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The Effect of Physical Environment (Gamma Radiation) on Changes in Enamel Structure in Teeth

Elanda Fikri[1,2], Tantin Retno Dwijartini[3], Evan Puspitasari[3], Enny Chalimah[4], Siti Amaliyah[3], Yura Witsqa Firmansyah[5,6,*]

[1]Department of Environmental Sanitation, Bandung Health Polytechnic, Indonesia
[2]Center of Excellence on Utilization of Local Material for Health Improvement, Bandung Health Polytechnic, Indonesia
[3]Directorate of Research and Innovation Infrastructure Partnership Strengthening,
National Research and Innovation Agency, Indonesia
[4]Deputy for Research Facilitation and Innovation, National Research and Innovation Agency, Indonesia
[5]Department of Health Information and Medical Record, Vocational Faculty of Universitas Santo Borromeus, Indonesia
[6]Environmental Science Doctoral Program, Graduate School of Universitas Sebelas Maret, Indonesia

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Abstract This research is needed to know and get clear information about the effects of radiation on enamel, in the form of density, microstructure, morphology, and element content in the tooth enamel layer. This type of research is correlated with a before-and-after with control group experimental design that is in vitro the effect of ionizing radiation on tooth enamel samples (n = 18). The samples were irradiated with doses of 20Gy, 30Gy, 40Gy, 50Gy, and 70Gy. Then the density of tooth samples, the content of calcium and phosphorus constituents, as well as surface morphological analysis and pores on the teeth were observed. The results density in enamel samples has a significant average difference with the t-paired test in the pre-irradiated and post-irradiated groups of 30 Gy radiation dose (P = 0.035), and 40 Gy (P = 0.001). The content of calcium and phosphorus constituents in molar tooth enamel samples has a significant average difference with the One-way Anova test in the control and treatment groups (P = 0.001) and (P = 0.006). Reactions that occur in water molecules and organic substances produce more significant morphological changes in the interprismatic region. These microstructural changes result in a decrease in microhardness, and teeth become more brittle and cracks develop. Patients exposed to ionizing radiation must get specialized oral care to improve oral health.

Keywords Irradiation, Enamel, Density, Oral Care

1. Introduction

Radiation therapy is a commonly used form of treatment to treat malignancies in the head and neck region. However, the ionizing radiation used can also have significant side effects on oral health [1], [2]. The effects of ionizing radiation can cause tooth decay, either caused by factors external to the teeth with reduced saliva production or changes in the oral environment that can increase bacterial growth leading to dental caries, and enamel erosion. Internal factors can also occur due to damage to the enamel structure itself. So that damage can occur even though the patient has good dental hygiene [3], [4].

Radiation exposure can have several effects on the microstructure of tooth enamel. The following changes can occur in irradiated enamel. Radiation can cause hypomineralisation of tooth enamel, which refers to a reduction in the mineral content of enamel. This can result in weakened enamel making it more susceptible to necrosis and erosion of the enamel [5], [6]. Changes in the microstructure of enamel are due to disturbances in the structural units of enamel. This disruption can weaken the enamel and make it more susceptible to damage, such as fracture lines in the enamel [7]–[10]. Radiation can cause surface irregularities in tooth enamel resulting in roughness, deep pits, or roughness on the enamel surface [7], [11]. This can affect the aesthetics of the teeth and make them more susceptible to plaque accumulation and staining [7], [8], as well as an increase in tooth enamel porosity. This results in more microstrains or pores in the enamel structure. Increased porosity can make enamel more susceptible to acidity in the oral environment and bacterial invasion [8]. In primary teeth, radiation results in an increase in microhardness only on the enamel surface [9], in permanent teeth, other studies show a decrease in microhardness and an increase in mineral density in (post-irradiated samples) [7], [8].

