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RADIATION EFFECT ON STRUCTURE AND MECHANICAL PROPERTIES OF TEETH- AN INVITRO STUDY

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ABSTRACT

Objectives: Evaluating the effects of radiation therapy on enamel and dentin by performing microhardness test and Scanning Electron microscopy (SEM) studies. **Methods:** Vickers hardness test was performed for extracted teeth sections (n=16 hemisections) on outer enamel region and 3 regions on dentin layer (superficial, intermediary and deep). The hardness values were obtained before radiation therapy and after 70 Gy beam treatment. The values obtained were analyzed statistically by performing paired‘t’ test and one way ANNOVA followed by tukey’s test. SEM analysis (n=6 hemisections) was carried out to study the changes in morphology of Enamel and dentin after radiation. **Results:** The hardness values showed a steady decrease in each portion of enamel and dentin of the radiated teeth. The test values were found significant at $p < 0.001$. The SEM images showed demarcation of prisms, dentinal tubules in non-radiated teeth samples whereas the tissue differentiation was a critical task in radiated samples due to degradation of the components. **Conclusions:** The ionizing radiation is found highly effective in damaging the dental tissues at the dosage rate of 70 Gy. It reduces the mechanical strength of the tissues and also causes morphological changes developing cracks which can lead to variety of dental challenges. **Clinical significance:** Radiation Therapy can serve as an onset for development of dental caries or loss of whole

tissue. Hence preventive measures are necessary to be taken in case of high dosed radiation therapy among head and neck cancer.

KEYWORDS: Radiation therapy, Enamel, Dentin, Microhardness, Surface analysis.

INTRODUCTION

Ionizing radiations are one among the environmental factors widely used in various fields. Currently they have been highly used in radiation therapy; nuclear reactors, diagnostic centers etc and are known to cause severe impact on human health. Medical professionals have demonstrated that there is an increasing risk of dental issues among the people exposed to radiations.[1] Darchuk et al have shown dental tissues with increased level of abrasion and pitting on the enamel surface. [2] The process of degeneration of tissues serves to be a critical challenge in developing dental related diseases as not directly caused by irradiation.

World Health Organization has recently noted that oral cancers are to be given priorities on treatment modalities.[3] The head and neck cancers are usually treated by using ionizing radiation,[4] either as alone or as a combined therapy (surgery, chemotherapy).[5] The study on effect of radiation therapy provides limited resources for causing changes in the tooth structure.[6-7] Earlier studies have shown that the mechanical properties of teeth changes after radiation exposure. It is observed to develop abrasions on the smooth enamel surfaces damaging the quality of tooth.[8]

It is a well known fact that radiotherapy induces alterations in the healthy oral tissues. Similar study was performed were the subjects were treated with a radiation dosage of 50- 70 Gy over a period of 5–7 weeks. In conclusion, Damage to Oral cavity parameters were reported.[9] Invitro studies showed Salivary gland impairment in rats subjected to dosage rate between 2 and 60 Gy.[10] Morphological and structural changes of enamel and dentin of rats were observed in 30-70 Gy radiation treatment under scanning Electron Microscopy.[11]

The improvisation of treatment strategy is of utmost importance in this present era to reduce the effect of radiation on healthy dental tissues. Hence it becomes necessary to maintain the crystal structure of enamel and dentin to safeguard the dental tissues from hazardous effects. The influence of radiation in the development of dental complications can be assessed by studying the association of SEM and Microhardness of tissues. This paper details the change in mechanical properties supported by the SEM analysis to verify the hilarious effect of Ionizing radiation.

MATERIALS AND METHODS

The freshly extracted Intact molar teeth from healthy individuals (n=12 nos) were collected from the Dept. of Oral and Maxillofacial surgery, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore. These teeth specimens were washed thoroughly to remove particulates or debris and are stored in distilled water at 4°C until use (recommended usage time is within a month). The study has been performed under the consent of Nitte University Human Ethics Committee. The cleaned teeth were incubated in artificial saliva at 37°C initially at the start of the experiment. The teeth were then sectioned mesiodistally using Carbondom Disc (Micromotor, SCHICK, Master 1) obtaining 2 halves which were then used for the following experiments.

