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EFFECT OF GAMMA-RADIATION ON DE- AND RE-MINERALIZATION OF ENAMEL AND DENTIN (AN IN-VITRO STUDY)

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KEYWORDS

Demineralization; Enamel; Dentine; Gamma irradiation; Microhardness; Remineralization

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ABSTRACT

Introduction: Gamma radiation is usually used, as a primary or supplementary treatment, for oral cancer patients. **Aim**: The present work was undertaken to investigate, in vitro, the effect gamma irradiation of a cumulative dose of 60 Gy (20 Gy/day over three days) on enamel and dentin microhardness before and after demineralization and remineralization. **Materials and methods**: Ten healthy freshly extracted human third molars were used in this study. The teeth specimens were cut perpendicular to their occlusal surface into two halves. Half of the specimens were gamma-irradiated to a dose of 60 Gy (20 Gy/day). The data involved the measurement of VHN Vickers microhardness on both radiated and non-irradiated. **Results**: The results indicated that the mean microhardness values of the non-irradiated enamel samples were decreased after gamma irradiation, and were significantly decreased after demineralization; meanwhile, the microhardness values of the demineralized non-irradiated enamel samples were significantly increased after remineralization. The results showed that the microhardness of the demineralized non-irradiated dentin samples was increased, and that the microhardness of the gamma-irradiated dentin samples was slightly decreased. **Conclusions:** gamma-irradiation had significant effect on enamel and dentin microhardness values and had significant decrease effect on demineralization of enamel while had significant increase by remineralization.

INTRODUCTION

Gamma radiation therapy is still used in the treatment of patients with oral cancer[1,2], but it might have serious negative dental and oral effects. Orofacial tissues, oral mucosa, salivary glands, taste, dentition, periodontium, bone, muscles, and joints are all affected by radiation[3]. The impact of radiotherapy on the structure of the teeth is, however, a subject of scant research. It was discovered that after irradiation, the radiolysis of water, which oxidizes the organic components of dental structure, has an impact on the mechanical properties of teeth. Caries developments occur on enamel surfaces, which are typically resistant to decay[4,5]. The patients' pain response, atrophying pulpal tissue, and delayed treatment requests are common. Therefore, it is crucial to understand whether radiation caries results from a direct, indirect, or combined impact on teeth[6]. There are effects that follow the harmful effects of this kind of radiation on the head and neck area.

The majority of side effects temporary, including mucositis, sensitive or painful soft tissues, osteoradionecrosis of the jawbone, and taste loss[7]. Other effects, such as trismus, changes in the bacterial microflora and proteins, and muscles atrophy in the jaw region, are more long lasting. Patients who are recovering from radiotherapy must change their eating habits to include more soft foods in order to gain or maintain weight. The threshold for salivary gland damage is 30 Gy; after this point, the damage to the glands is irreversible[8,9]. It is still unknown what causes teeth to fall out: changes to the dental hard tissues, hyposalivation, modifications to oral hygiene, or high doses of radiotherapy are administered to patients with head and neck cancer. [10,11]. The volume and location of the radiation, the total dose, the fractionation, the patient's age, their clinical status, and any accompanying treatments all affect how severe these reactions are. These reactions can happen during the acute phase (during or within the first weeks of treatment) or in the chronic phase (months or years after the radiotherapy). There is little information available regarding how gamma radiation affects enamel microhardness and resistance to demineralization [12]. Many investigations were made on the effects of gamma irradiation on the demineralization and remineralization of enamel and dentine, in which polarized light microscope, mineral analysis and scanning electron microscope have been used to evaluate this effect. According to our knowledge, there is a shortage in the studies that evaluating the effects of gamma irradiation by the measurements of the mechanical properties in terms of microhardness. Thus, the importance of this work is the use of gamma radiation, as a new technique, evaluating its effect on the microhardness of enamel and dentin before and after demineralization and remineralization.

MATERIAL AND METHODS

The study was waived from ethical revision.

