Effect of Radiotherapy on Calcium Loss on Enamel Surface

Radyoterapinin Mine Yüzeyindeki Kalsiyum Kayıplarına Etkisi

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ABSTRACT Objective: Radiation induced caries is a major complication in the treatment of patients suffering from head and neck cancer. The objective of this study was to evaluate the correlation between calcium loss of human enamel and radiation for different given doses. **Material and Methods:** Thirty three premolars extracted for orthodontic purposes were sectioned buccalingually and longitudinally, so that four specimens were obtained from each tooth. The specimens were randomly assigned to one of the four groups. Three parts of prepared teeth were exposed to radiation for different levels of radiation: 20 Gray (2 Gy/day, 5 days/week), 50 Gy, 70 Gy. The specimens in the fourth group were used as a control group. The specimens were treated with an artificial caries solution (pH 4) for 16 days; the solution was replaced on days 4, 8, 12 and 16. Calcium concentrations were determined by an atomic absorption spectrophotometer. **Results:** At the end of day 16, calcium ions released per square milimeter were calculated cumulatively as follows: 20 Gy group: 16.63±1.84 μg mL-1; 50 Gy group: 18.13±1.72 μg mL-1; 70 Gy group: 22.81±2.43 μg mL-1 and control group: 16.24±1.61 μg mL-1. The loss of calcium was statistically significant when the 20 Gy irridation group compared to the 50 Gy and the 70 Gy irridation groups ($p<0.05$). It is observed that calcium loss from the enamel surface is related to radiation dose. The highest calcium loss was observed in 70 Gy irradiation group. **Conclusion:** Calcium loss in enamel tissue becomes significant as long as the received radiation dose increases to a certain level.

Key Words: Calcium; dental enamel solubility; radiation oncology

ÖZET Amaç: Baş ve boyun kanseri olan hastaların tedavileri sırasında uygulanan radyasyonun çürüğe neden olması önemli bir sorundur. Bu çalışmanın amacı, farklı radyasyon dozlarının, minede meydana gelen kalsiyum kayıplarıyla olan ilişkisinin araştırılmasıdır. **Gereç ve Yöntemler:** Ortodontik amaçla çekilmiş 33 adet premolar diş, bukkolingual ve longitudinal olarak bölündü; böylece her bir dişten dört örnek elde edildi. Daha sonra örnekler rastgele dört gruba ayrıldı. Bölünmüş olan dişlerin üç parçasına farklı radyasyon dozları uygulandı: 20 Gray (2 Gy/gün, 5 gün/hafta), 50 Gy, 70 Gy. Dördüncü gruptaki örnekler kontrol grubu olarak ayrıldı. Örnekler 16 gün boyunca yapay çürük solüsyonu (pH 4) içerisinde bekletildi ve 4., 8., 12. ve 16. günlerde solüsyon değiştirildi. Kalsiyum konsantrasyonları atomik absorpsiyon spektrofotometresi ile belirlendi. **Bulgular:** 16. Günün sonunda, milimetrekare başına düşen kümülatif kalsiyum miktarları: 20 Gy grubunda (Grup 1): 16,63±1,84 μg mL-1; 50 Gy grubunda (Grup 2): 18,13±1,72 μg mL-1; 70 Gy grubunda (Grup 3): 22,81±2,43 μg mL-1ave kontrol grubunda: 16,24±1,61 μg mL-1olarak belirlendi. 50 Gy ve 70 Gy radyasyon grupları, 20 Gy grubu ile karşılaştırıldığında, kalsiyum kaybının istatistiksel olarak anlamlı olduğu gözlendi ($p<0,05$). Mineden kalsiyum kaybının radyasyon dozu ile bağlantılı olduğu görüldü. En yüksek kalsiyum kaybının 70 Gy radyasyon dozunda olduğu belirlendi. **Sonuç:** Radyoterapi sırasında alınan radyasyonun dozu arttıkça, mine dokusundaki kalsiyum kaybı önemli olmaktadır.