Tooth decay causes patients with cancer of the head and neck region to have difficulty eating accompanied by a decrease in appetite [10], [12], so patients lack food and nutrient intake [13], [14], resulting in a drastic decrease in quality of life. In addition to the hard tissues of the teeth being damaged, radiation can cause damage to the periodontal tissue [15]. Periodontal tissue damage is accompanied by mobility and loss of bone structures supporting the tooth in >50% around root surfaces [16]–[18], requiring additional materials to stabilize the condition needs periodontal treatment, namely the use of splints [19], [20]. This study is needed to determine and obtain information about the effects of radiation on enamel, in the form of density, microstructure, morphology, and element content in the tooth enamel layer. As well as knowing whether these changes affect the attachment of splint materials to the enamel surface, especially in patients with moderate to severe periodontal disease [21].

2. Materials and Methods

Type and Design of Research

This type of research is correlated with a before-and-after with control group experimental design. The design was used to observe the effects of ionizing radiation on enamel microstructure in human teeth. Dental samples were irradiated by gamma-ray at the upgraded gamma cell-220 irradiator facility, with a dose rate of 3.2 kGy/hour at the Center of Radiation Processing Technology - National Research and Innovation Agency (BRIN). The study samples were incisive teeth (n=6), premolars (n=6) and molars (n=6). Dental samples were divided into 6 groups consisting of 1 control group and 5 gamma-irradiated dose treatment groups namely 20 Gy, 30 Gy, 40Gy, 50 Gy, and 70 Gy.

Sterilisation and Preparation of Dental Samples

The extracted tooth was soaked in H_2O_2 solution for 1 hour, then rinsed and cleaned using aquabides, then dried. The tooth was cut in the cervical section and the crown was cut transversely from the occlusal to the cervical direction, only the crown was used. Then the dentin part was cleaned and the enamel part was taken using a diamond bur. The tooth sample was rinsed again using running water to remove debris.

Clinical Photographs, Radiographs and Density Determination

Clinical and radiography photos were taken of tooth samples before and after irradiation. The radiograph was taken by determining the point and marked with a box on all elements of the same tooth to take the area to be irradiated. The distance between the cone and the sample in taking dental radiographs is ± 2 cm using a digital dental X-ray brand MyRay RXDC incisor keV. Density determination was carried out through radiographic images of samples analyzed for grayscale values and calculated with image G. Statistical analysis was carried out on all treatment groups before and after irradiation using a paired sample t-test with a 95% confidence value.

SEM Overview

Figure 1. Tooth Retrieval Results via SEM

Caries-free tooth enamel samples were cut into 5 x 5 mm pieces with a thickness of 1 mm using a diamond bur. Rinse using running water to remove dental debris attached to the surface of the enamel sample. The enamel samples (**Figure 1**) were analyzed for surface morphology, pores, and measuring mineral composition of the enamel microstructure using SEM (Scanning Electron Microscopy) at the Nuclear Advanced Materials Laboratory - BRIN. Analyses were carried out on the control group, and 5 gamma-irradiated dose treatment groups namely 20Gy, 30Gy, 40Gy, 50Gy, and 70Gy. The composition of enamel constituent elements of each sample was also observed and measured three times from the specified point. Enamel composition data (calcium and phosphorus) were analysed using one-way ANOVA test with 95% confidence value. Meanwhile, the density value of tooth enamel was analysed using the T-paired test. Ethical review of the study was conducted at the Indonesian National Nuclear Energy Agency (BATAN).

Results of Tooth Enamel Density Radiograph Test

The results of radiograph analysis of tooth enamel density with five variations of gamma-ray dose are presented in **Table 1** and **Figure 2**. **Figure 3** presents gamma radiation exposure with a variation of 30 Gy to molar tooth enamel. Meanwhile, **Figure 4** presents the exposure to gamma radiation with a variation of 40 Gy on molar tooth enamel.