The first experiment involves the testing of microhardness of tooth enamel and dentin regions (n = 18 hemi sections). The specimens were grouped as pre and post radiated samples. This was followed by another experiment were the morphological changes in Enamel and dentin area was defined by performing SEM analysis for non radiated and post radiated samples (n= 6 hemisections). Since the radiation therapy followed among oral cancers usually involves the cumulative dosage between 30-70 Gy,[22] we have used 70 Gy as dosage higher limit in our study. The teeth sections were placed in 2 ml eppendorf tubes filled with artificial saliva so that it is subjected to uniform radiation/ unit area.

Microhardness Evaluation of Enamel and dentin

The microhardness of teeth was initially tested prior to the radiation treatment. The hardness was evaluated by using Vickers hardness tester with help of diamond indenter for calculating Vickers hardness (VH). 20Kgf load was applied for 10-15 seconds; the average of diagonal indentations is calculated to obtain the hardness value. The teeth samples (Pre radiated samples) were fixed to the wooden blocks for the ease of the testing. Indentations were carried out in the outer enamel region, and 3 portions on the dentin region. The dentin portions involved for indentation are the outer superficial dentin: 50 µm from the dentin-enamel junction, Intermediary dentin, and deep dentin: 50 µm above from the pulp chamber. All the hardness testing was performed by the same investigator to reduce the error to be involved in the experiment. The average of each indention parameters of the specific group was used for the statistical analysis of the data. After the evaluation of initial microhardness, the same teeth 18 hemisections were placed in artificial saliva and were subjected for radiation using Electron Beam Radiator at microtron center (Mangalore University). A total dosage rate of 70Gy/ fraction is been used to study the direct effect at higher limit. The post irradiated samples are then evaluated for microhardness measurements as followed initially. The data obtained is statistically analyzed using SPSS software (version 16.0). Paired ‘t’ test was performed at a significance level of $p<0.05$

SEM analysis of Enamel and dentin

We used total of 6 hemisections for SEM analysis, grouped as Non radiated and Post radiated samples (N=3 sections each). These samples were used to study the morphological changes occurred after radiation by analyzing under SEM. Surface analysis using SEM was performed in NITK college (Department of Mettalurgy, National Institute of Technology, Mangalore, Karnataka). The specimens were initially fixed in a glutaraldehyde solution in cacodylate buffer, cleaned for 10 minutes using distilled and deionized water, and then dehydrated using increasing concentrations of ethanol (25%, - 100%), and finally immersed in hexamethyldisilazane (HMDS) for 10 minutes. They were dried completely in hot air oven prior to analysis to prevent any persisting humid environment. The dried samples are then fixed to the stubs with a double-sided adhesive carbon tape and placed in a gold sputtering vacuum-metallizing machine. Gold coating was being processed with a pressure of 0.01 mbar, current of 40 mA, working distance of 50 mm, coating time of 90 seconds, and mean coating thickness of 20 to 30 nm. Once the coating is complete, the stub is place inside the vacuum chamber of SEM for analysis.

RESULT

Microhardness evaluation of Enamel and dentin

The mean value of Enamel microhardness before radiation was found to be 184 VH (Vickers hardness Unit) and after radiation treatment it decreased to 145 VH. The mean difference between pre and post irradiated sample is 39.5 with the paired ‘t’ test value of 22.56 indicating that the test is highly significant at the p value <0.001 (Table 1). This decrease in microhardness can be due to Uni stroke therapy of 70 Gy. The Microhardness of superficial dentin region were found to be the hardest compared to intermediary and middle dentin regions with the mean value of 152 VH (Table 2). The hardness decreased to 122.8 and 90.34 VH as we proceeded to intermediate and deep dentin regions respectively. The lowest mean value of 40.84 VH was obtained in the deep dentin region after radiation treatment and was determined statistically significant with‘t’ value of 11.98 (p < 0.001). Whereas the microhardness of superficial and intermediate dentin decreased to 100.2 and 82.7 VH respectively after the radiation treatment signifying that 70 Gy radition is causing the damaging effect on these tissues. We found that the test was significant on the microhardness measurement of Enamel and dentin regions (p<0.001), concluding that radiation is effective in reducing the hardness of dental tissues (Fig 1-2)

