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## Research Article

### ASSESSMENT OF RADIODENSITIES OF DENTAL HARD TISSUES WITH FRACTIONATED COBALT-60 RADIOTHERAPY

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#### ABSTRACT

**Objective:** The aim of the study is to assess the radiodensities of teeth pre and post Cobalt 60 radiotherapy and to correlate it with different fractionation of radiations.

**Material and Method:** 20 freshly extracted human molars free from apparent caries, macroscopic cracks, abrasions, staining as assessed by visual examination were selected and mounted on an acrylic block. Radiographs using paralleling techniques were done with set standard parameters. Further teeth were irradiated in a Cobalt unit with 1.25 MV photons at dose rate of 1Gy/min, and at a source-surface distance of 80 cm. A dose of 2Gy/fraction (1fraction per day, 5 times/week) was used up to cumulative dose of 66Gy (33fractions in 6 weeks course).

After each fraction, radiographs were done. All images were evaluated for changes in density by digital subtraction radiography and pixel density assessment of enamel and dentin and were compared with fractionation of radiation.

**Results:** Average mean pixel density of teeth showed fluctuations following irradiation fractions. With subsequent radiation fractions a positive correlation between mean density and fraction of radiation at 10, 20, 30, 40, 50 grey of radiation exposure was obtained using Pearson's correlation test.

**Conclusion:** This study shows that irradiation affects radiodensities of hard tissues of teeth, which might contribute to increased risk of radiation tooth decay associated with salivary changes, microbiota shift and high soft and carbohydrate rich diet.

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## INTRODUCTION

Radiotherapy is widely employed treatment modality for head and neck cancers, either as an adjuvant to surgical treatment in conjugation with chemotherapy or as a palliative treatment for advanced or inoperable tumors [1]. Radiation Therapy has many adverse effects to oral tissues and functions. One such severe consequence of radiotherapy is deterioration of teeth, which can result in radiation caries with an incidence of about 28.1%. Hyposalivation, dietary changes with concomitant alteration in oral flora are considered to be the most important aetiological factors in development of radiation caries following radiotherapy [2].

Despite advancements in radiation therapy, cobalt -60 still continues to be the most widely used treatment modality in head and neck cancers.

Cobalt-60 uses high-energy radiation in fractionation. Several in vitro studies with cobalt-60 radiotherapy have demonstrated that radiation therapy can also exert direct effects on teeth like

changes in the crystalline structure, acid solubility of enamel, stability of dentinoenamel junction and microhardness of teeth [3]. Radiation caries can occur either because of direct effect on the teeth or due to indirect effect and a combination of these two factors.

Radiographic image analysis can be a non-invasive tool in assessing the subtle changes in teeth following irradiation. With the advancements in digital radiography and image analysis techniques, these subtle changes can be well appreciated.

However, no studies are available in literature assessing changes in radiodensity of teeth following irradiation. Hence realizing the paucity, this study was planned to assess radiodensities of teeth at different fractionation of radiation and correlating it at various fractions, understanding of which can add information about the density changes in the teeth, thus improves the restorative management of teeth, which can prevent further morbidity in Head and Neck cancer.

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## MATERIAL AND METHODS

This in vitro study was carried out on 20 extracted human molars at our institution with an ethical clearance obtained from institutional ethical board. Images of 20 freshly extracted human molars free from apparent caries, macroscopic cracks, abrasions were included in the study, while images of teeth with internal resorption, pulpal calcifications, endodontic treatment and other developmental anomalies were excluded from the study.

### Procedure for Radiographic Imaging

The selected 20 teeth were assigned numbers from 1 to 20 and were mounted in self cure acrylic blocks of 2x2 cm<sup>2</sup> and were stored in artificial saliva which was renewed on daily basis aintained at 37° C. Radiovisigraphic (RVG) images using paralleling technique with Kodak 51000 digital radiographic sensor, with paralleling instrument and with following exposure parameters kVp -65, 8 mA, 1.5 mm Al filter, 230 volts were taken at baseline (pre-irradiation) and after every fractionation of radiation [Figure 1].

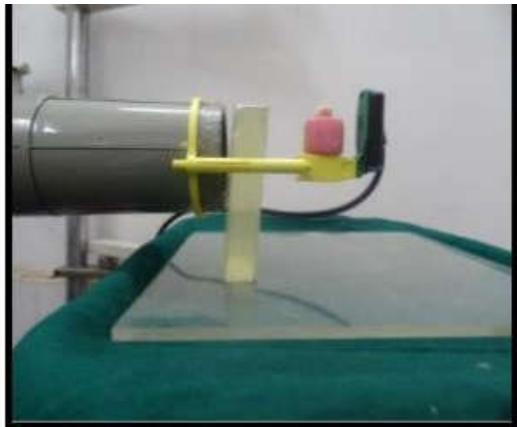


Figure 1 RVG imaging of mounted tooth using paralleling technique with 10 slides packed with cellophane tube to simulate buccal gingiva

### Procedure for Irradiation

Teeth were irradiated by a Cobalt unit (Theratron Phoenix 60 Cobalt Radiotherapy Treatment Unit) with 1.25 MV photons at dose rate of 1Gy/min, and at a source-surface distance of 80 cm and dose of 2Gy/fraction (1fraction per day, 5 times a week) was used up to cumulative dose of 60 Gy [Figure 2]. After each fraction, radiographs were done.



