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Histopathological changes in dental pulp of rats following radiotherapy

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ABSTRACT

Background: Radiotherapy is one of the oral and pharyngeal cancer treatment methods that can cause damage to the tissues in the radiation area; the purpose of this study is to evaluate the effects of radiotherapy on dental pulp tissue in rats.

Materials and Methods: In this interventional, experimental double-blind study, 30 rats were studied in three groups (n = 10 each). The first group received 12 gray (Gy), the second group received 18 Gy in one session, and the third group was not exposed to radiation (control group). The 5 μm sections of mandibular molar tooth were prepared and stained with hematoxylin and eosin. Samples were studied under optical microscope to evaluate and score inflammation, necrosis, hyalinization, and vascular congestion. The data were coded and analyzed by statistical tests of χ^2 and Fisher's exact tests. The significant level of P = 0.05.

Results: In Group 1, necrosis in two cases, inflammation in one case, hyalinization in one case, and vascular congestion in four cases were observed. In Group 2, inflammation in four cases, hyalinization in two cases, and vascular congestion in five cases were observed. In Group 3, inflammation was observed only in one case. In comparison between the groups, no significant differences were observed in inflammation ($P > 0.05$), necrosis ($P > 0.05$), and hyalinization ($P > 0.05$). However, the difference was significant for vascular congestion ($P < 0.05$).

Conclusion: Radiotherapy with doses of 12 and 18 Gy had no significant effect on inflammation, necrosis, and hyalinization in all groups; however, the difference was significant for vascular congestion.

Key Words: Dental pulp, histology, cancer, radiotherapy

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INTRODUCTION

The incidence of malignancies is increasing day by day in different societies. Malignancies have been known as the second leading cause of death in developed countries. Head and neck cancers account for about 4% of malignancies.[1]

Radiotherapy is a one of the methods for head and neck cancer treatment. In radiotherapy, cancer cells are destroyed and the malignant lesion is treated or stopped using ionized ray. Although radiotherapy has therapeutic effects, it can cause certain side effects as mucositis, candidiasis, and xerostomia. These symptoms include decrease in mouth opening and loss of the sense of taste that are potentially related to the increasing tooth decay occurrence.[2,3]

Furthermore, teeth often receive high doses of radiation during treatment for head and neck cancers.

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In several *in vitro* studies, the direct effects of irradiation on enamel and dentin were evaluated and showed the conflicting results of negative effects of radiation on the solubility of the enamel and the destruction of dentine collagen.[4] However, some studies showed the small direct effects of X-ray on dental pulp, most of which were based on animal studies and obsolete techniques of radiotherapy. When the effect of direct irradiation on dental pulp components was studied, different results were obtained.[4]

Clinically, the inflammation is a response to trauma, infection, and dental interventions caused the decrease in pulp pain even in the presence of severe decay and pulp exposure. Pulp status determination is a key process to evaluate pulp health or pathology, and it is an important factor for making necessary decisions on endodontic procedures such as pulpotomy or endodontic treatment.[5,6] Thus, lack of awareness of pulp health status may result in inappropriate treatment measurements. The purpose of this study is to evaluate the effects of radiotherapy on dental pulp tissue in rats.

MATERIALS AND METHODS

Sampling was done randomly among 30 rats kept in the animals Center of Babol University of Medical Sciences.

Inclusion criteria

1. Male and Wistar breed of rats
2. Age range of 7–11 weeks
3. Weight range of 160 ± 20 g at the exposure time.

Exclusion criteria

Rats death before the completion of radiotherapy.

Before the exposure, all rats were kept under the same environmental conditions (temperature of 22°C ± 2°C), light and darkness control (12 h light and 12 h darkness), and the same nutrition from the same foodstuff and the same water tank.

Thirty male rats were divided into three groups:

- The first group (n = 10) was irradiated 12 gray (Gy) in one session
- The second group (n = 10) was irradiated 18 Gy in one session
- The rats were anesthetized by ketamine (100 mg/kg) through intraperitoneal (IP) before the exposure. The rats were fixed on a specific plane and exposed to gamma rays (by Teratn 780 device made in Canada) with cobalt-60 and 1.25 million electron volts of energy in the radiotherapy center of Shahid Rajai Hospital, Babolsar. The status and position of the tube were in a way that the entire rat's cranium was placed in the radiation field. As Vier-Pelisser in his study showed no significant differences in inflammatory reactions after 30 days,[2] different period had been chosen, and all rats were killed 60 days after the completion of radiotherapy.