Scanning electron microscopy analysis of enamel and dentin

The cross sections of the enamel portion of the non irradiated teeth showed well organized prism crystalline structure with no cracks or damages and clear interprismatic regions (Fig 5). But this organization was broken after radiation treatment which showed deep cracks and amorphous substances on the surface completely destroying the alignment of the prismatic structure (fig 6). The electron micrographs show marked loss of defined structures as well as interprismatic space. The non irradiated groups showed clear dentinal tubules and inter tubular region in the cross section the tooth samples (fig 7). Unfortunately this systematic tubular organization had completely changed after radiation treatment showing amorphous porous like structures with widened pits. The identification of dentinal tubules was hardly an impossible task even at the highest possible magnification (fig 8).

Table 1: Vickers Microhardness value of tooth enamel before and after radiation

Group Enamel Hardness 70 Gy	Mean	Std. Deviation	Mean Difference	‘t’ value	P value
Before Radiation	184	26.51	39.7	22.564	<0.001
After Radiation	145	24.24			

Table 2: Vickers Microhardness value of superficial dentin region of teeth before and after radiation

Group Superficial dentin Hardness 70 Gy	Mean	Std. Deviation	Mean Difference	‘t’ value	P value
Before Radiation	152	27.69	52.8	27.141	<0.001
After Radiation	100.2	23.26			

Table 3: Vickers Microhardness value of Intermediate dentin region of teeth before and after radiation

Group intermediate Dentin Hardness 70 Gy	Mean	Std. Deviation	Mean Difference	‘t’ value	P value
Before Radiation	122.88	27.71	40.01	38.181	<0.001
After Radiation	82.79	26.49			

Table 4: Vickers Microhardness value of Deep dentin region of teeth before and after radiation

Group Deep Dentin Hardness 70 Gy	Mean	Std. Deviation	Mean Difference	't' value	P value
Before Radiation	90.34	27.58	47.8	11.987	<0.001
After Radiation	42.47	17.99			

