HISTOLOGICAL EVALUATION OF HUMAN PULP TISSUE IN RESPONSE TO CARIES PROGRESSION

(EVALUASI HISTOLOGIS RESPON JARINGAN PULPA GIGI MANUSIA TERHADAP TINGKAT KEPARAHAN KARIES)

Tetiana Haniastuti

Oral Biology Department
Faculty of Dentistry, University of Gadjah Mada
Denta street, Sekip Utara, Yogyakarta
Email: haniastuti@yahoo.com

Abstract

The dental pulp is protected by enamel, dentin, and cementum from the microbial rich oral environment. Carious lesion may provide pathways for microorganisms and their toxins to enter the pulp. This study aimed to evaluate the responses of human dental pulp to caries progression. Twenty volunteers who had been scheduled to undergo extraction for various therapeutic reasons, were enrolled in the study. Five intact third molars and 15 third molars with carious lesion involving *enamel, dentin, and pulp were extracted. Before decalcifying with 10% EDTA solution (pH 7.4), the lesion conditions* were confirmed with micro-computed tomography. The specimens were embedded in paraffin, sectioned serially and stained with Hematoxylin Eosin. The specimens were then evaluated under a light microscope. All normal intact teeth showed histological features of normal pulp tissue with no inflammation. All teeth with carious lesion confined to enamel revealed slight inflammation and disorganization of odontoblast layer under to the lesions. Most samples with caries confined to dentin showed slight inflammation, only 1 sample showed moderate inflammation. Odontoblast layer lost its continuity and could not be observed in the areas under to the lesions. Severe inflammation was observed in 3 out of 5 carious teeth with pulp exposure, while the remaining samples showed moderate inflammation. Numerous blood vessels were found in the tissue surrounding the intense cellular infiltration. In conclusion, caries may induce inflammation and disorganization of the pulp tissue. The severity of pulp inflammation and disorganization depends on the depth of the lesion.

Key words: dental pulp, caries, histological evaluation

INTRODUCTION

The pulp is a soft tissue of mesenchymal origin with specialized cells, the odontoblasts, arranged peripherally in direct contact with dentin matrix. Dental health is directly related to the pulp tissue since it has important interactions with other tissues such as dentin, periodontium, and central nervous system.[1,2]

The dental pulp resides in a rigid chamber comprising enamel, dentin, and cementum which provide strong mechanical support and protecttion from the microbial rich oral environment. However, when the integrity of this barrier is breached, noxious elements of external origin may gain entry to the pulp chamber and cause various pathogical reactions.[3]

Dental caries, the major disease in the oral cavity, is an infectious and transmissible disease. This disease develops when changes in the oral environment enhance the growth of cariogenic bacteria. Caries lesions may be caused by a range of bacteria, but principal among the cariogenic flora are Gram-positive bacteri a such as *Streptococcus, Lactobacillus,* and *Actinomyces* spp. It has been observed that Gram-positive bacteria are frequently found in dentinal tubules of teeth with deep caries lesions and in teeth with irreversible pulpitis.[4,5]

A variety of stimuli have been demonstrated to have an effect on the pulp. Caries, mechanical injuries, and open restoration margins provide pathways for microorganisms and their toxins to enter the pulp.[6,7] There had been several investigations regarding responses of the pulp to caries[8,9,10]; however, little information is available regarding the pulp tissue changes in response to carious lesion at varying rate of progression. The presentstudy aimed to evaluate histologycally the responses of human

dentika Dental Journal , Vol 15, No. 2, 2010: 165-169

dental pulp to caries progression.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by ethic committee of the Medical School of Universitas Gadjah Mada. Informed consent was obtained from all subjects after the proposed study was fully explained. Twenty volunteers ranging in age from 30 to 40 years who had been scheduled to undergo extraction for various therapeutic reasons, were enrolled in the study. Five vital intact third molars and 15 vital third molars with carious lesion involving enamel (n=5), dentin (n=5), and pulp (n=5) were extracted. The depth of the cavity was judged by clinical examination and further confirmed 3 dimensionally with micro-computed tomogramphy. After decalcifying with 10% ethylene diaminetetraacetic acid (EDTA) solution (pH 7.4), the specimens were then embedded in paraffin, sectioned serially, and stained with Hematoxylin Eosin. The sections were viewed under alight microscope and evaluated according to the criteria listed in Tables 1-2.[11]

