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HISTOLOGICAL CHANGES IN THE ODONTOBLASTIC LAYER OF DENTAL PULP FOLLOWING TOOTH PREPARATION

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Abstract

Introduction: The dental pulp is a highly specialized tissue because of its capability to regenerate. The pulp shows inflammation in response to insult and if untreated, it leads to pulp infection and pulp death.

Objective: Objective of this study was to compare the histological changes in the odontoblast layer of coronal and radicular pulp following 2 mm coronal tooth preparation.

Material and Methods: An experimental study was performed. Thirty premolar teeth intended for orthodontic extraction were categorized into two groups of fifteen each which were the control group and the experimental group (Teeth prepared up to 2mm using high-speed hand piece).

Results: There was a significant difference (p value <0.001) between control and experimental groups in the odontoblastic layer and presence of nuclei in its cells of coronal dental pulp. Whereas radicular area of dental pulp has not exhibited any significant difference between control and experimental group.

Conclusion: The results help us to understand the effects of the injury on the healthy dental pulp. Keeping in view the histological changes in tooth pulp following 2mm of coronal tooth preparation, the radicular pulp was normal while histological changes were seen in the coronal pulp.

Keywords: coronal pulp; odontoblast layer; radicular pulp; tooth preparation

INTRODUCTION

The dental pulp is a mesenchymal tissue, highly specialized that tends to regenerate. The primary function of pulp is the formation, induction, and nourishment of the developing tooth germ (Janjić et al., 2016).

In an adult tooth, it becomes reparative and protective. The unique nature of dental pulp is due to its low compliance environment, the occurrence of more sensory nerves to stimulate, and the massive circulation of blood in micro vasculature (Marrelli et al., 2018). The dental pulp is enclosed by three mineralized tissues which are, dentin, enamel, and cementum. These barriers protect the pulp from the microbes(Daud et al., 2016). Histologically, the dental pulp is divided into two parts: pulp proper and odontogenic zone. The odontogenic zone is composed of cell-free zone (Weil's zone), the cell-rich zone (odontoblasts and undifferentiated mesenchymal cells) and nerve plexus. On the other hand, the pulp proper contains fibroblasts, extracellular matrix, blood, and nerve supply. In coronal pulp, the odontoblasts are columnar, whereas, in the radicular pulp, the odontoblasts are cuboidal (Nanci, 2017).

The pulp shows inflammation in response to microbial invasion and traumatic injury, and if it is not treated, it will lead to pulp infection or pulp death (Fleig et al., 2017). The functional cells of pulp, the undifferentiated mesenchymal cells and odontoblasts are important because they form dentin continuously, till death. This allows the healthy pulp to partially compensate for the loss of dental hard tissue caused by mechanical wear, caries, and operative procedures, thus establishing a barrier that protects the remaining pulp tissuefrom irritants(Bakhtiar et al., 2018). In response to operative procedures involving cavity and crown preparation, these odontoblasts may form either reparative dentin or reactionary dentin, depending upon the extent of the injury. Pre-existing odontoblasts are responsible for the formation of reactionary dentin, whereas reparative dentin is laid down by newly differentiated odontoblasts derived from the undifferentiated mesenchymal cells (Pisciotta et al., 2015). The flexible ground substance of the pulp limits the intrapulpal pressure to the site of irritation, thus restricting the inflammatory response to a localized area of affected pulp and is not spread throughout the pulp space (Ricucci et al., 2018). This response is beneficial for unaffected areas as localized repair keeps them alive and normal. The success of the pulpotomy procedure was established on the indication of inflammatory responses and vascular changes that are limited to the coronal pulp and radicular pulp remains normal. Protection of vital pulp is essential to maintain the longevity of teeth (Soni, 2016)[10].The study aimed to understand the effects of tooth preparation procedure on the health of the dental pulp.

