre-exposure	43.51	1.15	0.000*				
		20th day	14.483	1.15	0.000*				
	20th day	Pre-exposure	29.03	1.15	0.000*				
		10th day	-14.48	1.15	0.000*				
CBCT (SA)	Pre-exposure	10th day	-49.24	1.22	0.000*	36449.58	18224.79	836.98	0.000*
		20th day	-32.79	1.22	0.000*				
	10th day	Pre-exposure	49.24	1.22	0.000*				
		20th day	16.44	1.22	0.000*				
	20th day	Pre-exposure	32.79	1.22	0.000*				
		10th day	-16.44	1.22	0.000*				

* Statistically significant at p<0.05

Table 3-Between the group comparison of exposure values of OPG, CBCT (SV), CBCT (SA) at Pre-exposure, 10th day and 20th day time interval using Kruskal Wallis ANOVA and Tukey's Post Hoc test

Dependent Variable			Mean Difference	Std. Error	Tukey HSD	Sum of Squares	Mean Square	ANOVA (F)	Significance
Pre-exposure	OPG	CBCT (SV)	-1.03	1.33	0.71	28.75	14.37	0.56	0.57
		CBCT (SA)	-1.34	1.33	0.57				
	CBCT (SV)	OPG	1.03	1.33	0.71				
		CBCT (SA)	-0.31	1.33	0.97				
	CBCT (SA)	OPG	1.34	1.33	0.57				
		CBCT (SV)	0.31	1.33	0.97				
10th day	OPG	CBCT (SV)	-13.82	1.37	0.000*	6013.81	3006.90	109.43	0.000*
		CBCT (SA)	-19.86	1.37	0.000*				
	CBCT (SV)	OPG	13.82	1.37	0.000*				
		CBCT (SA)	-6.03	1.377	0.000*				
	CBCT (SA)	OPG	19.86	1.377	0.000*				
		CBCT (SV)	6.03	1.377	0.000*				
20th day	OPG	CBCT (SV)	-13.72	1.196	0.000*	5041.19	2520.59	121.55	0.000*
		CBCT (SA)	-17.79	1.196	0.000*				
	CBCT (SV)	OPG	13.72	1.196	0.000*				
		CBCT (SA)	-4.06	1.196	0.000*				
	CBCT (SA)	OPG	17.79	1.196	0.000*				
		CBCT (SV)	4.06	1.196	0.000*				

* Statistically significant at p<0.05

IV. DISCUSSION:

Evaluation of Mn in exfoliated cells of buccal mucosa is widely used to detect cytotoxic and genotoxic effect of ionizing radiation.[6] Since the buccal mucosa is under direct x-ray exposure during radiographic examination nuclear alterations are seen in the cells. To identify the nuclear changes in exfoliative cytology is a simple and cost-effective method to analyse the changes in the cells of the buccal epithelium.[8]

In the present study we evaluated frequency of Mn in exfoliated buccal mucosa cells on exposure to OPG, CBCT(SV), CBCT (SA). The frequency was noted prior to exposure, 10 days and 21 days post exposure. The period of 10 days and 21 days post exposure was observed as

turnover of oral epithelium is approximately 21 days, whereby the cells reach the surface mucosa and exfoliate. So, this period allowed basal layer to mature and thus collect the cells effectively by cytological swabbing techniques.

According to the results obtained frequency of Mn in exfoliated buccal cells showed significant increase post exposure to CBCT and OPG. Frequency of pre-exposure Mn are attributed to environmental changes that is gases released from earth's crust, sun, cosmic rays, food containing radionucleotides and many more such things.[3] Though, percentage of Mn after twenty days is less than the percentage after ten days of exposure, it is still higher than the pre-exposure values. Bashaet al.[6] compared pre and post exposure values between OPG and CBCT, and Doua H. Altoukhi et al.[2] who exposed to patients to CBCT considered two follow ups after pre exposure that is on tenth day and one month later. Results obtained by both studies revealed that number of Mn post exposure were more in the patients who were exposed to CBCT as well as OPG and were reduced in the second follow-up. Results of the present study were consistent with studies carried out by Bashaet al.[6] and Doua H. Altoukhi et al.[2]These results of this study are of great value as none of the previous studies compared OPG with CBCT (SV) and CBCT (SA).[2] These facts thus call for additional study to examine the long-term effects of radiation using a bigger sample size and a longer follow-up time. Concerning these genotoxic effects, it indicates that OPG induces lesser nuclear alterations than CBCT but still induces a significant effect than the non-exposed ones.[6]

Angelieri et al. suggested that OPG may not cause chromosomal damage, it can induce cytotoxic nuclear alteration.[9] On the other hand, Cerqueira et al. found that X-ray radiation emitted during panoramic dental radiography can lead to genotoxic effects on epithelial gingival cells, increasing the frequency of chromosomal damage and nuclear alterations.[10,9] Additionally, Kesidi et al. conducted a study that revealed full mouth radiographs can generate cytotoxic and genotoxic effects on oral mucosa cells.[11] The research also, indicates a significant increase in the number of micronuclei following CBCT exposure, signifying the accumulation of such genetic damage in epithelial It is widely acknowledged that these alterations serve as the fundamental cause of the development of premalignant lesions and cancer. The results of this study suggest that the use of CBCT should be approached with caution to minimize the risk of adverse health consequences. [12,13]

Here, the research indicates that though utilization of CBCT is an important diagnostic tool, it has raised concerns regarding its potential to cause genetic damage. It indicates a significant increase in the number of Mn following the exposure, signifying the accumulation of such genetic damage in epithelial cells. Similarly, OPG also causes a significant genotoxic and cytotoxic effects on nuclear alteration. Therefore, it can be concluded that, routine use of CBCT and OPG for dental diagnosis or screening must be avoided, it must be validated by history and clinical examination of patient, regarding potential risks that come along with the benefits. [14]

V. CONCLUSION:

The use of diagnostic radiographs should be exercised with caution and only when necessary. It is recommended that the "ALARA (As Low as Reasonably Achievable)" principle be employed to minimize radiation exposure and its inevitable adverse health effects. Future studies can be carried out to expand the sample size and establish the molecular level alterations occurring after exposure to diagnostic radiation.

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Nil.

VII. CONFLICTS OF INTEREST:

There are no conflicts of interest.

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