Anahtar Kelimeler: Kalsiyum; diş minesi çözünürlüğü; radyasyon onkolojisi

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Radiotherapy plays an important role in the management of head and neck cancer where it usually results in complex oral complications affecting the salivary glands, oral mucosa, bone, masticatory musculature and dentition in the head and neck region.[1,2] Surprisingly little is known of the direct effects of ionising radiation on the teeh.[3]

Radiation caries, is a rapidly developing and highly destructive form of teeth decay.[4] Hyposalivation which is induced by irradiation and dietary changes with concominant alteration of the oral flora is considered to be the most important etiological factor.[5-7] Dental caries may become evident as early as three months following the initiation of radiotherapy. In severe cases, a previously healty dentition can be completely lost within a year.[1]

Radiation caries frequently occurs on buccal and oral smooth surfaces as well as on occlusal or incisal edges of the teeth. However, these areas generally are considered as relatively caries-resistant in nonirradiated patiens suggesting the possibility of another cause as well as reduced salivary flow and altered microbial composition.[7-9]

Physical and chemical changes of enamel after radiotherapy as a direct result of the irradiation have been documented. Radiation damage and crystalline restructing of the mineralized tissues of teeth may account for the post radiation changes of the dentition. However this explanation has generated controversy in the dental literature.[7,10-12]

The techniques involved in administering radiotherapy are concerned with delivering the correct dose to a specific portion of the patient. Most orofacial complication are dose-dependent phenomena. The situation in which the patient is most likely to experience severe side effects occurs when high doses (> 4.5 Gy) are administered in larger fields including both sides of the mouth, jaws and associated salivary glands.[13]

The objective of this study was to evaluate the correlation between calcium loss of human enamel and radiation for different given doses.

MATERIAL AND METHODS

Thirty three premolars extracted for orthodontic purposes were rinsed in tap water and cleaned of plaque and debris with a dental handpiece and a brush. The buccal, lingual and occlusal surfaces were checked under a stereomicroscope, and teeth with enamel defects or cracks were rejected. Selected 33 teeth were stored in 0.9% saline solution for 1 week and then rinsed in distilled water. Each tooth was sectioned buccalingually or buccopalatinally into two parts with a diamond disk. These halves were then sectioned longitudinally into two parts. So that four specimens were obtained from each tooth. These specimens were randomly assigned to one of the four groups, on the condition that the four specimens of each tooth were divided equally among the groups (one specimen per tooth per group).

Three parts of prepared enamel surface of the teeth were irradiated using a linear accelerator (Elekta, Crawley-England) with different level of radiation. The first group was irradiated fractionally up to 20 Gray (Gy) with a total of 10 doses (2 Gy/day, 5 days/week), the second groupwas irradiated 50 Gy with a total of 25 doses (2 Gy/day, 5 days/week) and the third group was irradiated 70 Gy with a total of 35 doses (2 Gy/day, 5 days/week). For the homogenity of the irradiation specimens were stored in daily renewed saline solution (0.9% NaCl; 2 cm in height). The field size was 20x20 cm^2 and radiation was performed at room temperature. The specimens in the fourth group were used as a control group and were kept in artificial saliva during the test period (Table 1).[14]

After the irradiation, the specimen were rinsed with distilled water and dried. Enamel surface of the teeth were then covered with wax, except for around window area (6.83 mm^2). Care was taken to keep the experimental enamel sides free from wax. Acetic acid buffered with 0.34 M sodium acetate (pH=4) was used as a demineralization solution. Salt of calcium monohydrate [$Ca(H_2PO_4)2H_2O$)] was dissolved to obtain 10 mmol L^{-1} Ca^{2+} and 20 mmol L^{-1} PO_4^{3-} in the solution. Each specimen was treated with 50 mL of solution in the

polyethylene test tubes. The specimens were treated with buffer four times for four days. At the end of the fourth day each specimen was taken out from each test tube and placed in new tubes which contain fresh buffer solution (4^{th}, 8^{th}, 12^{th} and 16^{th} days).[15,16]