The third group (control group) (n = 10) was not exposed to radiation and was killed at the same time of the first and second groups of rats.

The rats' heads were transferred to a pathology laboratory. In the laboratory, two mandibular molar teeth with minimal trauma were removed by a forceps; then, the apical third of root was cut by the short diamond bur to facilitate the entry of the formalin into the pulp space, and then, teeth were transferred into 10% buffered formalin. After 24 h, teeth were removed from the formalin and transferred to a 5% nitric acid solution for decalcification. The tooth tissues' softening rate was evaluated regularly each day. When the tooth tissues softening reached the right amount for cutting, the teeth were taken out of the acid and transferred again to the 10% formalin.

The 5 μm sections of each tooth were prepared and stained with hematoxylin and eosin. The lamella was prepared from the samples and examined using an optical microscopy (Olympus BX51, Tokyo, Japan) with a magnification of ×40 and ×400.The pulpal space of coronal and radicular area was totally evaluated.

- The amount of inflammation, necrosis, hyalinization, and vascular congestion in coronal and radicular pulp of the samples were analyzed[7]
- Inflammation is divided into the following levels based on the presence of inflammatory cells:
  - Absent (0): Absence of inflammatory cells
  - Slight (1): <20% of pulp space is occupied by inflammatory cells
  - Moderate (2): 20%–40% of pulp space is occupied by inflammatory cells
  - Intense (3): >40% of pulp space is occupied by inflammatory cells.[7]
- Necrosis is divided into the following levels:
  - Absent (0): No necrosis
  - Slight (1): <20% of pulp space is necrotic
  - Moderate (2): 20%–40% of pulp space is necrotic

- Intense (3): >40% of pulp space is necrotic.[7]

- Hyalinization is divided into the following levels:
  - Absent (0): No hyalinization
  - Slight (1): <20% of pulp space is hyalinized
  - Moderate (2): 20%–40% of pulp space is hyalinized
  - Intense (3): >40% of pulp space is hyalinized.[7]
- Vascular congestion is divided into the following levels:
  - Absent (0): No congestion
  - Slight (1): 3 congestive blood vessels
  - Moderate (2): 3–5 congestive blood vessels
  - Intense (3): >5 congestive blood vessels.[7]

At the end, the data were coded and analyzed using the statistical software SPSS (IBM, New York, USA) and the statistical tests of χ^2 and Fisher's exact tests. The significance level of $P = 0.05$ and $P \leq 0.05$ was considered as statistically significant.

The study was reviewed and approved by the Ethics Committee of Babol Medical University, Babol, Iran (ORN: 1066, August 1, 2015).

RESULTS

Dental pulp of the 30 male rats whose ages were ranged between 7 and 11 weeks with the average weight of 160 ± 20 g exposed to ionized radiation (first group 12 Gy, second group 18 Gy, and third group without radiation) was studied 60 days after radiotherapy the criteria of inflammation , necrosis, hyalinization, and vascular congestion were evaluated [Tables 1-4].

All necrosis, inflammation, hyalinization cases observed in the study were slight, and none of the samples showed moderate or intense levels [Figures 1 and 2].

In comparison between the three groups, χ^2 and Fisher's Exact tests showed no significant differences for inflammation ($P = 0.310$), necrosis ($P = 0.310$), and hyalinization ($P = 0.754$). However, this difference was significant for vascular congestion ($P = 0.038$).

Table 1: The frequency of inflammation

Level	Inflammation			
	Absent (0)	Slight (1)	Moderate (2)	Intense (3)
Group 1 (12 Gy)	9	1	0	0
Group 2 (18 Gy)	6	4	0	0
Group 3 (control)	9	1	0	0

Table 2: The frequency of necrosis

Level	Necrosis			
	Absent (0)	Slight (1)	Moderate (2)	Intense (3)
Group 1 (12 Gy)	8	2	0	0
Group 2 (18 Gy)	10	0	0	0
Group 3 (control)	10	0	0	0

Table 3: The frequency of hyalinization

Level	Hyalinization			
	Absent (0)	Slight (1)	Moderate (2)	Intense (3)
Group 1 (12 Gy)	9	1	0	0
Group 2 (18 Gy)	8	2	0	0
Group 3 (control)	10	0	0	0

Table 4: The frequency of vascular congestion

Level	Vascular congestion			
	Absent (0)	Slight (1)	Moderate (2)	Intense (3)
Group 1 (12 Gy)	6	4	0	0
Group 2 (18 Gy)	5	5	0	0
Group 3 (control)	10	0	0	0