3. Result and Discussion

Table 1. Mean test of Tooth Enamel Density Values before and after Irradiation

Doses	Pre-Irradiation Mean ± SD	Post-Irradiation Mean ± SD	Correlation value	CI: 95%
20 Gy	51752.911 ± 8461.511	52894.403 ± 4413.973	0.682	0.62
30 Gy	50657.587 ± 8043.946	48327.296 ± 7370.565	0.743	0.035
40 Gy	52430.914 ± 5480.039	51451.829 ± 6169.761	0.943	0.001
50 Gy	55914.154 ± 3437.974	52038.311 ± 5905.735	0.378	0.356
70 Gy	48813.811 ± 7343.894	46750.835 ± 9955.781	-0.128	0.763

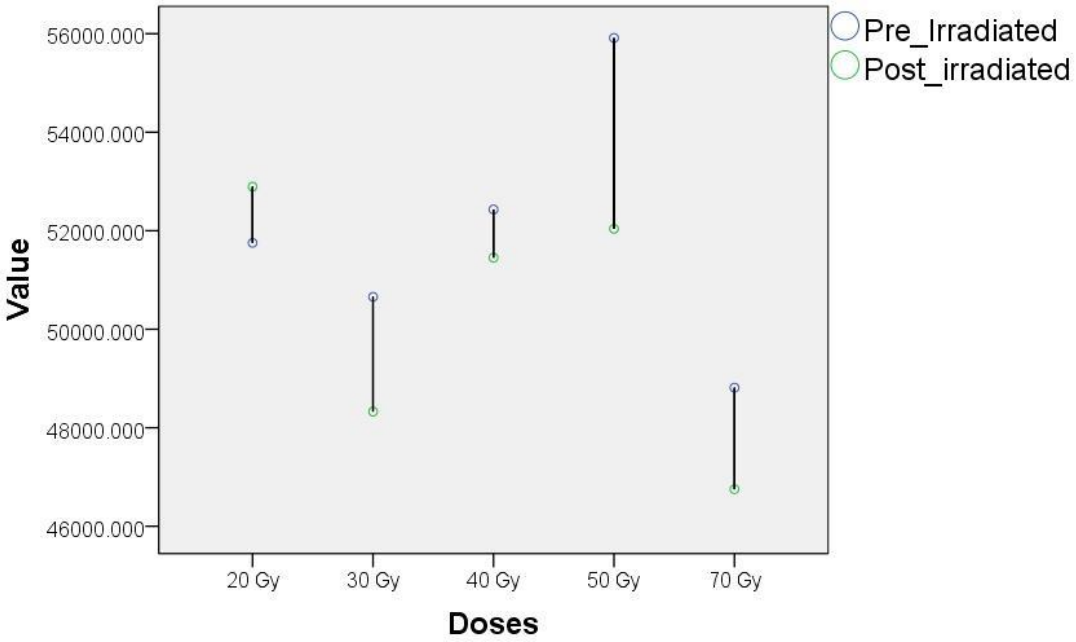

Figure 2. Graph of Mean Density Value of Tooth enamel before and after irradiation

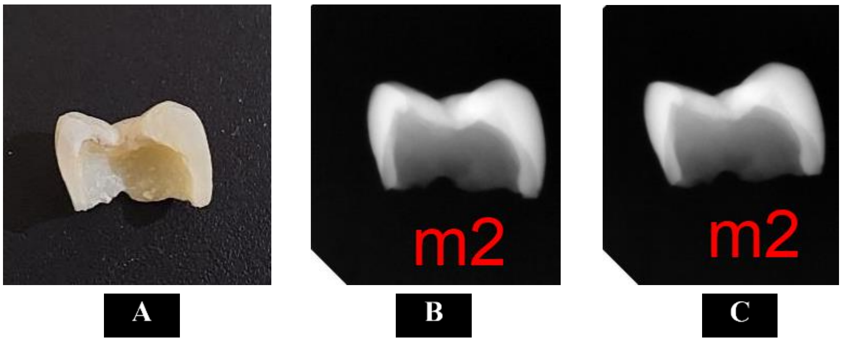

Figure 3. Sample with 30 Gy irradiation (A) Clinical photograph, radiograph image of pre-irradiated Tooth Enamel of Molar and (C) post-irradiated