The previous solutions were kept in their tubes to be tested afterwards for their Ca^{2+} loss with atomic absorption spectrophotometer. For calcium analysis 0.1 mL of each demineralization solution was diluted with 4.9 mL of distilled water. Because the calcium concentrations of each buffer solution in each test tube were too high to be measured. To prevent the interaction of magnesium and phosphate ions, 50 000 mg of lantana chlorine ($LaCl_2$) was added to each test tube to end up with 10% of $LaCl_2$ in each buffer solution. The same procedure was applied to blank and standard solutions of calcium. The amount of calcium concentration of the samples was detected with an atomic absorption spectrophotometer, *Varian Spectra-10 plus AA* (wavelength: 422.7 nm; slit: 0.5 nm) (Varian, Victoria, Australia). The calcium quantities released to the buffer solution were measured on 4^{th}, 8^{th}, 12^{th} and 16^{th} days and were compared using repeated measured analysis of variance (ANOVA) and Bonferroni as Post Hoc Tests.

TABLE 1: The distrubition of tooth according to study group (n= 33).

Groups	Irradiation	Dose (2 Gy/day, % days/week)
1	20 Gy	10 Dose
2	50 Gy	25 Dose
3	70 Gy	35 Dose
4	N/A	N/A

N/A: not applicaple.

RESULTS

The amount of released calcium from specimen to buffer solution after treatment with radiotherapy (Control, 20 Gy, 50 Gy, 70 Gy irradiation) was evaluated separately and cumulatively in every 4 days (4^{th}, 8^{th}, 12^{th} and 16^{th} days), and at the end of the 16^{th} day, 16.24±1.61 μg mL^{-1}, 16.63±1.84 μ μg mL^{-1}g/mL, 18.13±1.72 μg mL^{-1}, and 22.81±2.43 μg mL^{-1}were obtained in total, respectively (Table 2, 3).

A statistically significant difference was observed among the groups of the 4^{th}, 8^{th}, 12^{th} and 16^{th} and in total ($p<0.05$) when the loss of calcium in each of the test groups was compared with that of the control group using the "Repeated Measures ANOVA". In addition, Bonferroni test was used as

TABLE 2: Release of Ca2+ from the specimens after treatment with irradiation (in μg mL-1).

Days	1-4 Mean±Std	5-8 Mean±Std	9-12 Mean±Std	13-16 Mean±Std	Total Mean±Std
20 Gy	3.25±0.83	3.86±0.80	4.40±1.12	5.12±1.01	16.63±1.84
50 Gy	3.90±0.87	4.39±0.94	4.39±0.68	5.45±0.98	18.13±1.72
70 Gy	4.89±1.75	4.54±0.94	6.23±1.78	7.15±1.54	22.81±2.43
Control	3.30±0.66	3.64±0.51	4.13±0.74	5.15±0.82	16.24±1.61

n: 33 for each group.

TABLE 3: The cumulative results of calcium release from the specimens on days 4, 8, 12, and 16 (μg mL-1)(n=33 each group).

Days	1-4 Mean±Std	5-8 Mean±Std	9-12 Mean±Std	13-16 Mean ± Std
20 Gy	3.25±0.83	7.11±1.81	11.51±1.68	16.63±1.84
50 Gy	3.90±0.87	8.29±1.47	12.68±1.59	18.13±1.72
70 Gy	4.89±1.75	9.42±2.19	15.66±2.23	22.81±2.43
Control	3.30±0.66	6.95±0.76	11.08±1.19	16.24±1.61

n: 33 for each group.

Post-Hoc test to identify the significance between the groups (Table 4).

When the groups were analyzed separately, the amount of calcium released from specimens to buffer solution from control and the 20 Gy irridiation group was statistically insignificant ($p>0.05$). However, the calcium loss was significant between control and groups of 50 Gy and 70 Gy irradiation ($p<0.05$) (Figure 1). The statistical analysis of the groups was shown in Table 4. The loss of calcium was also statistically significant both when 20 Gy irradiation group was compared with 50 Gy and 70 Gy irradiation groups and when 50 Gy was compared with 70 Gy irradiation group ($p<0.05$).