Samples collection and preparation

Total number (n=10) of Tooth was collected; it was made sure that the used teeth had any caries, restorations, surface defects or cracks. The teeth were then thoroughly washed, scraped to remove shreds of periodontal ligament, ultrasonically scaled to remove plaque and calculus and then the teeth were polished, and water coolant. The teeth were kept in de-ionized water at 5°C in a refrigerator. Specimens were cut perpendicular to their occlusal surface into two halves. The twenty halves were divided into two groups, ten halves for enamel treatment and the remaining halves for dentin treatments. The dentin surfaces were further polished using a silicon abrasive paper for 20 seconds to standardize the smear layer [13]. Desensitizing materials (potassium nitrate plus sodium fluoride gel) was mixed in a separate cleaned and dry glass slabs with standard measure (3 grams of powder and 2mm^3 liquid. The teeth were brushed with mixed desensitizing materials by a brush and kept on enamel and dentine surfaces for five minutes and after that, they were washed with distilled water for 15 seconds [14].

Gamma irradiation

Half of the specimens (n=10) specimens, for both enamel and dentin exposed to gamma irradiation dose of 60 Gy (20 Gy day after day), where the other half not exposed to gamma irradiation. The irradiation was performed at National Centre for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo, Egypt, using 137 Cesium Gamma Cell 40 at a dose rate of 1.657 kGy/h at the time of experiment.

Preparation of deminerlizing solution

The deminerlizing solution contains 2.2% mM (Ca), 2.2% mM (PO_4), 50 mM (acetic acid) and the solution was adjusted at pH 4.4. Each sample was placed in 50 ml of deminerlizing solution and solution is changed every 12 hours [15], all the specimens were stored in the deminerlizing solution for 2 days.

Preparation of remineralizing solution

The remineralizing solution was prepared according to a standard reported method at pH 9.9[16]. The procedure can be summarized as follows:

1. In a Teflon container, 0.856g KCl powder, 0.1525 g $MgCl_2$-$6H_2O$, 2.776 g NaCl powder, 1.1341g $NaHCO_3$ and 0.3549g Na_2HPO_4 powder were added to 500 mL of deionized water and stirred until completely dissolve. Then, 0.2505 g Ca metal was added and stirred at 500 rpm for 20 minutes at room temperature.
2. The formed precipitate was filtered by using a No. 42 filter paper and the precipitates were washed with about 750 mL of deionized water.
3. The precipitates were dried at room temperature for 48 hours.
4. Amorphous calcium phosphate powders consisting of nano-spheres was ready.

Microhardness measurement

The microhardness of the teeth' samples were measured before and after demineralization using digital display Vickers microhardness Tester, Shmadzu, HMV.2 series, Japan. A load of 100 gm was applied to the surface of enamel or dentin for 30 seconds. The diamond shaped indentations were carefully observed under the microscope. Vickers microhardness values MHV according to the following equation:

$$\mathbf{MHV=0.1891 \times (F/d^2)}$$

Where **MHV** is the microhardness in Kgf/mm^2, **F** is the load and **d** in μm is the average of the two diagonals length in mm.

Statistical analysis

The analysis of variance using ***ANOVA tests*** of significance was applied to compare variables, which affecting the mean microhardness of enamel and dentine samples.

When the significance level (p) value was $P > 0.05$ = non-significant, $P < 0.05$ = Significant and $P < 0.001$ = highly significant.

The second T in dependent test was used to compare between sound tooth (T_o) structure and between each group (T_1, T_2 and T_3) separately.

RESULTS

The mean microhardness of the non-irradiated enamel sample (ER_0) was 239.90 (kg/mm^2). After gamma irradiation, the mean microhardness value of enamel samples (ER) was 227.55 (kg/mm^2). Thus, the mean microhardness value of enamel samples was deceased by 5.15% after gamma irradiation. The results showed that the mean microhardness value of the non-irradiated enamel samples after demineralization (Me_1R_1) was decreased to 215.70 (kg/mm^2), Meanwhile, the mean microhardness value of the gamma- irradiated enamel sample (ER) was decreased from 227.55 (kg/mm^2) to 210.64 (kg/mm^2) after demineralization (Me_1R_1). Meanwhile, the gamma- irradiated enamel samples (ER) was decreased to 217.72 (kg/mm^2) after remineralization (Me_2R_1). These data indicate that the mean microhardness of non-irradiated enamel sample was decreased by 10.09 and 1.79% after demineralization and remineralization, respectively.

