

Original Article

Histological evaluation of pulp tissue from second primary molars correlated with clinical and radiographic caries findings

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ABSTRACT

Background: Managing dental caries in young children is demanding due to the elusions present on the right diagnostic criteria for treatment. The present study evaluated the histological status of pulp tissues extracted from primary second molar with caries involvement. Histological findings are correlated with clinical and radiographic assessment.

Materials and Methods: Simple experimental study was conducted on upper or lower second primary molars with occlusal (22 teeth) or proximal (22 teeth) dental caries. Selected children were below 6 years of age. Percentage of caries involvement, residual dentin thickness (RDT), radiographic assessment of interradicular and periapical areas, clinical caries depth and signs and symptoms are the parameters considered for comparing with the histological findings. The specimens were grouped based on the nature of the inflammatory process as acute or chronic. The data were analyzed by Student *t*-test to compare histological types of inflammation with clinical parameters. *P* value < 0.05 was considered as significant.

Results: Four cases revealed severe acute inflammation in coronal and relatively mild acute inflammation in radicular pulp. In the rest of the specimen coronal and radicular pulp had similar acute or chronic inflammatory changes. Histological evidence of pulpitis correlated with dental caries depth of $\geq 80\%$, RDT of ≤ 1 mm, radiographic rarefactions in the interradicular regions and symptoms of pain.

Conclusion: Primary second molars with more than two-third caries involvement with symptoms of pain histologically showed inflammation of both coronal and radicular pulp tissues in all cases.

Key Words: Caries, deciduous, dental pulp, immunohistochemistry, inflammation

Received: December 2013

Accepted: May 2013

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INTRODUCTION

Diagnosis of inflammation of pulp in primary teeth due to dental caries is eluding based on the clinical and radiographic assessment. Accurate diagnosis of inflammatory statues of the dental pulp in primary teeth and selecting the right treatment based on the diagnosis enhances success of restorative/endodontic

treatment.^[1] This is important while treating young children with limited ability to co-operate as the need for re-treatment can be drastically reduced.

Pulpal inflammation in carious primary teeth is classified according to the clinical signs and symptoms. However, in young children this information is not reliable. Hence, we have to rely on the radiographic assessment. Furthermore, the only way to assess the correct inflammatory status of the pulp is histopathology.^[2]

Managing dental caries in young children is demanding due to the elusions present on the right diagnostic criteria for treatment. There is a need to provide evidence-based treatment to primary teeth with dental caries involvement.^[3] The treatment

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modalities such as indirect pulp capping, pulpotomy, pulpectomy and extraction should be based on the evidence of inflammatory changes in the dental pulp.^[4] There is insufficient evidence in the existing literature, which correlates signs, symptoms and histopathological status of the dental pulp tissues in carious primary teeth with the right treatment choices.

Hence, the present study is carried out to evaluate the histopathological status of pulp tissues retrieved from primary second molar with caries involvement. Histological findings are correlated with clinical and radiographic assessment.

MATERIALS AND METHODS

Simple experimental study was conducted on 44 children 6 years and below with no relevant medical condition that could affect pulp histology attending University Dental Hospital Sharjah (UDHS). Teeth selected were restorable with less than 1/3 root resorption and no radiographic evidence of calcification, internal or external resorption. Upper or lower second primary molars with occlusal (22 teeth) or proximal (22 teeth) dental caries were selected. This study was carried out after obtaining approval from the Ethics Committee, College of Dentistry, University of Sharjah, United Arab Emirates as per Helsinki declaration. The parents of the subjects involved in this study were informed regarding the procedure with the written consent.

Children reported to UDHS in 2 weeks of onset of pain. Nine children presented within 2-3 days of onset whilst the remaining children in the study presented with persistent pain for more than 10 days. On clinical examination, all selected teeth were firm with healthy gingiva. Intraoral periapical radiographs were taken to assess the percentage of caries involvement to the total hard tissue thickness, residual dentin thickness (RDT) and the presence of interradicular/periapical rarefaction. Radiographs were mounted on the grid mount (Nix Company Ltd., Tokyo, Japan) and viewed on the X-ray viewer. Grid mount was used to measure the RDT and caries depth. Since measurements were calibrated with a grid the recordings were accurate and repeatable as tested by the principal investigator using the same procedure within a short time. The caries depth was calculated as a percentage of whole dentin thickness as previously reported.^[3]

Clinical procedure involved local anesthesia, rubber dam, caries removal, opening pulp chamber,

amputation of coronal pulp with spoon excavator. In the entire sample bleeding did not stop with 5 min of pressure with saline moist cotton pellet on the radicular pulp. Following which radicular pulp tissues were removed with barbed brough's. Single visit pulpectomy procedure was completed and the tooth restored. The removed coronal and radicular pulp tissues were placed in 10% buffered formalin and sent for histopathological examination.