Table 1. Scores for inflammatory cell response [11]

Score	Characterization
0	Absence of inflammatory cells
1	Slight inflammatory cell infiltration with polymorphonuclear or mononuclear leukocytes below the lesion
2	Moderate inflammatory cell infiltration involving less than 2/3 of the coronal pulp
3	Severe inflammatory cell infiltration involving more than 2/3 of the coronal pulp or abscess present

Table 2. Scores for tissue disorganization [11]

Score	Characterization
0	Normal tissue
1	Odontoblast layer disorganization beneath the lesion, but central pulp normal
2	Disorganization of the pulp tissue in less than 2/3 of the coronal pulp
3	Pulp necrosis in the coronal pulp

Table 3. Number of samples and degree of inflammation

Lesion	Inflammatory response			
	0	1	2	3
Intact	5	0	0	0
Enamel	0	5	0	0
Dentin	0	4	1	0
Pulp	0	0	3	2

Table 4. Number of samples and degree of tissue dis organization

Lesion	Tissue disorganization			
	0	1	2	3
Intact	5	0	0	0
Enamel	0	5	0	0
Dentin	0	4	1	0
Pulp	0	0	5	0

RESULTS

Allsamples showed histological features of normal pulp with no observable evidence of inflamemation (Figure1). The odontoblasts exhibited a tall columnar shape and established a continuous layer with a clear epithelioid appearance. The odontoblast layer was aligned along the periphery of the pulp. Cell free zone was present subjacent to the odontoblast layer.

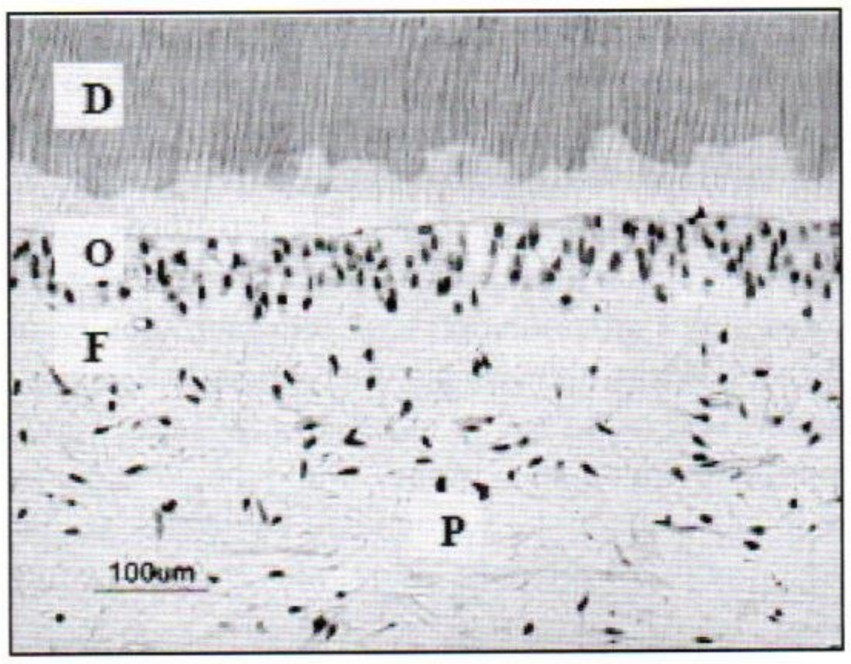

Figure 1. A specimen of normal intact tooth. Histological features of normal pulp tissue is shown. The odontoblasts exhibit a tall columnar shape and establish a continuous layer. Cell free zone is present under the odontoblast layer. (D=dentin; O= odontoblast layer; F= cell free zone; P= pulp proper)

All samples showed slight inflammation in the areas corresponding to the lesions (Figure 2). A few number of polymorphonuclear leukocytes (PMNS), macrophages, and lymphocytes were observed in limited area under the lesion. Odontoblast layer was maintained its continuity and observed aligned along the pulp. Some vacuolizations were observed in the odontoblastic layer subjacent to the lesion. Cell free zone was not observed in all samples. The core of the pulp tissue revealed a normal histologic appearance.