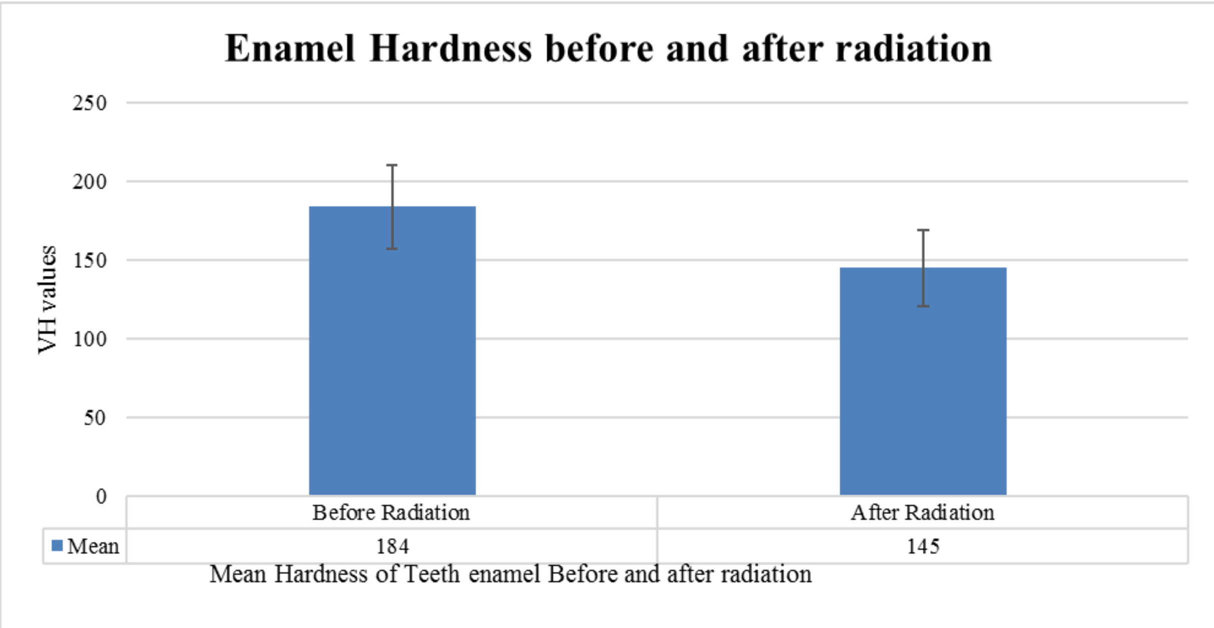

Fig 1: Graphical representation of Enamel Hardness Before and after radiation

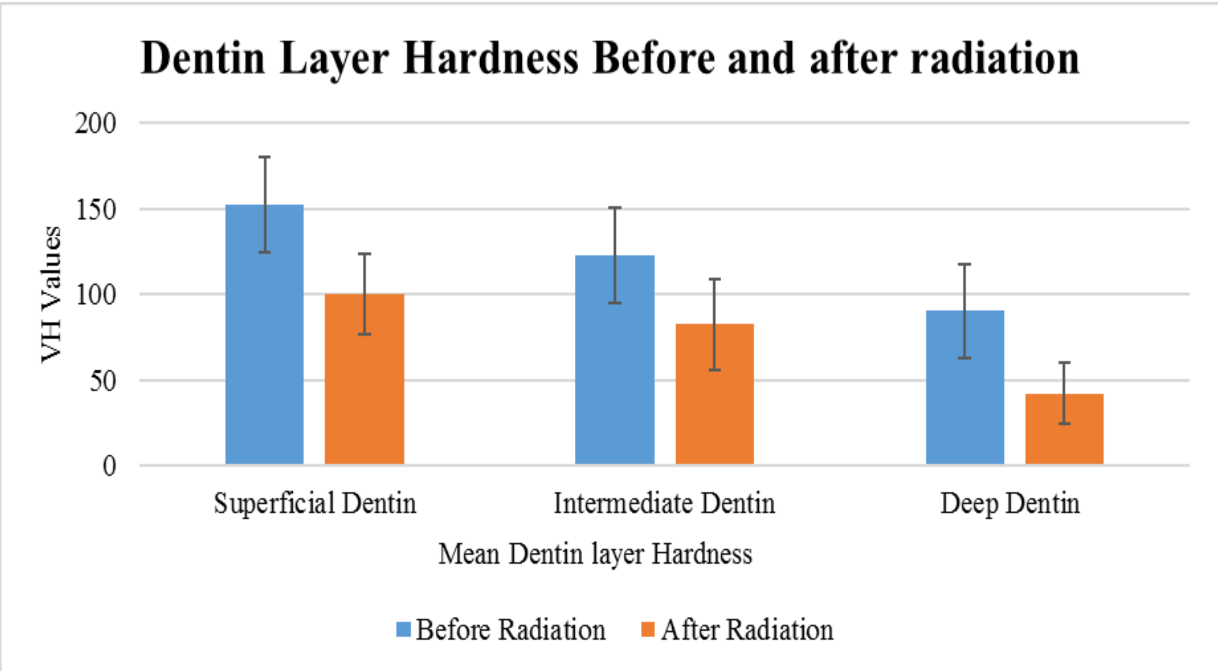

Fig 2: Graphical representation of Dentin Hardness Before and after radiation

SEM Images

Fig 3: Tooth Structure before radiation at 40 X magnification

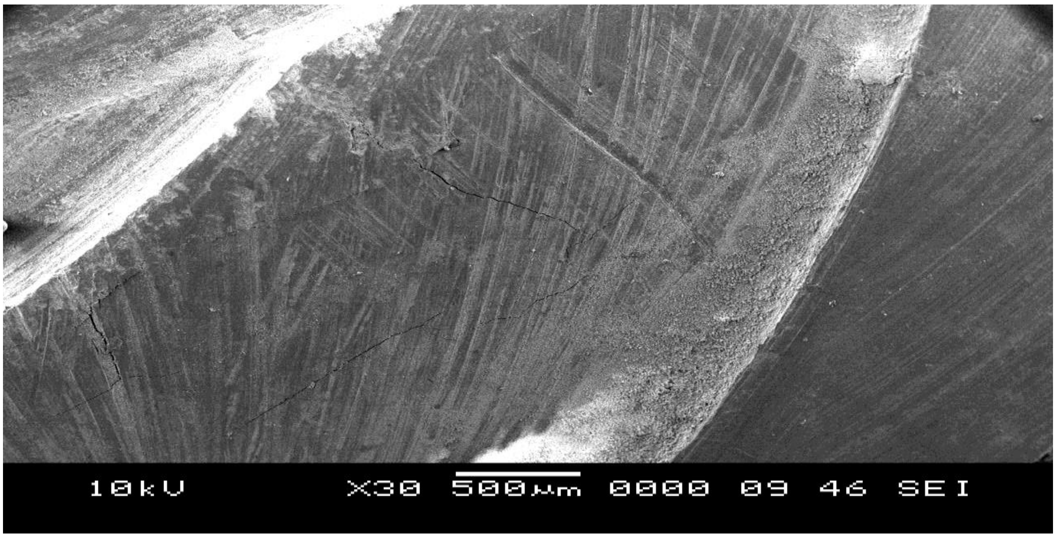