MATERIALS AND METHODS

The entire work was conducted in accordance with the code of ethics of World Medical Association(*WMA - The World Medical Association-WMA International Code of Medical Ethics*, n.d.). Thisclinical study was conducted at the Histology laboratory of Anatomy Department, Postgraduate Medical Institute Lahore and de 'Montmorency College of Dentistry, Lahore. The study was performed between May 2018 to May 2019.Thirtypatients with age of 18-25 years with permanent premolars that were scheduled for orthodontic extraction were included in the study. Written consent was taken from each patient. The study was approved by the institutional review board (ERB/No-5001/15). Only vital fully erupted teeth, in occlusion with the opposing teeth were chosen for this study. Carious and cracked teeth with incomplete roots, periapical infection, and premolars with artificial crowns were excluded from the study.

The specimens were divided into control and experimental group of fifteen tooth each. Control group consisted of tooth

without preparation and the study group with tooth preparation up to 2mm.The teeth specimens were processed under the guidance of clinical supervisor.

Tooth Preparation Procedure

Local anesthesia (lignocaine-5mg/kg body weight) was injected with 27-gauge needle to anesthetize the premolar to be prepared and extracted for orthodontic reason. Tapered needle edge diamond bur No. 021 (According to ISO nomenclature) was used to break the inter-proximal contact by using NSK high speed hand piece (Up to 400,000 rpm and air pressure of 2.2 bar) with water spray. Guiding grooves of 2.0 mm were made for axial reduction using diamonddepth cutting bur ISO No. (834.FG). Later these grooves were joined to complete the axial preparation by using standard diamond grit, tapering fissure bur ISO No. (846R.FG). Similar guiding grooves of 2.0 mm were made on the occlusal surface and were joined in the similar way to complete the occlusal reduction using the same bur.

Tooth Extraction Procedure

Tooth extraction procedure was performed in both groups. Periosteal elevator was used to reflect the gingival tissue from buccal and palatal sides and the force was applied through the universal forceps. The tooth was extracted, and the root was examined to make sure that it was not broken and intact. Immediately after tooth extraction the socket was irrigated with normal saline and digital pressure was applied to squeeze the extraction socket. The socket sterile gauze piece with saline was placed at the extraction site and patient was asked to bite on it gently. Post extraction instruction was given, and patient was discharged.

Tissue Processing

Immediately after tooth extraction, the teeth were placed in the 10% formalin for 72 hours to complete the fixation of dental pulp. After fixation the specimen was cleaned, dehydrated in increasing percentages of alcohol, and finally embedded in paraffin wax(*1557923707-105-Bancroft-s-Theory-and-Practice-of-Histological-Techniques-8th-Edition-Kim-s-Suvarna-Frcpath-Christopher-9780702068645-Elsevier-2019-574-209.Pdf*, n.d.). A longitudinal section of 4µm thickness was cut from the coronal as well as radicular pulp. The specimen was stained with Haematoxylin-Eosin (HE) staining and was seen under the light microscope(Nikon C1 eclipse) with 100x, 200x and 400x magnification to see the histological features of dental pulp.

Statistical Analysis

The data was entered and analyzed using SPSS 20 Mean ± S.D for quantitative variables. Independent t-test was applied to observe the mean differences in the odontoblast layer of coronal and radicular pulp following 2 mm coronal tooth preparation between control and experimental groups. P-Value of < 0.05 was considered statistically significant with a power of study at 90%.

RESULTS

In this study, thirty patients, who are nineteen males and eleven females required orthodontic extractions of their premolars were included.

Odontoblast cells layer: Layer of odontoblastic cells was normal in coronal area of all the 15 specimens of control group and was abnormal in coronal area of all the 15 specimens of experimental group. The result was significant with *p*-value <0.001. (Table: 1. Fig. 1& 2)

Layer of odontoblast cells was normal in radicular area of all the 15 specimens of control group and normal in 11 specimens in radicular area in experimental group.

However, it was abnormal in 4 specimens in radicular area in experimental group. The result was not significant (Table: 1. Fig. 3 & 4).

Table 1: Comparison of Odontoblast cells layer among groups.