The gamma- irradiated enamel sample was deceased by 7.43 and 4.32 % after demineralization and remineralization, respectively **Figure 1**.

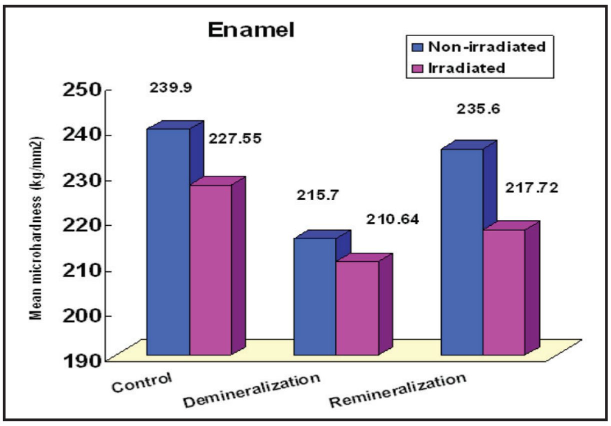

Fig. (1) Mean microhardness of enamel before and after gamma irradiation and followed by demineralization and remineralization.

The mean microhardness of the non-irradiated dentin sample (DR_0) was 58.78 (kg/mm^2). After gamma irradiation, the mean microhardness value of dentin samples (DR) was 55.05 (kg/mm^2). Thus, the mean microhardness value of dentin samples was deceased by 6.55% after gamma irradiation. The results showed that the mean microhardness value of the non-irradiated dentin samples after demineralization (Md_1R_1) was decreased to 54.63 (kg/mm^2), Meanwhile, the mean microhardness value of the gamma- irradiated dentin sample (DR) was decreased from 54.63 (kg/mm^2) to 50.18 (kg/mm^2) after demineralization (Md_1R_1). Meanwhile, the gamma- irradiated dentin samples (DR) was decreased to 52.08 (kg/mm^2) after remineralization (Md_2R_1). These data indicate that the mean microhardness of non-irradiated dentin sample was decreased by 7.06 and 2.09% after demineralization and remineralization, respectively. The gamma-irradiated enamel sample was deceased by 8.84 and 5.40 % after demineralization and remineralization, respectively **Figure 2**.

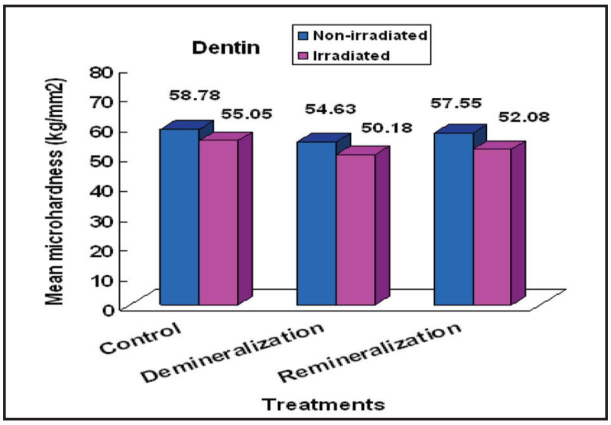

Fig. (2) Mean microhardness of dentin before and after gamma irradiation and followed by demineralization and remineralization.

DISCUSSION

Gamma radiation is frequently used to sterilize and treat medical supplies as well as to treat food[17,18]. It has also been used to sterilize bone allograft in orthopedics [19–21]. Gamma radiation was applied to non-caries extracted teeth at doses up to 2 kGy to sterilize. Higher radiation doses might be required if carious teeth are used[22,23]. With therapeutic advances in head and neck surgery and radiotherapy, the quality of life for patients with head and neck tumors is improving[5]. The mechanical strength of enamel and dentin can therefore eventually be altered by radiotherapy[6]. Patients receiving head and neck treatment should be aware of the serious harm gamma radiation causes to the organic dentin components (collagen matrix, odontoblastic, and pulp complex). The increased risk of radiation tooth decay linked to changes in salivary function, a shift in the microbiota, and a diet high in carbohydrates may be a result of these effects on the dental substrate, particularly in the dentin [24].