Dental pulp tissue specimens were embedded in paraffin and thereafter sectioned. The specimens were prepared for staining at two levels. Sections were cut parallel to the long axis to a thickness of 5 μ m and stained with hematoxylin and eosin (H & E) in order to determine the type of inflammatory cells, vascular proliferation and fibrosis. H & E findings were confirmed by masson trichome (for fibrosis) and immunohistochemistry for CD (cluster differentiation) 45 leukocyte common antigen and CD34 for endothelial cell markers (DAKO, Denmark). All staining procedure protocols were performed as described in the previous study.^[5] The degree of pulp inflammation was graded for CD45 as: 0=normal, +(mild)=1-50 cell/20 high power field (HPF), ++ (moderate) = 51-100 cells/20 HPF, +++ (severe) = >100 cells/20 HPF. CD34 was graded as + = up to 50 blood vessels with inflammatory infiltrate/20 HPF, ++ = 51-100 blood vessels with inflammatory infiltrate/20 HPF and +++ = >100 blood vessels with inflammatory infiltrate/20 HPF. The lymphocyte counting procedure was same as in one recent study^[6] using Olympus BX51 microscope (Hamburg, Germany). The extent of fibrosis was subjectively graded as mild, moderate and severe depending on the density of collagen in Masson trichrome stained sections. The numbers of cells/20 HPF were counted by the co-investigator and accuracy was checked by repeating the counting by the same investigator using the same procedure within a short time. The data were analyzed by Student *t*-test to compare histological types of inflammation with clinical parameters. *P* value < 0.05 was considered as significant.

RESULTS

Children's ages were below 6 years. Twenty two teeth each for occlusal and proximal caries were assessed. Pulp tissues taken from upper or lower primary second molars from 44 children were subjected to histological analysis [Table 1]. Correlations of histological findings with clinical parameters are shown in Table 2.

The results presented in Table 1, show various histological pulpal conditions in caries teeth with occlusal or proximal lesions. The specimens were grouped based on the nature of the inflammatory process. Group A revealed marked focal or diffuse acute inflammatory infiltrate predominantly comprising of neutrophils admixed with variable amount of lymphocytes, plasma cells and occasional eosinophils [Figure 1]. Rest of the groups (B1, B2 and C) showed mild to severe chronic inflammatory

infiltrate predominantly composed of lymphocytes, macrophages and plasma cells along with the proliferation of blood vessels and variable amount of fibrosis [Table 1, Figure 2]. Groups B1, B2 and C were designated based on the extent of fibrosis and degree of angiogenesis. The histological findings of coronal and radicular pulp were same in all cases except four in group A. These four cases in group A revealed severe acute inflammation in coronal and

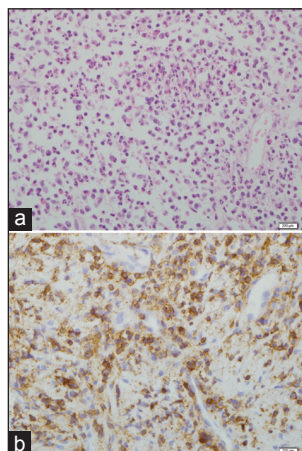


Figure 1: Representative histological sections from group A with acute inflammation revealing marked infiltration of neutrophils admixed with variable amount of macrophages, lymphocytes and plasma cell and scattered dilated blood vessel (a: Stained with H & E/20 HPF Olympus BX51) and the same section stained with leukocyte common antigen-CD45 staining predominantly cell membrane (b)