		Abnormal	Normal	p-value
Coronal Pulp	Control group	0(0%)	15(100%)	<0.001
	Experimental group	15(100%)	0(0%)	
Radicular Pulp	Control group	0(0%)	15(100%)	0.100
	Experimental group	4(26.7%)	11(73.3%)	

Nuclei of odontoblasts

Nuclei of odontoblasts were presented in coronal area of all the 15 specimens of control group and were absent in coronal area of all the 15 specimens of experimental group. The result was significant with *p*-value <0.001(Table: 2. Fig. 1 & 2).

Nuclei of odontoblast cells were presented in radicular area of all the 15 specimens of control group and in 11 specimens in radicular area of experimental group but they were absent in 4 specimens in radicular area of study group (*p*-value ≤0.100) (Table: 2. Fig. 3 & 4)

Table 2: Comparison of number of Odontoblast Nuclei among groups.

		Absent	**Present**	**p-value**
Coronal Pulp	Control group	0(0%)	15(100%)	<0.001
	Experimental group	15(100%)	0(0%)	
Radicular Pulp	Control group	0(0%)	15(100%)	0.100
	Experimental group	4(26.7%)	11(73.3%)	

Light microscopic images of coronal and radicular pulp of control and experimental group showed normal morphology of dental pulp demonstrating each of the four zones and their particular cells as well as morphological changes in dental pulp and its particular cells.

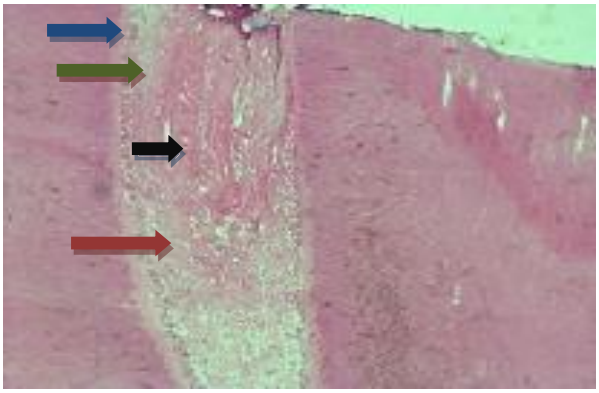

Figure 1: Photograph of longitudinal section of coronal area of dental pulp of control group with normal morphological patterns.

Note: Blue arrow shows normal odontoblasts with nuclei present. Green arrow shows cell free zone. Red arrow shows cell rich zone. Black arrow shows pulp core (100X).

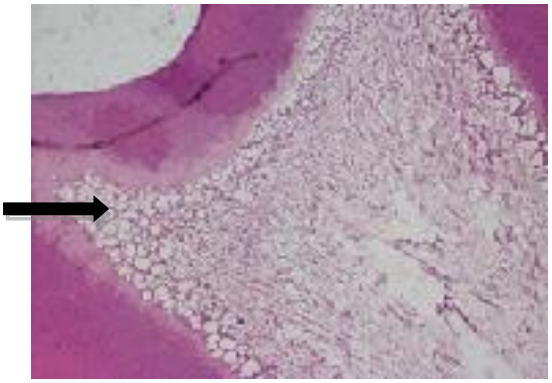

Figure 2: Photograph of longitudinal section of dental pulp of coronal area of study group showing changes in morphological pattern.

Note: Black arrow shows vacuolated odontoblast cells layer with absent nuclei (100X).

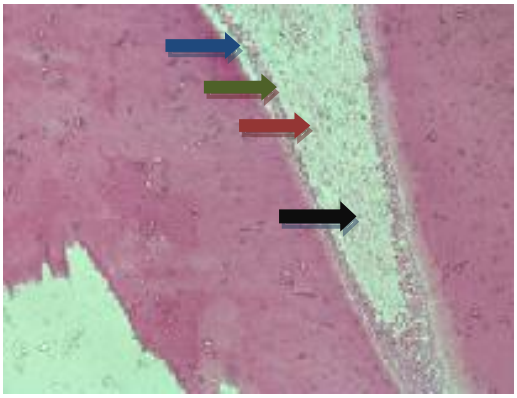

Figure 3: Photograph of longitudinal section of radicular area of Dental pulp of control group with normal morphological patterns.

