


Inflammatory response of the human tooth pulp tissue to dental caries

Respuesta inflamatoria del tejido de la pulpa dental humana producida por caries

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ABSTRACT

Introduction: Dental caries is a chronic infectious disease resulting from the penetration of oral bacteria into tooth hard tissues. Microorganisms subsequently trigger inflammatory responses in the dental pulp and the stem cells provide a source of cells to replace the damaged cells and facilitate repair. These events can lead to pulp healing if the infection is not too severe and treated in a short time. Remaining pulpal pathosis in severe form without treatment induces permanent loss of normal tissue due to limited repair capacities in response to large damage. The importance of the depth of inflammation has been underestimated in pulpal healing. The purpose of this study is to investigate the pulp tissue response to dental caries and to find out the association of different distributions of the inflammatory characteristics among a different depth of dental caries. **Materials and Methods:** Pulp tissue samples were collected from 118 extracted teeth from the Privet dental clinics and dental health centers in Duhok government, from April/ 2016 to August/ 2017 (16 months period). Each section prepared and stained with hematoxylin and Eosin (H&E). Inflammatory infiltration, fibrosis, calcification, and necrosis were the main features that have been examined histopathologically and assessed with the presence of dental caries at a different depth. **Results and Discussion:** Inflammatory features were identified in 88 of the samples examined. Inflammatory infiltration and fibrosis were the most frequent features among the deep caries teeth compared to the shallow caries teeth. Single and group of calcification were observed in 57 samples, most of them (48 samples) were in deep caries sections. **Conclusion:** the histopathological observations of pulp tissue in response to caries process provide useful information for the clinical aspect and how to decide and select the best strategy in the treatment of dental caries at a different depth to preserve the pulp tissue vitality for a longer time, and strength of the tooth hard tissue will maintain.

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INTRODUCTION

The dental pulp is a loose connective tissue, present in a hard tissue chamber of dentine surrounded by enamel in crown and cementum in the root, which provides mechanical support and protection from the microbial irritation from within the oral environment. Dental pulp is externally separated from dentine by odontoblasts and by Höhl's subodontoblastic cells which are preodontoblasts (Goldberg M., et al. 2004). Adjacent to this layer, the pulp is rich in collagen fibers and poor in cells. Then a more vascular internal layer contains nerves, progenitor cells, and undifferentiated cells, some of which are considered stem cells (1). Any destruction or loss to this mechanical protection will expose the pulp to many harmful stimuli as bacteria with their toxins that enter the pulp via caries, cracks and fractures (2).

Dental caries is a chronic disease, affecting a large number of populations. The carious process is the destruction of the mineralized dental hard tissue of the teeth, enamel, dentine, and cementum, and caused by the action of the acidic by-products from the microorganisms on fermentable carbohydrates in the diet (3). Microorganisms subsequently trigger inflammatory responses in the dental pulp.

The pulp inflammation or the Pulpitis in human teeth occurs most commonly due to dental caries, attrition, abrasion, or trauma; chemical irritation, thermal damage, and iatrogenic pulpal exposure (3). These inflammatory events can lead to pulp healing if the infection is not too severe following the removal of diseased enamel and dentin tissues and clinical restoration of the tooth. The complete tooth healing makes a barrier to distance and protects the pulp. However, chronic inflammation often with persist irritation to the pulp inducing permanent loss of normal tissue and reducing innate repair capacities (4).

As caries-related bacteria invade deeply into dentin and come into close proximity to the pulp, inflammatory cells infiltrate into the pulp tissue and consequently, pulpitis develops. The initial inflammatory cell infiltrates consists principally of lymphocytes, plasma cells and macrophages (5). Fibroblasts, which are a major cell type in the dental pulp, have the capacity to produce pro-inflammatory cytokines, (4) and adhesion molecules that are responsible for the initiation and progression of pulpitis. The aim of this study was to evaluate the inflammatory response to dental caries at different caries depth.

MATERIALS AND METHODS

In this study, the ethical approval was given by the research ethical committee of Duhok directory of the general health center.