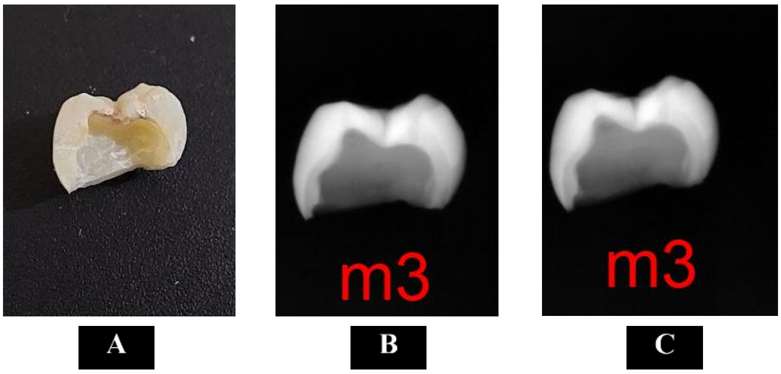

Figure 4. Sample with 40 Gy Irradiation (A) Clinical photograph, Radiograph Image of pre-irradiated Tooth Enamel of Molar and (C) post-irradiated

Results of Calcium and Phosphor Composition Analyses on Premolar and Molar Tooth Enamel Samples

The results of the analysis of calcium and phosphate composition in premolar tooth enamel are presented in **Figure 5** and **Table 2**, while the results for molar tooth enamel are presented in **Figure 6** and **Table 3**. The results of morphological analysis of the surface and pores of tooth enamel are presented in **Figure 6**. The following analysis results were obtained.

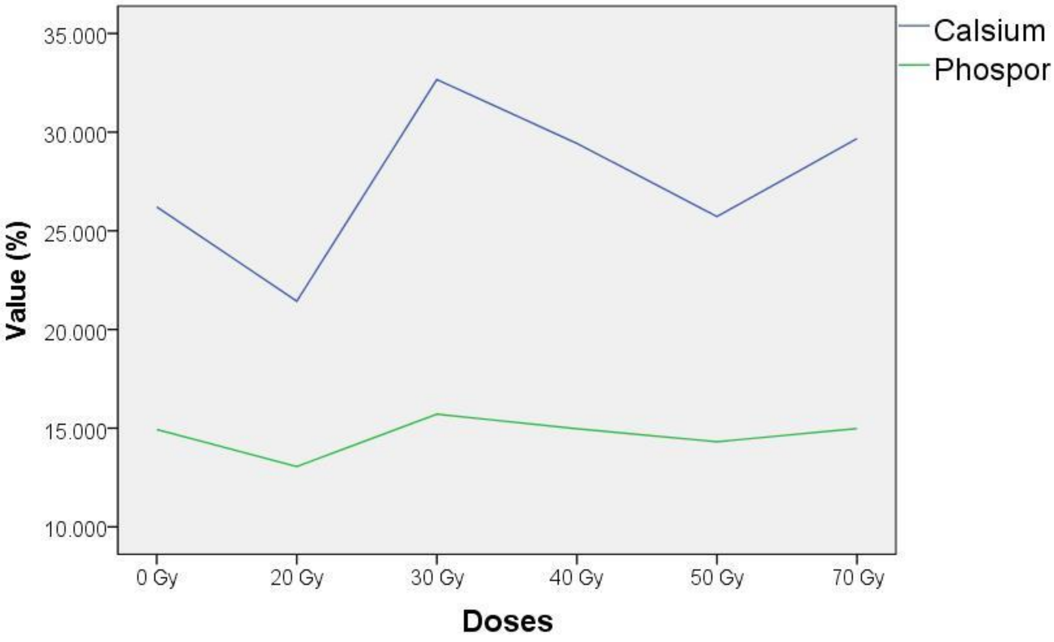

Figure 5. Results of Analysis of Calcium and Phosphorus Composition in percent (%) of Premolar Tooth Enamel Samples

Table 2. One-way ANOVA of Calcium (Ca) and Phosphorus (P) composition in per cent (%) of Premolar Tooth Enamel Samples

Doses	Content of Ca (%) Mean ± SD	CI: 95%	Content of P (%) Mean ± SD	CI: 95%
0	26.213 ± 3.806	P: 0.001	14.93 ± 1.650	P = 0.06
20 Gy	21.433 ± 0.291		13.057 ± 0.382	
30 Gy	32.667 ± 0.441		15.71 ± 0.387	
40 Gy	29.43 ± 0.963		14.97 ± 0.533	
50 Gy	25.723 ± 0.297		14.31 ± 0.168	
70 Gy	29.67 ± 4.135		14.98 ± 1.299	