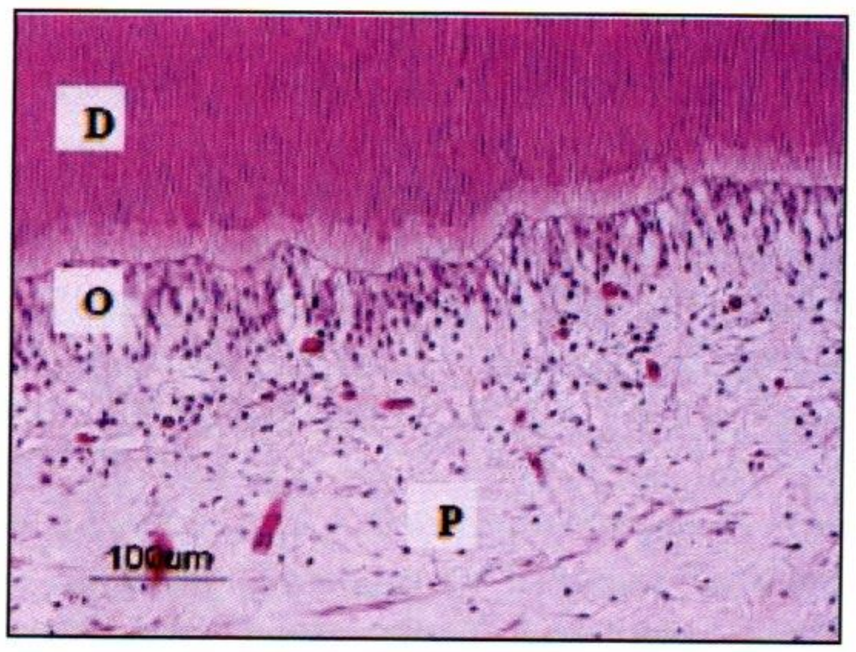

Figure 2. A specimen of tooth with carious lesion con fined to the enamel. Slight inflammation is ob - served under the lesions. Some vacuolizations a reseen in the odontoblastic layer subjacent to the lesion. (D=dentin; O=odontoblast layer; P= pulp proper)

Most samples (4 out of 5 teeth) showed slight inflammation characterized by infiltration of pmns, macrophages, and lymphocytes in approximately 1/3 of the pulp chamber. Odontoblast layer beneath the lesions was disrupted. The core of the pulp tissue revealed a normal histologic appearance. Tertiary dentin formation was observed beneath the lesion.

Moderate inflammation occured in 1 sample, characterized by an intense infiltration of pmns, macrophages, and lymphocytes in approximately 1/2 of the pulp chamber (Figure 3). Numerous blood vessels were found in the tissue surrounding the intense cellular infiltration. Odontoblast layer lost their continuity and could not be observed in the areas subjacent to the lesions; however, some individual odontoblast cells were discernible adjacent to the lesion. In uninflamed areas, odontoblast layer and pulp tissue were found showing normal morphology.

Severe inflammation characterized by an intense infiltrations of pmns, macrophages, and lymphocytes in approximately 2/3 of the pulp chamber (Figure 4) was observed in 3 out of 5 teeth, while the remaining samples showed moderate inflammation. An abscess within the coronal portion of the pulp under the region of the exposure was also seen in samples with severe inflammation. Numerous blood vessels were found in the tissue surrounding the intense cellular infiltration. Odontoblasts adjacent to the affected dentin were destroyed. Some odontoblast cells in other areas was observed. The remainder of the pulp was uninflamed showing normal histology cappearance. Tertiary dentin formation was seen in all samples.