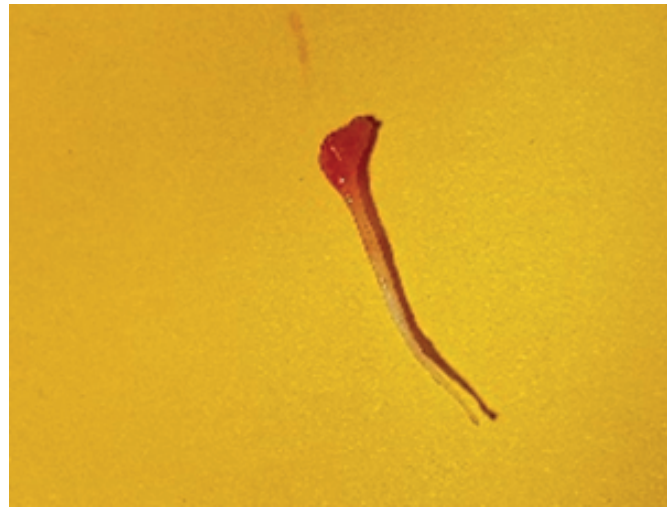
Sample collection

The study included 118 extracted teeth with their pulp tissue collected from April/2016 to August / 2017 from Dental health centers in Duhok in addition to private dental clinics. Among the extracted teeth, 88 teeth were diagnosed as caries teeth and classified as deep and shallow caries types. The remaining 30 teeth were without dental caries and were extracted for orthodontic and prosthetic purposes (representing the control group).

Sample preparation

After the pulp extirpation from the tooth (Figure 1), the samples were fixed in formalin, and processed in accordance with the standard protocol for the histopathological processing and examination (6). From the paraffin blocks, 4 μ m thick sections were cut and stained with Hematoxylin and Eosin (H & E) with an automated strainer in Vajeen private hospital. Each section was examined under a light microscope.

Figure 1. Human dental pulp tissue from lower right first premolar (without caries).



The evaluation of each specimen was performed microscopically, analyzed at different magnifying power (10x and 40x). Histological criteria used for evaluation of the pulp inflammation based on (6) were: (i)

Inflammatory infiltrate, (ii) Collagen deposition, (iii) Calcification of pulp, and (iv) Necrosis. The values are shown in (Table 1) reflect the features.

Table 1. The score values meaning used in this study.

Features	Score		
Inflammatory infiltrate	0 = Absent (No inflammation)	1 = Mild Fields had <35% spaces filled by inflammatory cells	2 = Intense Fields had > 35% spaces filled by inflammatory cells
Deposition of the Collagen*		1 = Mild Fields had <35% spaces filled by collagen	2 = Intense Fields had >35% spaces filled by collagen
Calcification of the pulp	0 = Absent	1 = Mild 1-3 small areas	2 = Intense >3 areas or large collections
Necrosis	0 = Absent	1 = Present	

*The collagen deposition characterized by eosinophilic area with reduced cellularity and blood vessels density (6).

Statistical analysis

The data were analyzed by Cross tabulation test using SPSS software (Version 16), with *P* value at <0.001 used to indicate a significant difference.

RESULTS

The age of the patient ranged from 7 - 69 years with a mean of 31.3 years. The age group of 16 - 26 years showed a higher frequency of dental caries (36.4%) among others, whereas the age group of ≥ 56 years showed the lowest one (2.5%). The teeth with caries, $n = 89$ (75.4%) are more than those without caries, $n = 29$ (24.6%), as shown in (table 2).

Table 2. The age group and tooth status.

Age Group	Number	Percentage
5–15	15	12.7
16–25	43	36.4
26–35	32	27.1
36–45	16	13.6
46–55	9	7.6
> = 56	3	2.5
Tooth status	Number	Percentage
Sound	30	25.4
Caries	88	74.6
Total	118	100.0

The Histopathological findings of the pulp tissue of both case and control group are summarized in table 3. Intense inflammation was seen in 57 cases (48.3%). Intense fibrosis and calcification were seen in 65, (55.1 %) and 62 (52.5 %) respectively whereas necrosis was only present in 37 cases (31.4). Among

these cases, the results were significantly high in teeth with caries for the intense inflammation (n= 57), intense fibrosis (n= 60), intense calcification (n=57), and Necrosis (present in n=37) when compared with the samples of teeth without dental caries.