Figure2 Cobalt Irradiation Unit

### Image Analysis

The RVG images of selected 20 teeth mounted on acrylic block at baseline and after every fraction of radiation were stored as JPEG file in 8bit format with file size of 1.8 MB, resolution of 1200x1600 pixels. Similar RVG images of all the 20 acrylic blocks were obtained. All images thus obtained were analyzed for mean pixel density by using ImageJ 1.43 program (US National Institute of Health, Bethesda, MA) followed by which the mean pixel density of each respective acrylic block was subtracted from mean pixel density of combined tooth and acrylic system so as to procure the mean pixel density of the region of interest that is the tooth at every fractionation of radiation [Figure 3].

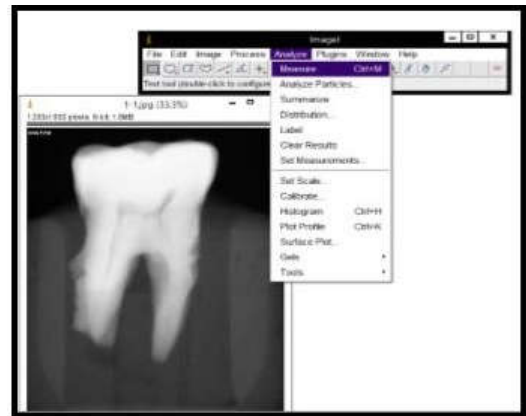


Figure 3 Analysis of the image using Image J software

### Statistical Analysis

The data thus obtained is stored and statistically analyzed by using paired t test for comparison of mean pixel density of teeth before irradiation and after every fractionation of radiation up to 60 Gy. Correlation of mean pixel density with fractionation of radiation is done by Pearson's correlation test.

Table 1 Mean Pixel Density of 20 teeth before and after completion of fraction.

Toothno	Baseline(preirradiation)	At60gy
1	14	16
2	17	13
3	19	19
4	16	16
5	15	17
6	11	14
7	29	28
8	14	15
9	15	19
10	17	16
11	21	23
12	16	27
13	12	9
14	14	15
15	11	12
16	11	15
17	7	9
18	15	14
19	9	11
20	14	11
Average mean pixel density	14.85	15.95

## RESULTS

Correlation was obtained between the mean pixel density of tooth with different fractions of radiation as mean density was presented on

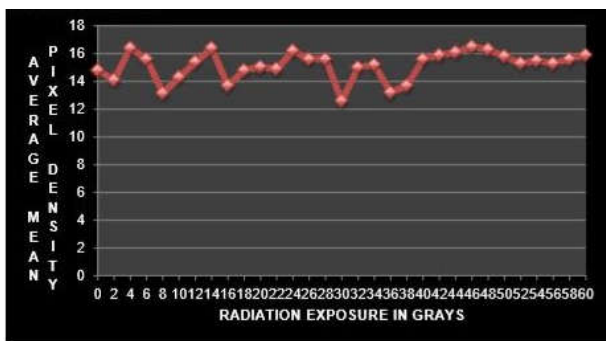
vertical axis and fraction of radiation on horizontal axis. It was evident that at baseline mean pixel density of tooth had strong correlation with all different fractions of radiation used 10, 20,30,40,50,60 Gy. With subsequent radiation fractions also a positive correlation between mean density and fration of radiation at 10,20, 30, 40, 50 grey of radiation exposure was obtained [Table 2].

**Table 2** Correlation of Mean Pixel Density with fractionation of radiation

Variables	Baseline	10	20	30	40	50	60
Baseline		0.78**	0.87**	0.73**	0.70**	0.70**	0.79**
10			0.72**	0.73**	0.53**	0.59**	0.79**
20				.86**	0.91**	0.85**	0.80**
30					0.72**	0.72**	0.75**
40						0.87**	0.67**
50							0.82**
60							

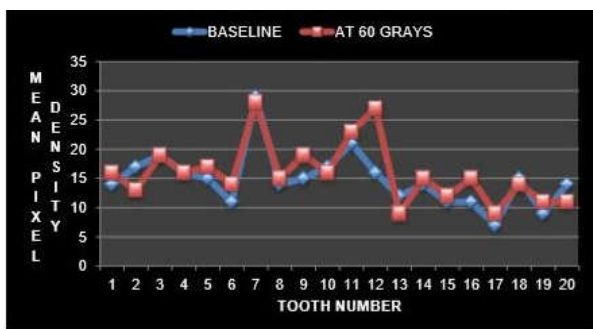
\*\*Correlation is significant at the 0.01level (2-tailed).