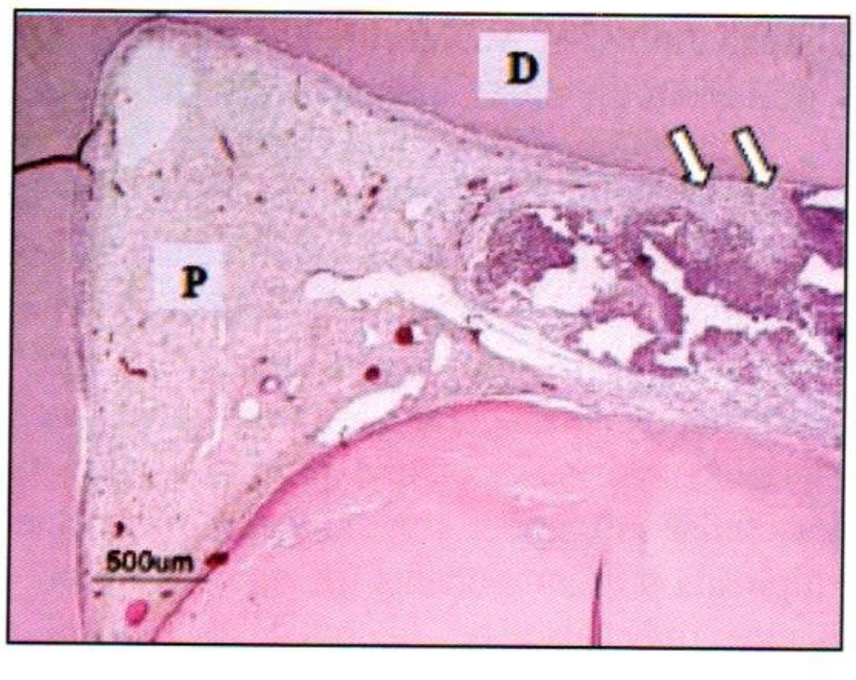

Figure 3. A specimen of tooth with carious lesion Confined to the dentin showing moderate inflammation. Odontoblast layer lost their continuity in the areas subjacent to the lesions (white arrows). (D=dentin; P=pulp proper)

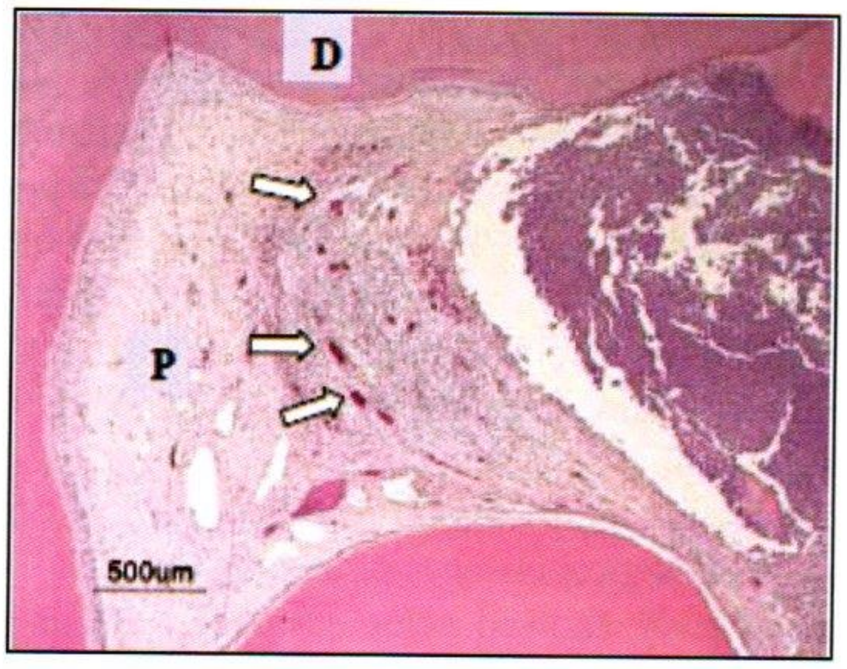

Figure 4. A specimen of tooth with pulp exposure. Severe inflammation is shown adjacent to the exposed area. Numerous blood vessels are found in the tissue surrounding the intense inflammatory cells infiltration (white arrows). Odontoblasts adjacent to the affected dentin are destroyed. (D=dentin; P=pulp proper)

DISCUSSION

This study showed dental pulp tissue changes in response to carious lesions at varying rate of progression.In normal intact teeth, no inflammatory cells infiltration was found in the pulp chamber. In those cases, the dental pulps were reside in a rigid chamber protected by enamel and dentin from the microbial oral environment.[3] Odontoblast layer was observed aligned along the periphery of the pulp; thus, playing a role in the maintenance of the tooth integrity owing to their capacity of depositing new layers of dentin throughout life.[12]