Enamel, the body is toughest and most mineralized tissue. Dentin, which is mineralized tissues that exhibit the bony inorganic content, forms the root. The nearly 96 percent mineral content of enamel makes it very hard and brittle, prone to cracks and fractures [25,26]. Dentin, on the other hand, has a variable elasticity modulus. In contrast to enamel, dentin has a lower microhardness and a unique response to tensile strength due to the collagen content and orientation of the tubules. Crystalline calcium phosphate occupies a substantial portion of the inorganic enamel matrix, setting it apart from other mineralized tissues [4] and the remaining 4 percent is made up of water and protein. Biological apatite is a family of minerals that includes the minerals that are present in human teeth. It was produced by the mineral calcium hydroxyapatite ($Ca_{10}(PO_4)6(OH)_2$) [25, 26].

The hardness of enamel and dentin has been measured using a variety of techniques, including abrasion, pendulum, scratching, and indentation techniques[27]. The Knoop diamond indenter is one common type. However, a hardness test is an inexpensive, repeatable, and relatively simple test. In the current study, the microhardness of the teeth was assessed both before and after exposure to gamma radiation at a cumulative single dose of 60 Gy.

It was observed that the dentin samples are less sensitive to gamma irradiation than the enamel samples based on the data obtained in the current work. This result is consistent with earlier studies[28]. In this regard, after receiving a cumulative dose of 60 Gy of gamma radiation, it was -recorded that the mean microhardness of enamel that had not been exposed to radiation had decreased from 239.90 to 227.55 (kg/mm^2) with loss in the mean microhardness value 4.31%. The non-irradiated dentin's mean microhardness, dropped from 58.77 to 55.05 (kg/mm^2) with loss 6.33%, the results are consistent with earlier studies that inferred this effect to denaturation of the organic dental substrate substrates [29–31]. In the dose, range of 0.2-6.0 Gy, the impact of gamma radiation on the hard dental tissues (enamel surface, dentinal tubules, and cementum surface) was assessed using scanning electron microscopy for doses up 0.5 Gy. No signs of cracks on the enamel surface were observed, with a 1.0 Gy dose of irradiation, cracks were easily seen in specific locations, whereas with a 2.0 Gy dose, the enamel displayed morphological changes as disturbed prismatic and interprismatic areas [32]. Impact on the mechanical properties of dentin or enamel was observed for the radiation doses used in the present study. The non- irradiated enamel and dentin samples, which reached 239.90 and 58.78 kg /mm2, has significant difference when compared to gamma irradiated enamel and dentin samples, which reached 227.55 and 55.05kg/mm^2, respectively. Meanwhile, there is significance between non- irradiated enamel and dentin samples followed by demineralization, which reached 215.70 and 54.63 kg/mm^2, respectively. In addition, significant difference was seen when comparing non-irradiated enamel and dentin samples after remineralization, which reached 235.60 and 57.55kg/mm^2, respectively.

In the current study, because of demineralization, the samples of gamma-irradiated enamel and dentin microhardness values reached 210.64 and 50.18 kg/mm^2, respectively. This revealed a significant difference in these samples. Additionally, a difference of 217.72 and 52.08 kg/mm^2, respectively, was observed between enamel and dentin microhardness values that had undergone gamma irradiation following remineralization. The impact of radiation on the enamel structure surface microhardness of irradiated and non-irradiated enamel was assessed utilizing a 25-kGy dose of gamma radiation[33]. Furthermore, to test enamel resistance to demineralization, both irradiated and nonirradiated teeth were subjected to a pH-cycling model.

According to the findings, the 25-kGy medical gamma radiation dose has no impact on the enamel's hardness or resistance to demineralization. The structural alteration seen in the hydroxyapatite crystals of the radiation treated enamel and dentin was used to explain this behavior because it would increase the solubility in the mouth. There may be a gradient in the solubility or rate of dissolution of the enamel, which could account for the flattening of the enamel surface and the increased mineral loss seen in the abraded enamel slabs. This behavior was also attributed to the higher mineral content in the outer enamel as well as the concentration and distribution of some trace elements found in the enamel [34,35]. Additionally, these results are consistent with earlier studies[36,37].