The average mean pixel density of 20 teeth was approximately 15, which after first irradiation fraction of 2 Gy decreased to value of 14. Further the effect of tumor therapeutic irradiation showed an increase in average MPD of about 16 at 4 Gy fraction, immediately followed by dramatic decline attaining a value of 13 at 8 Gy fraction. For the range of 8-14 Gy, a dose dependent increase in density was found with value of average mean pixel density of 16.Followed by again decrease in average mean pixel density at 16 Gy that approximated the baseline value of pixel density. Maximum mean pixel density value of 16 was achieved at 4Gy, 14Gy, 24 Gy, and at 46 Gy, while 30th Gy fraction showed lowest mean pixel density of 12. Similar fluctuations were observed during the entire course and finally attained value of 16 on completion of 60 Gy [Figure 4].



**Figure4** Comparison of average Mean Pixel Density of teeth before irradiation and after every fractionation of radiation up to 60 Grays

Mean pixel density of certain teeth like 1,5,6,8,9,11,12 increased upon completion of radiation fractions, while that of tooth number 14,15,16,17,19,20 showed decline in the mean pixel density whereas tooth number 3,4 showed nearly the same values of mean pixel density at baseline and at 60 Gy [Figure 5].



**Figure5** Comparison of Mean Pixel Density of 20 tee that base line and at 60G rays.

## DISCUSSION

The effect of irradiation of malignant head and neck tumors results in changes in mechanical properties of teeth. Tooth defects have been identified immediately and after first fraction of irradiation.

Radiation related caries is one of the highest indirect and late effect of radiation in head and neck region [4]. These changes in tooth are usually the aggravating conditions of mechanical properties; cracks and increasing surface roughness [5]. Studies have demonstrated that late effects also depend on fractionation dose [6]. Understanding the effects of radiation on teeth and correlating it with fractionation of radiation, helps us to arrive at a better preventive and restorative protocol which aids in reducing the radiation associated morbidities.

According to the methodology used in this study several aspects were taken in account to standardize the evaluation and to approximate the in vitro reality closer to clinical conditions. The protocol used for radiation therapy was the same as that normally used for the treatment of patients with head and neck cancer (2 Gy /day, 5 times week interspersed by 2 days without radiation) [7].

Additionally, the samples were placed in artificial saliva during irradiation to simulate, as precisely as possible, the conditions that are found in the oral cavity [8]. Although artificial saliva does not exactly mimic the characteristics of natural saliva, but is still considered the most suitable storage medium [9].

The Average Mean Pixel Density of all 20 freshly extracted teeth before irradiation was 14.8 and following first irradiation dose of 2 Gy, the MPD decreased to a value of 14.1. Similar observation was noted in a study conducted by W. Franzel, R. Gerlach where they found dramatic decrease of the mechanical parameters of enamel and dentin after .5 Gy of therapeutic irradiation [6].

Also in our study the MPD of majority of teeth increased upon completion of radiation fractions. A possible explanation for this could be that radiation causes reduced water content in tissues, and tissue dehydration leads to increased organic matrix stiffness and, consequently, to increased microhardness [5]. This is in agreement with the study conducted by Joyson-Bechal and Jasma *et al* that showed increase in resistance to demineralization [10]. Ionizing radiation may cause restructuring of the crystal structures of mineralized tissues and thereby modify their physical properties, including the structural microhardness [11].

While certain teeth also showed decrease in MPD on completion of 60 Gy, probably due to micro-morphological changes in dentin in the form of alterations in their secondary and tertiary structures, with harmful effects on the hydration of collagen fibers by the action of free radicals that could explain

the progressive decrease of microhardness with the increase of radiation dose. Radiation promotes side chain decarboxylation and a loss of acidic phosphate groups. The loss of acidic phosphate groups lead to the formation of new calcium ion bridged phosphate groups. Additionally, the mineral-organic interaction is reduced and the development of carbon dioxide may induce micro cracks in the hydroxyapatite mineral. As a consequence of the decarboxylation the hardness and elasticity in enamel as well as in dentine are dramatically decreased [12].

These varied responses of teeth following irradiation could be due to differences in environmental factors during the time of tooth formation and individual tooth mineralization [12]. New studies concerning the changes in dental hard tissues, even on molecular level, must be undertaken to give more understanding about the tooth damage in irradiated teeth.

## CONCLUSION

Image analysis can reveal subtle changes in teeth following irradiation, depending upon which we can plan the restorative management of teeth.

### Future scope

Hence, this in vitro study prompts the conductance of in vitro study to guarantee the benefits and effectiveness of quantitative assessment of pixelate density in clinical practice.

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