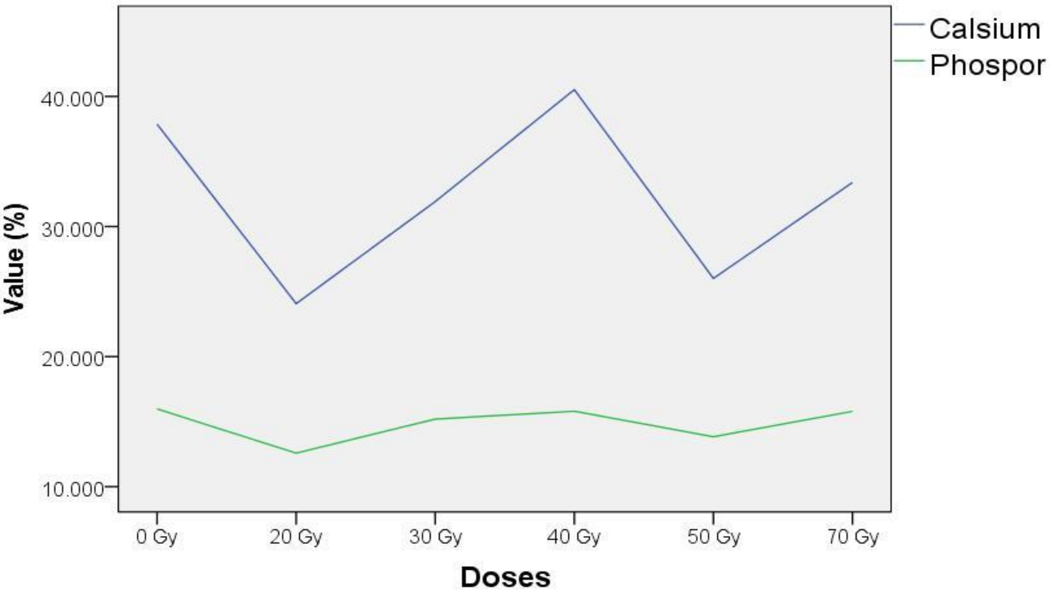

Figure 6. Results of Analysis of Calcium and Phosphorus Composition Elements in percent (%) of Molar Tooth Enamel Samples

Table 3. One-way ANOVA of Calcium (Ca) and Phosphorus (P) constituents in per cent (%) of Molar Tooth Enamel Samples

Doses	Content of Ca (%) Mean ± SD	CI: 95%	Content of P (%) Mean ± SD	CI: 95%
0	37.87 ± 4.126	P: 0.001	15.977 ± 0.906	P = 0.006
20 Gy	24.073 ± 1.203		12.577 ± 1.172	
30 Gy	31.917 ± 3.018		15.197 ± 0.791	
40 Gy	40.523 ± 0.955		15.800 ± 0.431	
50 Gy	26.013 ± 0.889		13.837 ± 0.875	
70 Gy	33.3867 ± 2.981		15.797 ± 1.401	