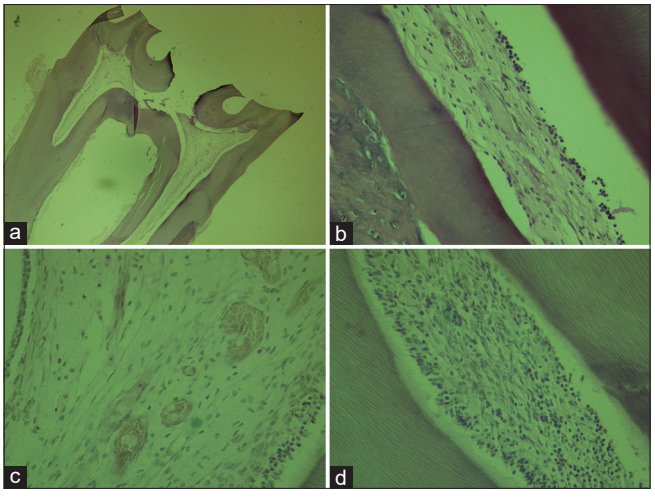

Figure 1: (a) Histological section photomicrograph of the Group 1 (12 GY, H and E, ×40). (b) Slight congestion and hyalinization (×400). (c) Slight congestion (×400). (d) Slight infiltration of inflammatory cells (×400).

DISCUSSION

The present study was carried out to evaluate the effects of radiotherapy on dental pulp tissue in rats. Head and neck cancers are the sixth most common cancer in the world. Currently, radiotherapy is one of the selected treatment methods in early diagnosed cases.[8] Ionizing radiation causes chemical damage in tissues, and radiotherapy may cause chemical changes in microcirculation. There is evidence that

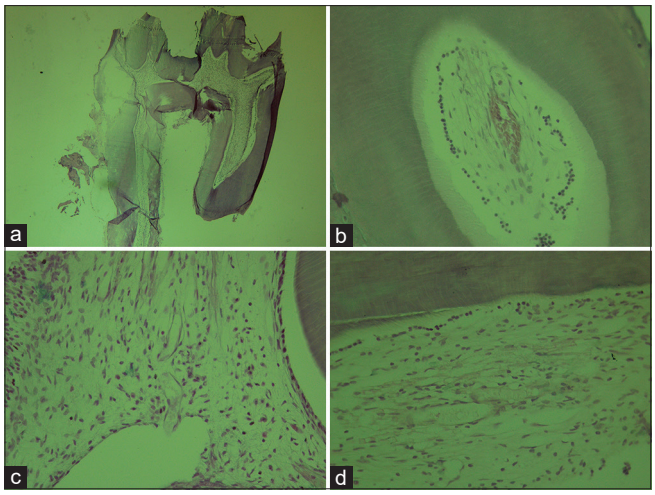

Figure 2: (a) Histological section photomicrograph of the Group 2 (18 GY, H and E, ×40). (b) Slight congestion (×400). (c) Slight infiltration (×400). (d) Slight necrosis (×400).

radiotherapy creates changes in dental pulp. It has also been suggested that radiotherapy may cause perfusion reduction, fibrosis, and atrophy of dental pulp.[4,9] The determination of pathological status is a key process to assess dental pulp health or the pathology, and it is an important factor for making necessary decisions on the endodontic processes such as pulpotomy or endodontic treatment.

Three groups of rats underwent radiotherapy for one session (the first group 12 Gy, the second group 18 Gy, and the third group without irradiation) were studied.

In the present study, no significant difference was observed between the groups in comparison of the studied criteria of necrosis, inflammation, and hyalinization. However, there was a significant difference in vascular congestion.

In the Faria *et al*. study, no difference was observed regarding the morphology of blood vessels and nerves, presence of odontoblasts, inflammation distribution pattern, and amount of calcification or fibrosis between the control group and the patients who had undergone head and neck radiotherapy.[10] In another study conducted by Faria *et al*. on the teeth of 23 patients who had finished head and neck radiotherapy, it was determined that direct effects of the radiotherapy were not capable of causing morphological changes in the microcirculation and the innervation of the extracellular matrix components of the patients' dental pulp with head and neck malignancies.[6] In addition, the immunohistochemical studies were done to validate the morphogenic findings. All samples showed CD34 expression indicating the vasculogenesis preservation.[6]

Finally, Faria *et al*. suggested that the direct effects of radiotherapy on the dental pulp cannot cause detectable morphological changes that could explain pulp sensitivity changes mentioned in some studies.[6]