Fig 4: Tooth structure at 40 X magnification after radiation showing breakage

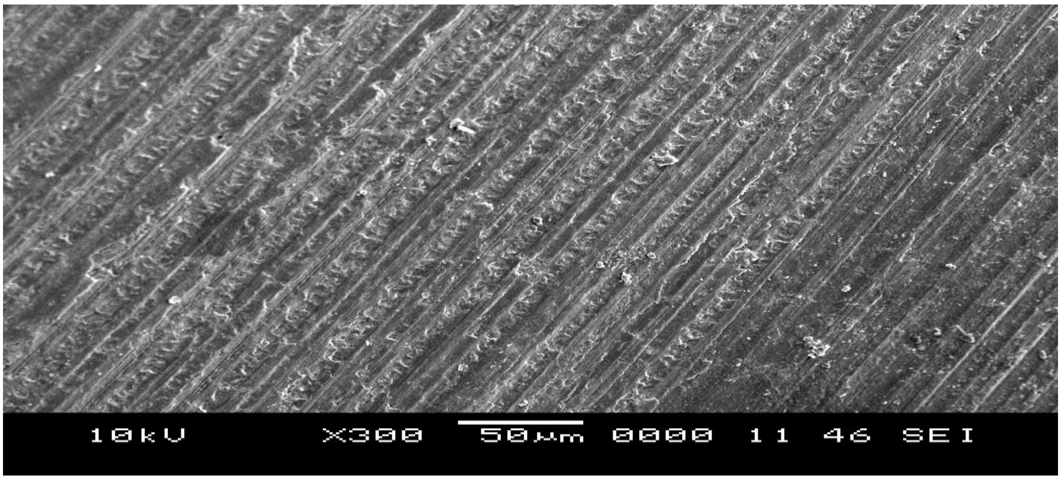

Fig 5: Enamel structure before radiation at 100 X magnification showing clear orientation of tubules

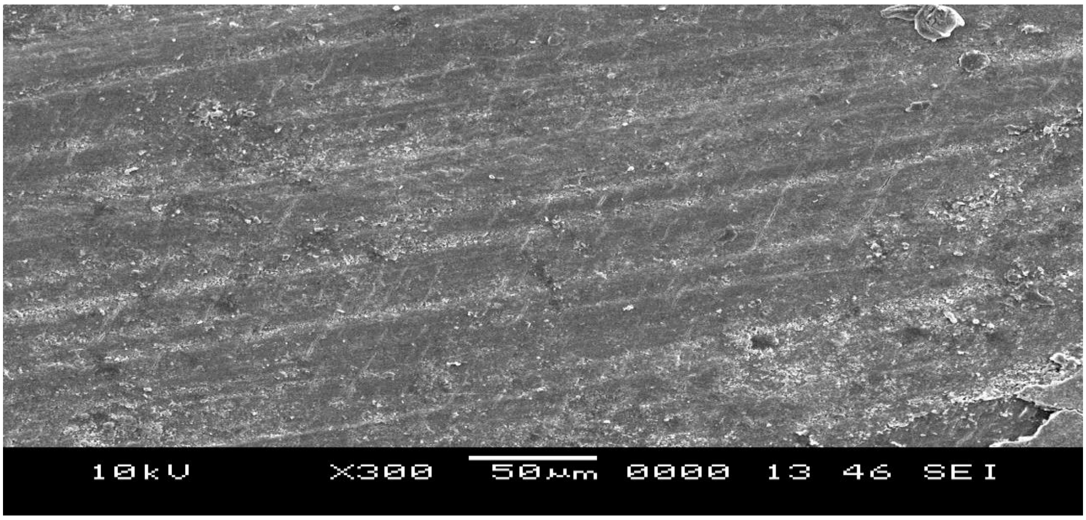

Fig 6: Enamel Structure at 100X magnification after radiation showing amorphous structure