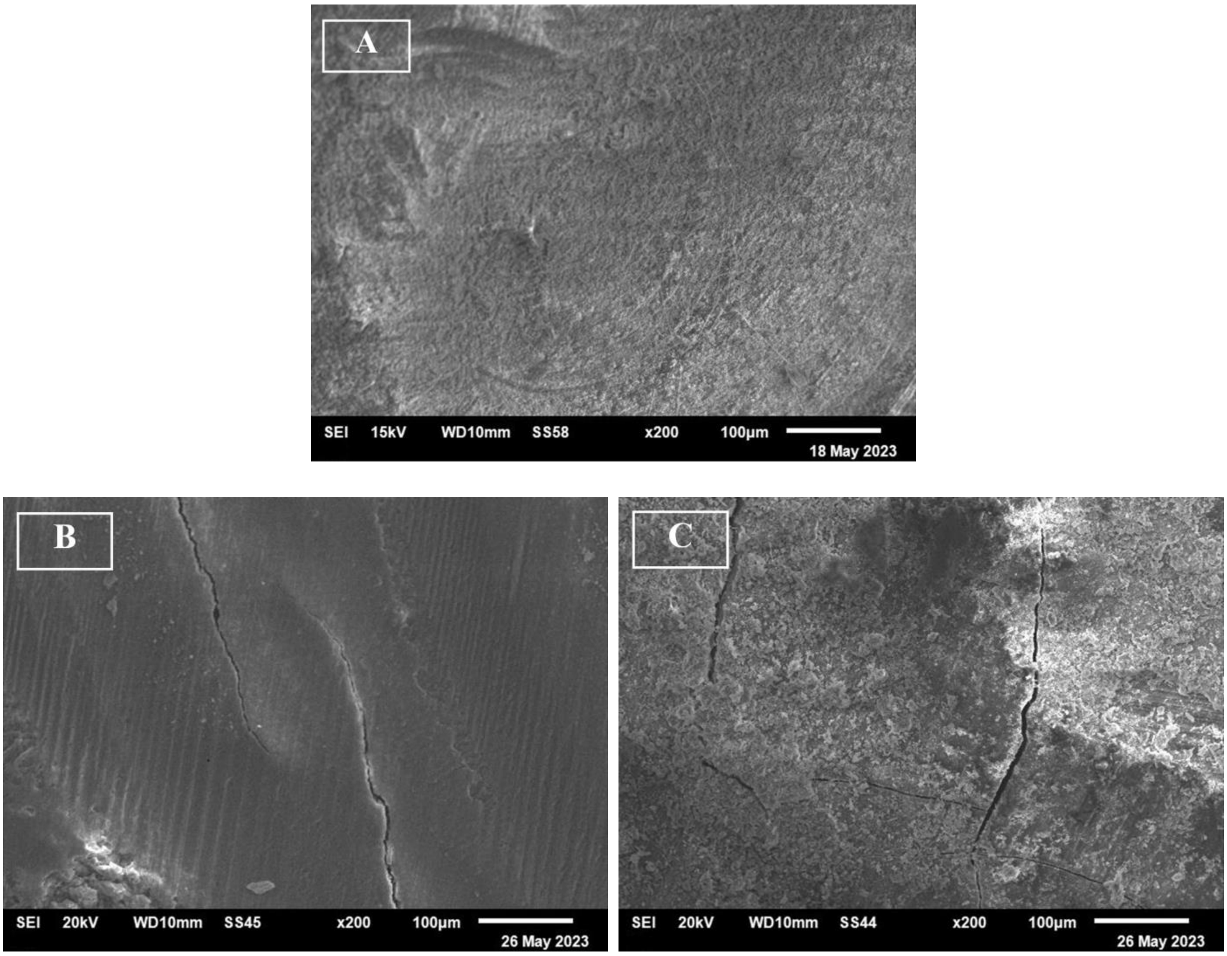

Figure 7. SEM micrographs of permanent tooth enamel samples at 200x magnification (A) Non-irradiated enamel samples on the surface no crack/fracture lines found (B) 40 Gy irradiated enamel samples on the surface cracks/fracture lines found (C) 70 Gy irradiated enamel samples on the surface cracks/fracture lines found.

Results of Calcium and Phosphor Composition Analyses on Premolar and Molar Tooth Enamel Samples

The results of the analysis of calcium and phosphate composition in premolar tooth enamel are presented in **Figure 5** and **Table 2**, while the results for molar tooth enamel are presented in **Figure 6** and **Table 3**. The following analysis results were obtained

Enamel density measurements (**Table 1**) were performed pre and post - irradiated on the same samples in each group. The enamel density values decreased after 30Gy (P = 0.035) and 40Gy (P = 0.001) cumulative dose irradiation when compared to pre-irradiated enamel. While **Figure 2** visualizes a decrease in the average density value of enamel samples pre and post - irradiated, it does not have a statistical correlation with a 95% confidence index. There was no linear relationship between radiation dose and mean density of enamel (P = 0.283).