In Vier-Pelisser study conducted on rats in 2007, regarding the inflammatory responses, no significant difference was observed between rats (1) immediately after radiotherapy (immediate effects), (2) after 30 days (delay effects), and (3) without radiation. In this study, the rats received radiation with fractionated doses of 200 cGy for 30 days (equivalent of 60 Gy). However, the Groups 1 and 2 showed greater changes which are statistically significant in the cell nucleus.[2]

The study of Havelek *et al*. on human dental pulp stem cells (DPSCs) showed that the radiation-induced causes differentiation of immature DPSCs to odontoblast and osteoblasts categories. The DPSCs are beside the pulp blood vessels; recovery after radiotherapy needs stem cells' cooperation. The DPSCs respond through differentiation to odontoblast-like cells during the injury, which synthesizes *in vivo* tubular dentin. Havelek *et al*. concluded that ionizing radiation starts the differentiation of DPSCs odontogenic/osteogenic and these cells' capability to live is not affected by getting old.[11]

Kataoka *et al*. showed that the amount of pulp oxygen will be reduced during the radiotherapy. However, pulp tissue oxygen and blood supply after the radiotherapy can return to normal levels.[12] However, in another study, Kataoka *et al*. showed that the patients' response to a cold test was reduced after passing 4–5 months after radiotherapy.[13]

However, the pulp innervations may be compromised after radiation, as a result, the teeth sensitivity in the area of radiotherapy or the adjacent areas is reduced. The issue of the oxygen pressure dropping in some stages of the study is not evidence of pulp necrosis during radiotherapy; one of its reasons could be partial necrosis. Another reason is the microcirculation decrease due to vascular damage by radiation in the healthy pulp of the necrosis areas.[12]

The congestion of the damaged blood vessels (as observed in the present study) during radiotherapy can also lead to ischemia, which causes reduction blood stream and/or undeleted metabolites.

In the study of Kataoka *et al*., after oxygen pressure decreased during the radiotherapy process, oxygen

pressure was increased again 4–5 months after the initiation of radiotherapy. This suggests the repairing capacity and remodeling of dental pulp after radiotherapy. These facts suggest a potentiality due to the vascularization in pulp because of inflammatory changes.[12]

The results of the present study and other studies were compatible and show that the inflammatory processes in pulp tissue begin after the initiation of the radiotherapy. However, these processes cannot lead to the significant changes in the morphology of pulp tissue. One reason could be that radiotherapy has no effect on microscopic structures of dental pulp, but regarding the results obtained by this study and the observation of the inflammatory initiation processes, the lack of effectiveness may be related to the high potential of pulp tissue in self repair. As Havelek *et al*. noted that the repairmen potential is not affected by the radiation because the pulp tissue, contains stem cells. These cells can be the factor of tissue repair potential.[11]

In the present study, vascular congestion observed in the groups which underwent the radiation was significant; this could explain the decrease in the sensitivity of the pulp seen in other studies.[12,13]

Considering that pulp sensitivity and also oxygen pressure due to radiotherapy would be decreased,[12,13] it is suggested that dental pulp therapy for patients who have received radiation should be done with caution as no pulp necrosis has been observed in the present study.

According to the formula "BED = nd (1 + d/[α/β])" (BED = biologically effective dose, n = number of fractions, d = dose per fraction), the amount of radiation is equivalent to the therapeutic radiation doses of 60 Gy (high dose) and 30 Gy (low dose) in studies with fractionated doses in several sessions (for example, 30 sessions 200 cGy).

In this study, different doses of 18 Gy (high dose) and 12 Gy (low dose) were used in one session and the effects of the radiation on rats were evaluated 60 days after radiotherapy. The reason for selecting 1 session of treatment in this study was to avoid long-term treatment for the rats not being suffered and decreasing the risk of losing them during the sessions. The required equipment of such studies is available in hospitals; it would be difficult to do research on animals in the hospitals which are specialized solely for human patients.[2]

Some issues should be considered while conducting such experiments, such as the difficulty of keeping and transferring the rats and also the probability of the rats' death, which would be higher as the duration of the study gets longer. Fortunately, there was no sample loss in the present study.

It is suggested to perform a similar study to evaluate the histopathologic changes of the pulp tissue under different duration of time. It is also recommended to carry out a human study to assess the vitality of the dental pulp and pulp blood supply by means of laser Doppler flowmetry, before and after radiotherapy

CONCLUSION

This study showed no significant differences between the rats that received radiation of 12 and 18 Gy and those who received no radiation, in the histological measures of inflammation, necrosis, and hyalinization, but this difference was significant for vascular congestion.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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