In the cases of carious lesion involving enamel,

dentika Dental Journal , Vol 15, No. 2, 2010: 165-169

slight inflammation was occured in the pulp chamber limited to the areas subjacent to the lesion. In those areas, some pmns, macrophages, and lymphocytes were observed. Caries can exert its effects on the dental pulp even before an infection breaches the dentin enamel junction.[13,14] Microorganisms within a carious lesion may produce a variety of tissue irritating substances. Various products such as bacterial enzymes, metabolic products, and other-extracellular substances are released during bacterial multiplication. Components of the bacterial cell structure may also be liberated after lysis and disintegration of bacterial cells. Those components have capacity to penetrate the dentinal tubules and act as a chemotactic agents to attract the leukocytes to the sites of infection.[15] In addition, odontoblasts exposed to bacteria and their by-products produce interleukin (IL)-8, a potent chemotactic factor for pmns.[16]

Macrophage and pmns play an important role in combating infection by phagocytosis and killing of the bacteria.[17] Inflammation is a protective response of the body to injury. Whenever the pulp is subjected to injury, the immune system will trigger an inflammatory response to limit tissue damage by eliminating and digesting invading organisms and cellular debris.[9,10]

The earliest signs of pulp reaction to dental caries are morphological changes of odontoblast cell.[12] The present study showed some vacuolizations in the odontoblast layer in the areas subjacent to the enamel lesion indicating early damage of the odontoblast cells; however, odontoblast layer was maintain edits continuity indicating that odontoblasts still survive and kept their vitality and functions. Therefore, they may upregulate their synthetic and secretory activities to secrete a reactionary dentin matrix as a part of repair in the dentin pulp organ.[18]

The present data showed that in most cases of the teeth with caries confined to the dentin, slight inflammation was occured. In those cases, inflammatory cell sinfiltration (pmns, macrophages, and lymphocytes) which was involving approximately 1/3 areas of the pulp chamber was observed. When enamel are lost as caries progressses, the exposed dentinal tubules provide diffusion channels from the surface to the pulp. Following exposure to the oral environment, bacterial macro molecules may penetrate the tubules and provoke an inflammatory response in the pulp. Once the caries lesion with its bacterial front has penetrated the dentin and to the pulp tissue proper, amassive mobilization of the inflammatory cells will take place.[19,20]

In the cases of caries with pulp exposure, the teeth showed moderate to severe inflammation. In this case, the pulp was exposed directly to the oral environment; therefore, the bacteria and their toxins and products may stimulate more severe pulp inflammation compare to caries with out pulp exposure. All teeth showed pulp tissue disorganization in approximately 2/3 of the pulp chamber. Pulp tissue injury can occur both as the result of the direct effects of microbial products and as indirect consequences of microbial activation of nonspecific host immune responses.[6] As the size of the exposure enlarges and an everincreasing number of bacteria enter the pulp, the defending forces are overwhelmmed. The pulp has a finite blood supply. There fore, when the demand for inflammatory elements exceeds the ability of the blood transport them to the site of bacterial penetration, the bacteria become too numerousforthe defenders and are able to proliferate without constraint. If the inflammatory reactions become uncontrolled, pulpal cell injury will occur and compromise pulpal vitality and survival.[20]

An increase in the severity of the pulp tissue destruction was noted in this study as the caries progressedtowardsthepulp. As the caries progresssed, an increase of bacterial products, activation of complement system due to the development of a local immune reaction, and the accumulation of arachidonic acid metabolism with the destruction of cellular components would occur. These might provoke an increase of macrophages and pmns in the advanced stage of immune reaction in human dental pulp. Although macrophages and pmns are important protective cells, they might cause damage to the host tissues. During phagocytosis, their cytoplasmic granules contain lysosomal enzymes that, on release, degrade the structural elements of tissue cells and extracellular matrices leading to pulpal damage.[21]

The present study showed that tertiary dentin formation was observed in most samples of carious teeth with dentinal lesion and pulp exposure. This data is in agreement with the previous study by Bjørndal and Darvann.[22] Tertiary dentin formation is the most common feature of pulp repair to prevent invasion of the pulp chamber by bacteria.[23]

In conclusion, caries may induce inflammation and disorganization of the pulp tissue. The severity of pulpal inflammation and disorganization depends on the depth of the lesion.