The average calcium composition (in percent) of enamel microstructure in premolar tooth samples (**Table 2** and **Figure 5**), showed a significant difference between the control group and all treatment groups (P = 0.001). The relationship between the amount of dose received and the decrease in calcium composition did not show linearity (P = 0.190) and phosphorus (P = 0.570). The Phosphor composition (in percent) of enamel in premolar tooth samples, showed no significant difference between the control group and the treatment group (P = 0.06). The highest value of calcium and phosphorus composition is contained in premolar tooth enamel samples irradiated by 30 Gy and the lowest at 20 Gy.

The average Calcium composition (in percent) of enamel microstructure in molar tooth samples (**Table 3** and **Figure 6**), showed a significant difference between the non irradiated group and all treatment groups (P = 0.001). The relationship between the amount of dose received and the decrease in calcium composition did not show linearity (P = 0.805) and phosphorus (P = 0.651). The Phosphor composition (in percent) of enamel microstructure in molar tooth samples, showed a significant difference between the non-irradiated group and the treatment group (P = 0.006). The highest value of calcium and phosphorus composition in premolar tooth enamel samples has been irradiated by 40 Gy and the lowest at a dose of 20 Gy.

Our study describes the density, microstructure, morphology, and mineral composition of gamma-irradiated tooth enamel samples. The tooth samples used were non-vital teeth extracted due to severe periodontal disease that affected the alveolar bone loss on >50% bone structures supporting the tooth around root surfaces with Grade 3 of mobility (Carranza's classified) [22], [23], caused tooth loss. Another reference states that there is no difference in the structure and content of enamel in vital and non-vital teeth, the difference is the low water content in non-vital teeth [24]. Enamel is avascular, meaning it does not contain blood vessels and nerves [25]. In the case of high-dose radiation exposure, saliva from the parotid glands is reduced to 20% while the other salivary glands are reduced to 50% after 2 weeks of therapy [26], [27]. The salivary glands are permanently damaged and no salivary remineralization occurs [28]. So in this case, non-vital teeth can be used as in vitro samples.

The results of the analysis of pre and post-irradiated enamel sample density measurements showed no significant difference in the paired t-test in the 20; 50; and 70 Gy groups. And significant in the 30 Gy radiation dose group (P = 0.035), and 40 Gy (P = 0.001). The constituents of enamel consist of Ca ions, PO_4 ions, and OH ions [29], [30]. The ionization effect caused by gamma radiation can cause OH- withdrawal, causing the OH aisle to become empty and there will be a decrease in the dimensions of the unit cell [31], [32], increasing microstrain. The increase in strain accumulatively on the entire enamel can cause internal pulling forces [33]. As a result of the buildup of energy, the position of the atoms that make up the apatite crystal structure in the irradiated tooth enamel sample changes. Changes in the strength of interatomic bonds after irradiation can also result in increased tensile stress or excessive elongation, brittleness or embrittlement, and microstrain enlargement [14], [33]. The enlargement and increase of microstrain results in a low-density x-ray image. Harun A. Gunawan in his report mentioned that specimens that had been irradiated by 40 Gy, not only showed a decrease in apatite crystallinity but also the addition of β-TCP (beta-tricalcium phosphate) compounds. The addition of β-TCP compounds will be seen in the X-ray image with a denser density [33]. β-TCP compounds have higher acid solubility properties than apatite (Elliot in Harun A). A retrospective cohort study showed that radiation dose exposure was associated with calculus composition (R=0.503 p=0.025); dental calculus and gingivitis (R=0.555 p=0.001) [34]. This results in a higher risk of caries than patients not exposed to radiation.