Note: Blue arrow shows normal odontoblasts with nuclei present. Green arrow shows cell free zone. Red arrow shows collagen fibers and fibroblasts. Black arrow shows pulp core (100X).

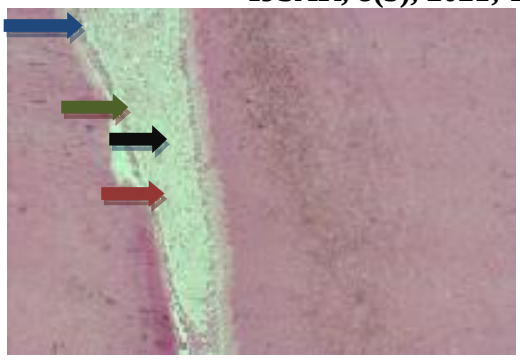

Figure 4: Photograph of longitudinal section of dental pulp of radicular area of study group showing normal morphological pattern.

Note: Blue arrow shows normal odontoblast cells layer with nuclei present. Green arrow shows cell free zone. Red arrow shows collagen fibers and fibroblasts are present in cell rich zone. Black arrow shows normal pulp core (100X)

DISCUSSION

In the current research, morphological changes in odontoblastic layer of dental pulp were compared in coronal and radicular pulp in control and experimental groups, after 2mm depth preparation. The impact of this iatrogenic procedure resulted in clinical symptom later in time. In the control group, the normal morphology of dental pulp has been seen in the slides demonstrating each of the four zones along with their cells. The odontoblastic cells (columnar) layer is continuous and prominent nuclei is a feature of the normal dental pulp. In the experimental group, the odontoblast cells layer is aggravated by the presence of vacuolization and the absence of nuclei in odontoblastic cells. These morphologic changes in dental pulp were also reported in a study and depicted that sudden degenerative changes in dental pulp were observed after various tooth preparations (Vitalariu et al., 2005).

A study was conducted to evaluate the immediate changes in the pulp-dentin complex that resulted from crown preparation, and their correlation with the thickness of remaining dentin and the preparation technique (with or without water spray cooling). The most severe changes appeared after profound preparation without water-cooling. The group that received water spray as coolant also depicted affected odontoblastic cells and vascular reactions in pulp core[12]. Another study revealed that there were also histological changes in the pulp and dentin following complete crown preparation occur, even the adequate technique of preparation was used(Farah, 2018). These results also support the results of the present study.A study done by Ahmed *et al*., to know that under standardized tooth preparation procedure what immediate changes were observed leading to the pulp necrosis. The results showed that the acute inflammatory infiltration and necrosis were absent in all the specimens of the experimental group. However, the vacuolated odontoblasts and the absence of nuclei were observed(Ahmed et al., 2017).

In the present study, none of the specimens of the dental pulp, showed any irritation or presence of any intense heat injury. This resultimplies that the crown preparation technique up to 2 mm is considered within safe limit and it does not cause any reversible or irreversible pulp damage. The observations were confined to the immediate changes in the dental pulp. However, it is possible that acute inflammatory infiltrate could be a positive finding within 24 hours, which is very unlikely to develop immediately after crown preparation.

The limitation of this study was that specimens used were only healthy premolars and they were collected from the patients who came for orthodontic extractions.

Conclusion: The results help us to understand the effects of the injury on the healthy dental pulp. Keeping in view the histological changes in tooth pulp following 2mm of coronal tooth preparation, the radicular pulp was normal while histological changes were seen in the coronal pulp.

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Conflict of interest: Nothing to declare.

Contribution of authors:

AA and MSI conceived the idea and conducted main research,

HM, SS and MA contributed in data collection and write-up

AF and FF edited and proofread the final draft

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