Table 3. The correlation between the tooth status and the Histopathological findings

Inflammatory Infiltration	Without caries		With Caries		Total		P- Value
	Number	(%)	Number	%	Number	(%)	
Absent	28	(23.7)	19	(16.1)	47	(39.8)	*<0.001
Mild	2	(1.7)	12	(10.2)	14	(11.9)	
Intense	0	(0)	57*	(48.3)	57	(48.3)	
Total	30	(25.4)	88	(74.6)	118	(100)	
Fibrosis							
Mild	25	(21.2)	28	(23.7)	53	(44.9)	*<0.001
Intense	5	(4.2)	60*	(50.9)	65	(55.1)	
Total	30	(25.4)	88	(74.6)	118	(100)	
Calcification							
Absent	18	(15.3)	0	(0)	18	(15.3)	
Mild	7	(5.9)	31	(26.3)	38	32.2	
Intense	5	(4.2)	57	(48.3)	62	52.5	
Total	30	(25.4)	88	(74.6)	118	(100)	
Necrosis							
Absent	30	(25.4)	51	(43.2)	81	68.6	
Present	0	(0)	37	(31.4)	37	31.4	
Total	30		88	74.6	118	100.0	

* Correlation is highly significant at the <0.001 level

Figure 2 and 3 show some of the histopathological changes

Figure 2. Histological aspects of dental pulp tissue: A- Normal pulp tissue without inflammation and preserved odontoblastic layer from lower right 2nd premolar without caries. B- Mild inflammatory infiltration with the destruction of the odontoblastic layer, from upper left 2nd molar with deep caries. C- Intense inflammatory infiltration, from the lower right 1st molar tooth with deep caries. (Nikon – DigitalSight DS-L1 NIKON CORPORATION camera, No. 214774) H&E, 10X.

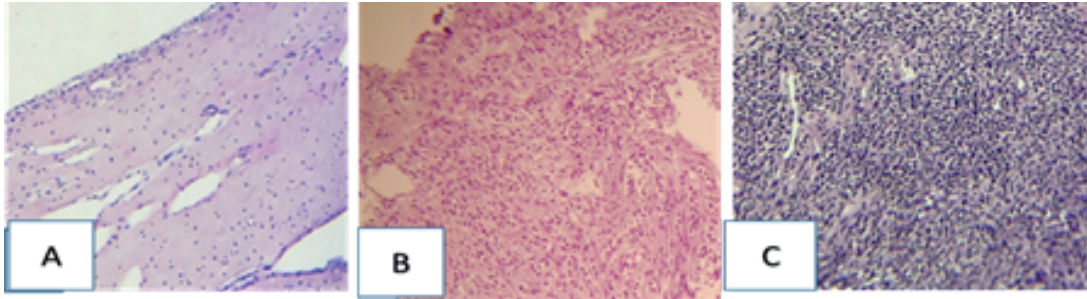
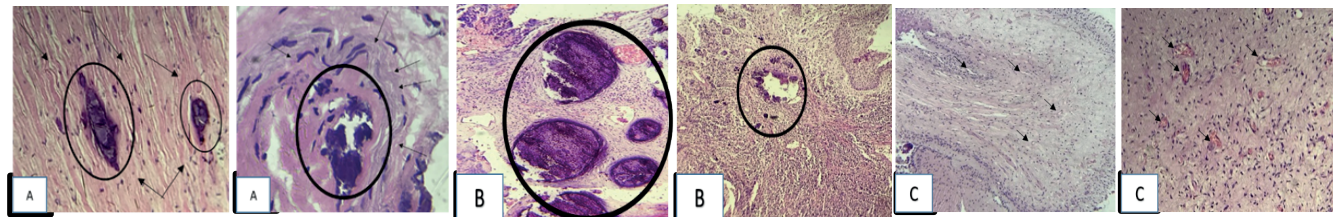
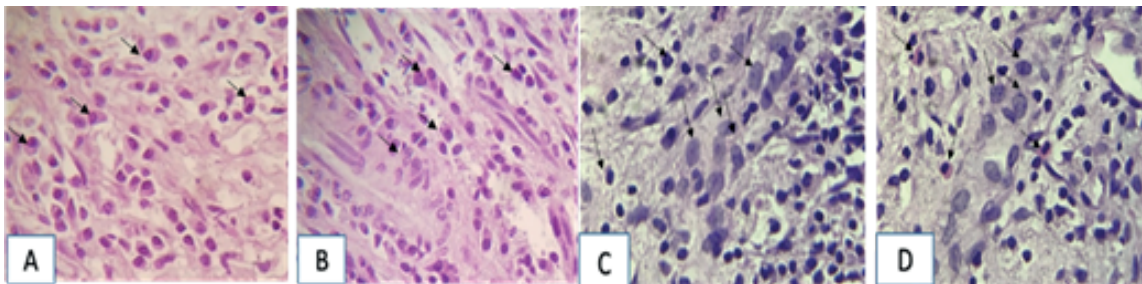