The mean ± SD values of calcium and phosphorus composition in premolar tooth samples are presented in **Table 2** and molar tooth samples in **Table 3**. There were statistically significant differences among the groups according to the elemental analyses. In molar teeth, the results of the observation of Calcium and Phosphorus composition showed statistically significant mean differences between the control group and all dose groups. In premolar teeth, significant differences only occurred in the content of calcium elements while the content of phosphorus did not show significant differences between the control and treatment groups. The analysis results showed significant differences in calcium content at every 10 Gy dose increase. The increase and decrease of calcium and phosphorus composition were not linear and irregular at each dose. Although the composition of enamel is essentially inorganic, the initial damage due to irradiation occurs in the organic part of the enamel, namely in the interprismatic space, through the oxidation of water molecules to hydrogen peroxide and hydrogen free radicals that denature organic components [24], [35], [36]. As a result, the mechanical properties and integrity of enamel are affected. This study shows that irradiation also causes changes in the prismatic structure of enamel, the clinically observed effects of radiation are due to changes in organic and inorganic compounds in enamel [34]. The results of the analysis of the increase and decrease of calcium and phosphorus elements were not linear and irregular at each treatment dose size, confirmed by Jagadish Kudkuli et al who stated that the simultaneous loss and increase of mineral density were due to the variation of different hydroxyapatite crystal matrices [6], [37], [38].

The results of SEM observations showed (**Figure 7**), that no fracture lines were seen in the control group, but fracture lines appeared on the surface of the enamel samples irradiated with doses of 40, 50, 60, and 70 Gy. Morphological changes in tooth enamel, indicate microstructural changes that occur in enamel that has been irradiated. Chemical properties of enamel consist of organic substances (30%), inorganic (58%) and water (12%). 58% of organic substances contained in enamel consist of enamelin protein [39] – [41]. While the main inorganic substance of tooth enamel is hydroxyapatite (HA) crystals. Enamel binds very tightly to the surface of apatite crystals as a fine, thin reticulum layer surrounding the uncalcified HA crystals that fill the space between prisms [25]. It has been reported that radiation does not exert a direct effect on the inorganic structure of human teeth, and the dental changes observed in patients with head and neck cancer after radiation therapy are due to changes in the enamel organic matrix [24], [35], [36]. Radiation results in poor adhesion between apatite crystals and collagen fibers [7], [24]. In addition, radiation also acts on the water content of enamel. Radiation breaks down water molecules in the tooth structure, producing free radical atoms that can affect the proteolysis of collagen and noncollagen proteins [7], [24]. The reactions that occur in water molecules and organic substances result in more significant morphological changes in the interprismatic region. These microstructural changes result in a decrease in microhardness, teeth become more brittle and cracks occur. It has been reported that irradiated teeth have a significant decrease in microhardness at all enamel and dentin sites compared to non-irradiated controls [7], [14], [24], [42]. Reinforced by the report of Ping Qing et al showing X-RD results, irradiation can reduce the crystalline content of enamel and increase the size of crystals in enamel so that it can cause cracks [14].

The manifestation of changes in the physical and chemical properties of the constituent structures of tooth enamel, due to radiation exposure, is a higher risk of caries [28], [33], crown fracture [14], [33] and can affect the attachment of restoration bonding materials [43]. Bonding materials are used for dental fillings and splinting for periodontal treatment.

4. Conclusions

Density in enamel samples has a significant average difference with the t-paired test in the pre-irradiated and post-irradiated groups of 30 Gy radiation dose (P = 0.035), and 40 Gy (P = 0.001). The content of calcium and phosphorus constituents in molar tooth enamel samples has a significant average difference with the One-way Anova test in the control and treatment groups (P = 0.001) and (P = 0.006). In premolar tooth enamel samples, significant mean differences with the One-way Anova test only occurred in calcium content but not in phosphorus. In the calcium element, there is an average difference every 10 Gy dose increase. Observation of surface morphology using SEM, no fracture lines were found in the non-irradiated enamel samples. Fracture/crack lines were found on the surface of the 40Gy, 50Gy, 60Gy, and 70Gy radiation dose group enamel samples. Reactions that occur in water molecules and organic substances produce more significant morphological changes in the interprismatic region. These microstructural changes result in a decrease in microhardness, teeth become more brittle and cracks develop. Furthermore, it is necessary to research effective dental care treatment for head and neck cancer patients who undergo radiation therapy and it is also necessary to do research on effect of different doses of radiation on morphological, mechanical and chemical properties of tooth samples for oral health safety under radiotherapy treatment of head and neck. Research needs to be done on composite resin attachment to teeth exposed to radiation. It is necessary to research the effects of ionizing radiation on tooth enamel in communities in high natural radiation areas, and occupational radiation.

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