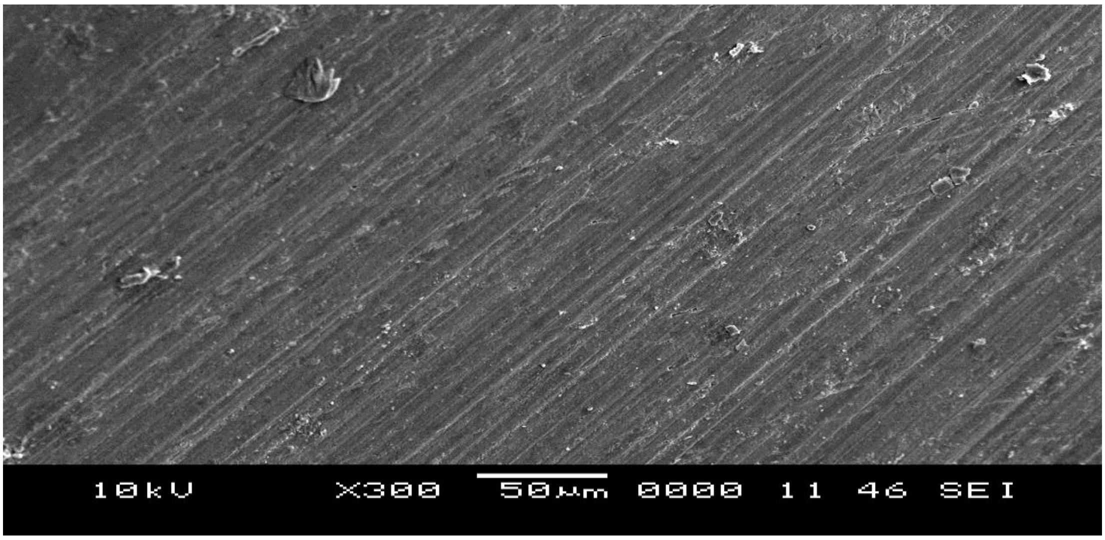

Fig 7: Dentin structure before radiation at 100 X magnification showing clear tubules

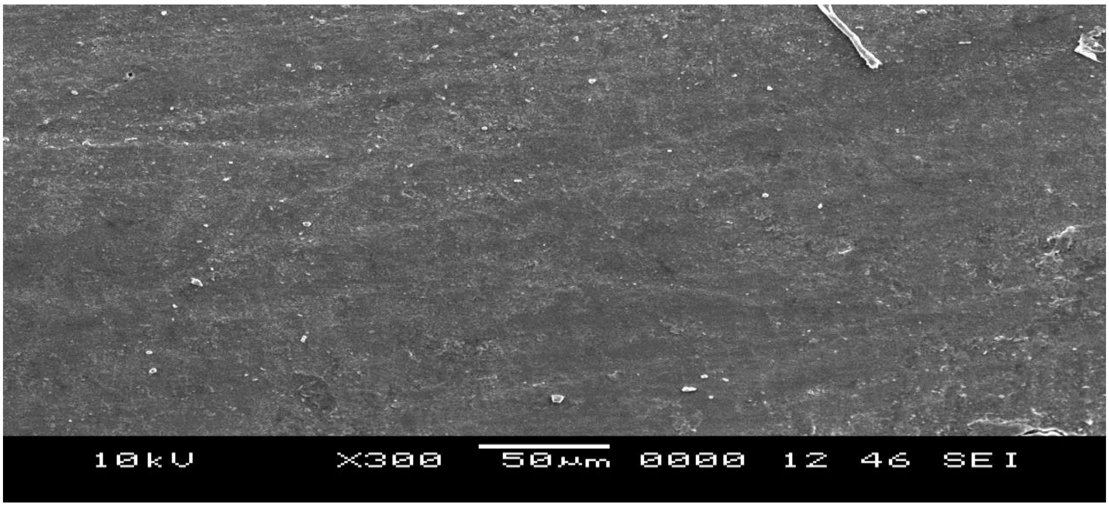

Fig 8: Dentin structure after radiation at 100 X magnification showing loss of dentinal tubules