Figure 3. H & E staining aspects of dental pulp tissue section observe histopathological features: A & B- Collagen fibers accumulation (arrows) around calcification in the central area of pulp (circle), taken from the upper left canine tooth and lower right 2nd premolar. C &D- Intense calcifications (circle) with inflammatory infiltration, taken from upper right 3rd molar and lower right 3rd molar with deep caries. E – Fibrovascular proliferation. F-congestion of blood vessels (arrows). (Nikon – Digital sight DS-L1 NIKON CORPORATION camera, No. 214774) H&E, 10X.



The main inflammatory cells that have been found in the histological slides of the dental pulp tissue taken from the deep caries teeth are Lymphocytes, plasma cells, macrophages and number of eosinophils, figure 4.

Figure 4- The histological slides staining with H & E at (40X) power, of dental pulp tissue obtained from teeth with deep caries, show inflammatory cells (black arrows): A & B-lymphocyte and plasma cell, C & D- Macrophages and eosinophil. (Nikon – Digital sight DS-L1 NIKON CORPORATION camera, No. 214774).



The statistical analysis of data from the teeth with both deep and shallow caries showed significant differences between the histopathological findings mainly in the inflammatory infiltration, calcification, and fibrosis, while the pulp necrosis shows no significant difference between the dental caries of deep and shallow cases (table 6).

Table 6: The correlation between the caries depth with the histopathological findings of the pulp tissue.

Caries Depth	Inflammation			Necrosis		Calcification			Fibrosis		P-Value
	Absent	Mild	intense	Absent	Present	Absent	Mild	Intense	Mild	Intense	
Shallow	17	3	0	20	0	0	11	9	12	8	<0.001*
Deep	2	9	57	31	37	0	20	48	16	52	
Total	19	12	57	51	37	0	31	57	28	60	
		88		88			88		88		

* Correlation is highly significant at the <0.001 P-Value level.

DISCUSSION

The dental pulp is a unique tissue which is under continues threat of the adverse stimuli from the mouth after distraction and demineralization of the protected mineralized layers (Enamel, dentine and cementum), such as caries, cracks, fractures and restoration with defected margins, all of which get pathways through the dentinal tubule for micro-organisms and their toxins to enter the pulp.

The current study revealed differences in the histological features in inflamed pulps of caries teeth compared with pulp tissue of the healthy teeth. This has been also indicated by others like (8); however, this study included the differences in histological changes in relation to the depth of caries.