DISCUSSION

It has always been a matter of debate whether radiation caries is due to a direct or indirect effect of irradiation on teeth, or to both. Several investigators have reported that the development of radiation caries was not dependent on the presence of teeth in the field of irradiation, but that the determining factor was whether the main salivary glands were within the radiation field.[5,17] Notwithstanding the study by Grotz et al., which showed that irradiation also resulted in dentinal changes in vital teeth, the current opinion still is that radiation caries is mainly due to salivary gland damage resulting in hyposalivation.[18-21] Thus, collectively, hyposalivation-related alterations in microbial, chemical, immunologic, and dietary parameters of cariogenicity contribute to an enormous increase in the caries challenge in irradiated patients.[22,23] This enormous caries challenge becomes even more obvious since both loss of enamel and severe destruction at the dentin-enamel junction can be observed within a few weeks of exposure of enamel slabs in the oral cavities of patients with radiation induced hyposalivation.[24] The changes observed were similar to the changes occurring in natural hyposalivation-releated dental caries.[8] So both the coronal enamel and the cervical area, where cementum or dentin is directly exposed to the oral environment, are areas at risk in dry mouth patients.

This study has been established on the thesis whether radiotherapy has had any effect on calcium loss on enamel surface. In some studies dealing with the effects of radiotherapy on tooth caries it has been suggested that radiotherapy might lead to the formation of caries since it causes xerostomia.[2,3] However radiotherapy oriented causes of the destruction on the enamel tissues of the patients having radiotherapy have not yet been documented. In some studies it has been suggested that changes in the junction of enamel dentin has been observed and that resistance against acid attack has decreased.[18,25,26]

In the present study, the specimens were kept wet during irradiation. This should be emphasized, since it is known that the apatitic crystals of dental hard tissue have incorparated some sodium, carbonate and magnesium by entrapment during their formation. In case of irradiation, these point defects could be mobilized from the surface layer of the crystals, thereby removing the entrapped ions. Wet conditions, on the other hand, have been supposed to stabilize the surface layers of the apatite phase

TABLE 4: Statistical evaluation of the test groups at the end of days 4, 8,12, and 16.

Irradiation	Days 1-4	Days 5-8	Days 9-12	Days 13-16	Total
20Gyx50Gy	*	-	-	-	*
20Gyx70Gy	*	*	*	*	*
20GyxControl	-	-	-	-	-
50Gyx70Gy	-	-	*	*	*
50GyxControl	*	*	-	-	*
70GyxControl	*	*	*	*	*

* Statistically significant differences between the groups ($p<0.05$).

- No statistically significant difference ($p>0.05$).

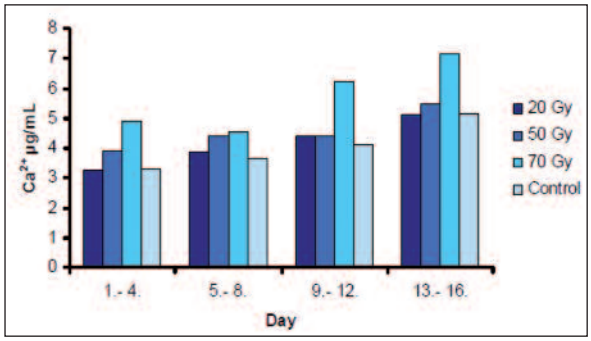

FIGURE 1: Release of calcium from the specimens to the buffer solution after treatment with radiotherapy (measured cumulatively).
(see for colored form http ://dishekimligi.turkiyeklinikleri.com/)

of dental hard tissue, thereby reducing the dissolution rate into slightly acidic environment.[27]

Several papers reporting on physical and chemical changes in irradiated enamel or octacalcium phosphate have been published in the past. The effects of these alterations have recently been discussed.[11,12,28]