CONCLUSIONS

Gamma-irradiation had significant effect on enamel and dentin microhardness. Gamma-irradiation had significant decrease effect on demineralization of enamel while had significant increase by remineralization in microhardness but not reach to original microhardness value. For dentin, gamma-irradiation had non-significant on both demineralization and remineralization processes.

Declaration of competing interest

The authors declare no conflict of interest regarding the publication of this paper.

REFERENCES

1. Hassan K. Effect of Therapeutic Gamma Radiation on Diametral Tensile Strength and Microhardness of Photo-cured Glass Ionomer Cements. Saudi Dent J 1997; 9(3): 120-4.
2. Berry R J. Therapeutic uses of X-rays. Inter J of Rad Biol 1987; 51: 891-902.
3. Konjhodzic-Prcici A, Keros J, Ajanovic M, Smajkic N, Hasic-Brankovic L. Incidence of Radiation Caries in Patients Undergoing Radiation Therapy in the Head and Neck Region. Pesq Bras Odontoped Clin Integer João Pessoa 2010; 10(3): 489-492.
4. Soares CJ, Neiva NA, Soares PBF, Dechichi P, Novais VR, Naves LZ and Marques MR. Effects of Chlorhexidine and Fluoride on Irradiated Enamel and Dentin. J Dent Res 2011; 90(5): 659-664.
5. Vissink A, Jansma J, Spijkervet F K, Burlage F R, Coppes R P. Oral Sequele of Head and Neck Radiotherapy. Cri Re in Oral Bio Med. 2003; 14(3): 199-212.
6. Andrews N, Griffits C. Dental Complications of Head and Neck Radioterapy. Austral. Dent J 2001; 46(3): 174-82.
7. Beumer J, Curtis Th A, Marunick MT. Maxillofacial rehabilitation, prosthodontic and surgical considerations, 1st Ed. Medico Dental Media International 1996; 21: 43–71.
8. Milano MT, Constine LS, Okunieff P. Normal tissue tolerance dose metrics for radiation therapy of major organs. Semin Radiat Oncol 2007; 17:131–140.
9. Dirix P, Nuyts S, van der Poorten V, Delaere P, van den Bogaert W. The influence of xerostomia after radiotherapy on quality of live. Sup Care Cancer 2008; 16:171–179.
10. Moore S, Burke MC, Fenlon MR, Banerjee A. The role of the general dental practitioner in managing the oral care of head and neck oncology patients. Dent Update. 2012, 39(10):694-698.
11. Thariat J, Ramus L, Darcourt V, Marcy PY, Guevara N, Odin G. Compliance with fluoride custom trays in irradiated head and neck cancer patients. Support Care Cancer 2012; 20(8):1811-4.
12. Lidiany K, Azevedo R, Jaime AC, Marines N. The Effect of Gamma Radiation on Enamel Hardness and Its Resistance to Demineralization in Vitro. J Oral Sci 2004; 46(4): 215-20.
13. Osmari D, Oliveira Ferrrera AC, Carlo Bello M, Sasin AH, Correa Aranha AC, Morquezon M, Silvereira BL. Micromorphological evaluation of dentin treated with different desensitizing agents. J laserers Med 2013; 4(3): 140-164.
14. Jansma J, Buskes JAKM,Vissink A, Mehta DM, Gravenmade EJ. The Effect of x-ray Irradiation on the Demineralization of Biove Dental Enamel. Caries Res 1988; 22:199-203.

15. Cuneyt ATAS. Amorphous calcium phosphate nanoparticles: Biomimetic Synthesis. Mater Sci Eng C: Mater Biol Appl 2012; 32 (5), 1097-1106.

16. Pinto SCS, Pochapski MT, Wombier DS, Pilatti GL, Santos FA. In Vitro and vivo analysis of the effects of desensitizing agents on dentin permeability and dentinal tubule occlusion. J. Oral Scien 2010; 52(1):23-32.

17. De Wald JP. The use of extracted teeth for in vitro bonding studies: a review of infection control considerations. Dent Mater 1997; 13(2):74–81.

18. Crawford LM, Ruff EH. A review of the safety of cold pasteurization through irradiation. Food Control 1996; 7(2):87–97.

19. Vastel L, Meunier A, Siney H, Sedel L, Courpied JP. Effect of different sterilization processing methods on the mechanical properties of human cancellous bone allografts. Biomaer 2004; 25(11):2105–10.