Normal Pulp is clinically symptom-free; however, the pulp may not be histologically normal. In this study pulp tissue of sound teeth of two clinically normal teeth showed mild inflammation in the microscopic examination, this may be due to the periodontal inflammation involvement. Same results have been observed by others (8). Literatures from previous studies on periodontal involved teeth observed that

infiltration of inflammatory cells including lymphocytes, plasma cells, and macrophages with a few leukocytes (9). Even the true incidence of pulpal calcifications of periodontally involved teeth is likely to be higher in histological examination because pulpal calcifications with a small diameter may not be seen on radiographs.

Inflammatory infiltration in the pulpal tissue sections ranging from minimal to marked inflammation. In this study, only three cases of shallow caries observed mild inflammation reverses to the response to deep caries that show high intense inflammation. In cases of minimum destruction of the tooth structure by caries lesion, the pulp tissue, initially responds to irritation as mild inflammation that results in protection by the formation of reparative dentin at the affected side. Therefore pulp healing is the first step, followed by regeneration, in this case, more inflammatory cells infiltration observed (lymphocytes and microphages) and no destruction to the odontoblasts cells (10). This explanation is in agreement with the present study in that the inflammatory infiltration is present even in the pulp tissue of shallow caries especially when the destruction is minimal.

In the present study, nodular and diffused calcification were present in the pulp tissue sections of the healthy and carious teeth at different age groups. These round or irregular calcifications were observed as single or in groups shows a reaction from the surrounding tissue as inflammatory infiltration and collagen deposition, review of the literature reveals a wide variance in the prevalence of pulp stones in different populations (13). Furthermore, the prevalence was also different in the literature shown by (14). Some investigators reported that the frequency of pulpal calcifications increases in elderly patients with periodontal disease. Pulpal calcification is evident as pathological manifestation due to local or systemic conditions, and may develop in different patterns and sizes (16).

The principle fibrous component of the dental pulp is typed I collagen, although type III is also present. The overall collagen content of the pulp increase with age (17). The greatest concentration of collagen generally occurs in the most apical portion of the pulp. Collagen deposition was seen in different pulp tissues in this study regardless of the age, gender, tooth position, and even dental caries. Fibrosis is, usually, seen with aging and chronic irritation of the pulp. However, studies have showed that age cannot be regarded as a causative factor for fibrosis (18). Pulp reduction and fibrosis, that have been always accompanied with the aging process due to compression also considered as adaptive changes (19) especially that more obvious in the radicular pulp than coronal pulp where the pulp first develops (20).

In the present study, 37 cases of necrosis were presented in pulp tissue of deep caries while none of shallow caries showed necrosis. This may be due to a large number of bacteria and their toxin within the pulp tissue with more destruction of the Odontoblastic cell layer that the inflammation cannot compensate. Under deep carious lesion, if unchecked, will progress through the dental hard tissues and

into the soft pulpal tissue and clinically it is difficult to determine if the pulp is still vital or not. In such case, the pulp tissue becomes fibrotic, inducing the formation of reactionary dentin very similar to bone-like tissue. The untreated caries lesion leads to the progression of inflammation to more severe that result in the pulp necrosis or apoptosis (2). Recently necrosis has been added to the process of dental pulp destruction as implicated in the (21). The development of pulp necrosis, as scientific thinking, due to pressure from the mobility of the periodontally involved teeth. At this stage, the pulp should be removed completely or partially (21).

Another mechanism that could be considered for the production of necrosis of the pulp was the interference of some of the blood supply through the lateral canals may become involved as a result of the periodontal lesion or curettage of pockets and planing of the root surfaces during periodontal treatment, this can lead to subsequent death of the pulp cells supplied by the affected capillaries (22).

CONCLUSIONS

Pulp tissue inflammation in teeth with deep carious lesions that extend through more than the dentine thickness appears to have more extensive histopathological pulpal changes than teeth without caries or with shallow caries. This finding may direct the clinical decisions in the management of teeth with deep carious lesions, to preserve the remaining vital pulp tissue and promote healing process by the elimination of the causative factor and changing the ecosystem of the caries cavity with the oral cavity environment. This study indicated that more attention should be paid to understand the progressive nature of the pulp disease processes and to use the appropriate and conservative treatment strategy for each caries condition.

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