The radiation dose needed for the treatment of cancer is based on location and type of malignancy, and whether or not radiotherapy will be used solely or in combination with other modalities. Most patients with head and neck carcinomas, treated with a curative intent, receive a dose between 50 and 70 Gy. This dose is usually given over a five to seven week period, once a day, five days a week, 2 Gy per fraction.[29]

In this study, radiation at 20 Gy, 50 Gy and 70 Gy has been applied to the samples. According to the results of this study, more calcium loss was observed on the enamel tissue as the radiation level increased. In this sense, the results of the study support the viewpoint that orofacial complications are a phenomenan increasing in relation to the dose. No difference has been determined between the 20 Gy radiotherapy applied group and the control group in none of the measurements on days 4, 8, 16 and those of the cumulative sums. This result has shown that the 20 Gy radiotherapy dose on the enamel tissue did not create a strong effect to help calcium to dissolve on enamel tissue. The samples, 50 Gy radiation applied, even a reasonable change is observed on the enamel tissue on 4th, 8th days, there is not considerable change on 12th and 16th. While radiation at this level causes calcium loss on the enamel tissue on the initial days, this loss has not occured significantly on the following days. This observation makes us think that the balance which seemed to have resulted against the favor of enamel at the beginning, has in time resulted for the benefit of enamel. However, in spite of the above mentioned benefit, when cumulative sum is concerned, the calcium loss on the samples to which 50 Gy radiation was applied has been determined to be significant enough to lead to the destruction of enamel tissue when compared with the amount of calcium in control group. This result leads us to think that the resistance of enamel has not yet completely been damaged at the above mentioned radiotherapy dose. However, when the 70 Gy radiation group was compared with the control group in terms of calcium loss measurements on 4th, 8th, 12th and 16th days and the cumulative sum; the fact that not any satisfactory result has been obtained in favour of enamel makes us think that the inorganic structure of the enamel tissue has suffered a serious detoriation at this radiation dose.

Jervoe demonstrated changes in the crystalline structure of enamel with X-Ray diffraction, but he irradiated at an extremely high single experimental dose of 10 Gy.[12] He concluded that the effect of X-Ray irradiation on enamel might not be exclusively a radiation-induced effect in crystal structure but that it might also be possible that the effect in the crystal is the result of a chemical reaction caused by radiolysis.

Several *in vitro* demineralization studies on irradiation effects either failed to show any differences between irradiated and nonirradiated human enamel, reported a reduced demineralization of irradiated specimens or revealed an increased dissolution of irradiated enamel.[30-32] On the other hand, it was observed an in situ demineralization model that irradiated human enamel is as caries susceptible as non irradiated. It was concluded that possible irradiation effects are not likely to be responsible for initial demineralization under clinical conditions.[7] An in vitro demineralization study that compared irradiated and nonirradiated bovine

specimens revealed that irradiated enamel had a reduced microhardness.[33]

Several studies could show that the microhardness of dentin will be affected by raidotherapy, a finding that also can be seen clinically.[25] These results could possibly explain the onset of radiation caries at the dentin-enamel border (cervical areas) since distruption of the dentin-enamel junction would lead to gap formation (resulting in enamel chips frequently breaking off) and subsequent microbial colonisation. Dentinal changes in irradiated vital teeth, with an increased obliteration of the dentin tubules, would support this hypothesis. The reduced microhardness of dentin would indeed be a direct factor leading to radiation caries, but more researches are clearly warranted.[2]

CONCLUSION

According to the results, calcium loss in enamel tissue becomes significant as long as the received radiation dose increases at radiotherapy. These findings we conclude that the damage and the loss in the dental tissues exposed to radiation should be studied at elemental level. Whether a direct effect of irradiation on teeth, other than the already mentioned dentinal changes in vital teeth, also contributes to the development of radiation caries has not been fully elucidated, and reports are contradictory. Because of this, radiation effects on salivary glands as well as poor oral hygiene techniques, in combination with radiation damage at the dento-enamel junction are considered to be the main causes of radiation caries.

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