20. Grieb TA, Forng RY, Stafford RE, Lin J, Almeida J, Bogdansky S, Ronholdt C, Drohan WN, Burgess WH. Effective use of optimized, high-dose (50 kGy) gamma irradiation for pathogen inactivation of human bone allografts. Biomater 2005;26 (14):2033–42.

21. Akkus O, Belaney RM. Sterilization by gamma radiation impairs the tensile fatigue life of cortical bone by two orders of magnitude. J Orthop Res 2005; 23(5):1054–8.

22. Habelitz S, Marshall GW, Balooch M, Marshall SJ. Nanoindentation and storage of teeth. J Biomech 2002; 35(7):9958.

23. Marshall GW, Balooch M, Gallagher RR, Gansky SA, Marshall SJ. Mechanical properties of the dentinoenamel junction: AFM studies of nanohardness, elastic modulus, and fracture. J Biomed Mater Res 2001; 54(1):87–95.

24. Novais VR, Soares PBF, Guimaraes CM, Schliebe LRSO, Braga SSLB, Soares CJ. Effect of Gamma Radiation and Endodontic Treatment on Mechanical Properties of Human and Bovine Root Dentin. Brazil. Dent. J, 2016; 27(6): 670-674.

25. Adhani R, Widodo BI, Sukmana, and Suhartono E. Effect pH on Demineralization Dental Erosion. Inter. ChemEng. App, 2015; 6: 138-141.

26. Hicks J, Godoy FG, and Flaitz C. Biological factors in dental caries enamel structure and the caries process in the dynamic process of demineralization and remineralization (part 2). J Clinic Ped Dent 2004; 28: 119-124.

27. Craig RG and Peyton FA. The Microhardness of Enamel and dentin. J. Dent Res 1958, 661-668.

28. Hui L, Qi Z, Jiang G, Binghui Z, Xinlin Y, Dongsheng Y, Wei Z. Direct radiation-induced effects on dental hard tissue. Rad Oncology 2019 ; 14: 5

29. Jansma J, Buskes JA, Vissink A, Mehta DM, Gravenmade EJ. The effect of x-ray irradiation on the demineralization of bovine dental enamel. A constant composition study. Caries Res 1988; 22:199-203.

30. Amaechi BT, Higham SM, Edgar WM., Efficacy of sterilisation methods and their effect on enamel demineralisation. Caries Res 1998 32, 441- 446.

31. Amaechi BT, Higham SM, Edgar WM. Effect of sterilization methods on the structural integrity of artificial enamel caries for intra-oral cariogenicity tests. J Dent 1999; 27: 313-316.

32. El-Faramawy N, Ameen R, El-Haddad K, Ahmed Maghraby A, El-Zainy M. Effects of gamma radiation on hard dental tissues of albino rats using scanning electron microscope – Part 1. Radiant Effects & Defects in Solids 2011, 166(12): 927–934.

33. Brauer DS, Saeki K, Hilton JF, Marshall GW, Sally J. Marshall SJ. Effect of sterilization by gamma radiation on nano-mechanical properties of teeth. Dent Mater, 2008; 24: 1137–1140.

34. Abdalla R, Omar A; Eid K, detecting demineralization of enamel and cementum after gamma irradiation using radiographic densitometry. Rad Environ Biophysics 2018; 57(3):293-299 (ISSN: 1432-2099).

35. Jagadish K, Ashish A, Om PG , Sunil DS, Rekha , Muhammed PD, Manzoor, AP, Balwant S , Rao, BS, Riaz A Demineralization of tooth enamel following radiation therapy; an in vitro microstructure and microhardness analysis. DOI: 10.4103/jcrt.JCRT, 8-19, 2020, 612-618.

36. Kielbassa AM, Hellwig E, Meyer-Lueckel H. Effects of Irradiation on in situ Remineralization of Human and Bovine Enamel Demineralized in vitro, Caries Res 2006;40:130–135 (DOI: 10.1159/000091059).

37. Rodrigues, LKA., Cury, JA., dos SMN. The effect of gamma radiation on enamel hardness and its resistance to demineralization in vitro, J Oral Sci 2004;46 (4):215-220.