References

1. Goodis HE. History of pulp biology. In: Hargreaves

KM, Goodis HE. eds. Seltzer and Bender's dental pulp. Chicago: Quintessence Publishing Co, 2002: 1-11.
2. Okiji T. Pulp as a connective tissue. In: Hargreaves KM, Goodis HE. eds. Seltzer and Bender's dental pulp., Chicago: Quintessence Publishing Co, 2002: 95-121.
3. Trowbrigde H, Kim S, Suda H. Structure and functions of the dentin and pulp complex. In: Cohen S, Burn RC, eds. Pathways of the pulp. 8th ed., St Louis: Mosby, 2002: 411-12.
4. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002; 13: 171-83.
5. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65: 1028-37.
6. Levin LG. Pulpal irritants. Endod Top 2003; 5: 2-11.
7. Yu C, Abbott PV. An overview of the dental pulp: its functions and responses to injury. Aust Dent J 2007; 52(Suppl.1): S4-16.
8. Huang FM, Tsai CH, Chen YJ, Liu CM, Chou MY, Chang YC. Upregulation of tissue-type plasminogen activator in inflamed human dental pulps. Int Endod J 2005; 38: 328-33.
9. Rodd HD, Boissonade FM. Immunocytochemical investigation of immune cells within human primary and permanent tooth pulp. Int J Paediatr Dent 2006; 16: 2-9.
10. Hahn, C, Liewehr FR. Innate immune responses of the dental pulp to caries. J Endod 2007; 33: 643-51.
11. Accorinte MLR, Loguercio AD, Reis A, Costa CAS. Response of human pulps capped with different self-etch adhesive systems. Clin Oral Invest 2008; 12: 119-27.
12. Arana-Chavez VE , Massa LF . Odontoblasts : the cells forming and maintaining dentine. Int J Biochem Cell Bio 2004; 36: 1367-73.
13. Bjørndal L. Dentin and pulp reactions to caries and operative treatment: biological variables affecting treatment outcome. Endod Top 2002; 3: 123-36.
14. Kidd EAM, Bjorndal L, Beighton D, Fejerskov O. Caries removal and the pulpo-dentinal complex. In: Fejerskov O, Kidd E, eds. Dental caries: the disease and its clinical management. Oxford: Blackwell Munksgaard Ltd, 2008: 371-2.
15. Cicchetti G, Allen PG, Glogauer M. Chemotactic signaling pathways in neutrophils: from receptor to actin assembly. Crit Rev Oral Biol Med 2002; 13:220-8.
16. Levin LG. Pulpal irritants. Endod Top 2003; 5:2-11
17. DeLeo FR. Modulation of phagocyte apoptosis by bacterial pathogens. Apoptosis 2004; 9:399-413.
18. Tziafas D. The future role of a molecular approach to pulp-dentinal regeneration. Caries Res 2004; 38: 314-20.
19. Olgart L, Bergenholtz G. The dentine-pulp complex: responses to adverse influences. In: Bergenholtz G, Horsted-Bindslev P, Reit C. eds. Textbook of endodontology., Oxford: Blackwell Publishing Ltd, 2003: 21-36.
20. Smith AJ. Pulpal responses to caries and dental repair. Caries Res 2002; 36: 223-32.
21. Nair PNR. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004; 15:348-81.
22. Bjørndal L, Darvann T. A light microscopic study if odontoblastic and non-odontoblastic cells involved in tertiary dentinogenesis in well-defined cavitated carious lesions. Caries Res 1999; 33: 50-60.
23. Mitsiadis TA, Rahiotis C. Parallels between tooth development and repair: conserved molecular mechanisms following carious and dental injury. J Dent Res 2004; 83: 896-902.

ISSN: 1693-671X)
Dentika Dental Journal, Desember 2010
Edisi Cetak Dentika Dental Journal