DISCUSSION

The effect of Radiation therapy varies among the population based on their normal condition of health and also the features of cumulative dosage applied. The doses for treatment of cancer range from 30 to 70 Gy, based on the size of tumor, its type and clinical measures.[12,13] In the present scenario health care professionals follow fractionation doses due to their prolonging late effects on the patients.[14,15] The maximum dose given to head and neck malignancies is upto 70 Gy,[12] hence we have chosen this dosage to study their effects on the dental tissues. In our study we maintained the samples in artificial saliva to ensure some conditions of oral cavity.[16] Researchers have used many other solutions such as saline,[17,18] distilled water, phosphate buffer etc,[18,19] however artificial saliva is termed as most suitable media for enhancing the oral conditions of tooth maintained in-vitro.[16,20]

The present study clearly signifies that irradiation is effective in causing alterations in the mechanical and morphological properties of teeth. The mechanical properties exhibit that radiation has damaging effect on teeth by decreasing its microhardness strength. Previously many investigators have suggested that the radiation treatment reduces the microhardness of enamel and dentin regions[21] on subjecting to the cumulative dosage of 60Gy. In contrast, some studies have explained that radiation at a higher dosage level show reconstructive property thereby increasing their physical hardness.[22]

The enamel layer of tooth is composed of prismatic crystalline structures that exhibit the anisotropic character thus enhancing its mechanical properties.[23] SEM is a modern technology which enables to study the surface property of any materials. Here in our study we observed that there was drastic change in the prismatic alignment of the crystalline structures. The morphological change observed after radiation treatment of 70 Gy depicted amorphous substances on the micrographs. These results are in accordance with the study described for bovine teeth.[24] The disintegration of the prism structures is been one of the critical for the development of caries.[25] Enamel is known to be highly inorganic, but the damages usually occur in the organic region, i.e in the interprismatic space causing oxidation of water molecules into hydrogen peroxide and hydrogen free radicals denaturing the organic components.[26] This is known to suppress the integrity of whole enamel region.[12] However, the micrographs do not show any variation in the prismatic or interprismatic region. The reason for such changes in the enamel can also be because of decarboxylation of dental tissues. The interaction between organic and inorganic components of the enamel occurs through calcium ion channel binding carboxylate ion to phosphate group. But irradiation impairs this mechanism reducing the interaction between the enamel components. Thus the damage is due to the changes caused both by organic and inorganic components of the enamel. This hypothesis is in correlation with the findings of pashley.[27]

The microhardness value of each dentin portion has shown decrease after radiation as compared with same portion on non irradiated teeth. Hence it is confirmed that every layer of the dentin has undergone reduction in its mechanical strength. This reduction is correlating with the studies of albeit et al where they irradiated the samples upto 70 Gy and found the similar results.[9,12] Basically, this kind of reduction in microhardness can be due to the presence of organic compounds within the tubules which undergo oxidation reducing the overall strength of dentin.[17] In general dentin is less organic, but contains around 10-12% of water content, where upon radiation produces free radicals[28] that denatures and enable them to lose their tubular stabitlity.[29]

The SEM analysis of Dentin regions showed clear tubular structure in non-radiated teeth and this tubular organization had lost its eternity after radiation with 70 Gy. The amorphous powder like particulates seen under SEM is known to be caused due to the degradation of collagen fibres framed in the dentinal tubules. This degradation of tissues is due to the function of free radicals released during the oxidation process initiated by irradiation.[30]

CONCLUSION

The radiation treatment is found to be effective in the decreasing the microhardness of the enamel and dentin regions. 70 Gy radiations as a Uni-dose is highly damaging to dental tissues and can have adverse effects. Morphological studies also showed degradation of intact tissue lines into amorphous porous form. This disruption caused by radiation signifies that it is damaging the mechanical property of dental tissues.

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