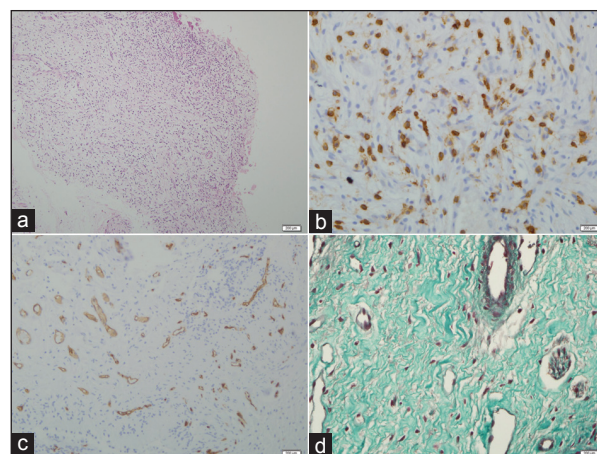


Figure 2: Representative histological sections from group B with chronic inflammation, revealing moderate infiltration of lymphocytes and plasma cells along with angiogenesis and fibrosis (a: Stained with H & E/ 20 HPF- Olympus BX51) and the same section stained with leukocyte common antigen-CD45 staining predominantly cell membrane (b) CD34 staining endothelial cell lining of blood vessels (c) and fibrosis stained with Masson trichrome (d)

Table 1: Histological findings of dental pulp tissues from primary second molar with occlusal and proximal caries

Group	Number		H and E findings	Trichrom	Immunohistochemistry							
	Occlusal	Proximal			Inflammation	Extent of fibrosis	CD 45			CD 34		
							+	++	+++	-	+	++
A	4	6	Acute	Negative	0	0	10	0	0	10	0	
B1	4	6	Chronic	Mild	0	10	0	0	0	4	6	
B2	14	6	Chronic	Moderate	0	20	0	0	6	14	0	
C	0	4	Chronic	Severe	4	0	0	2	2	0	0	

Table 2: Correlating histological groups with clinical parameters

Group	Number		Dental caries %					RDT (mm)					Caries depth (mm)					P value	
	Occ	Prox	100	95	90	80	0	0.25	0.5	1	2	Occlusal			Proximal				
												3	4	5	1	3	4		5
A	4	6	2	4	0	4	2	4	0	4	0	2	2	0	2	4	0	0	>0.05
B1	4	6	6	0	2	2	6	0	2	2	0	2	0	2	0	2	4	0	>0.05
B2	14	6	10	4	2	4	10	4	2	4	2	4	4	4	0	4	2	0	>0.05
C	0	4	2	2	0	0	2	2	0	0	0	0	0	0	0	0	2	2	>0.05

RDT: Residual dentin thickness; Occ: Occlusal; Prox: Proximal

relatively mild acute inflammation in radicular pulp. Nine cases presented within 2-3 days of onset of pain revealed acute inflammatory changes where in the rest when pain persisted for 10 days pulp tissues showed chronic inflammation except for one.

The results presented in Table 2, correlated histological findings with clinical parameters. All children participating in the present research presented with pain in the teeth, upon clinical examination healthy gingival tissues with the tooth being in the range of normal physiological mobility were observed in all cases. Intraoral radiographic examinations revealed minimal interradicular rarefactions in the absence of apical radiolucency in all cases. Depth of caries as determined by dental caries percentage $\geq 80\%$ and RDT ≤ 1 mm was associated with varying degree of inflammation of the pulp in all cases. Occlusal clinical caries depth of 2-5 mm and proximal break down of 1-5 mm were associated with inflammatory changes in the pulp. We could not find any significant difference ($P > 0.05$) between the histological types of inflammation and any of the clinical parameters assessed in this study.

DISCUSSION

Histopathological evaluation was carried out on pulp tissues taken from carious primary second molars with occlusal or proximal lesions of children less than 6 years. The criteria for age selecting was to make sure that physiological root resorption did not have any inflammatory effect on the dental pulp tissues.^[7] Nature of pain in the tooth being spontaneous was considered for selecting these teeth for the present work.^[8] Whilst interoral examination of teeth selected for the present study was firm with healthy gingiva indicating the teeth being vital.^[9] Radiographic examination of the teeth ruled out pathology such as internal or external resorption and calcifications in the pulp chamber.^[10,11]

Dental pulp tissues extracted from primary second molars with either occlusal or proximal caries were examined histopathologically. The specimens were grouped based on the nature of the inflammatory process as acute and chronic. We observed histological evidence of pulpitis in all cases as reported previously.^[3,12] Ten cases revealed acute pulpitis whilst the rest showed variable degree of chronic pulpitis.^[3,12] The nature and severity of inflammation was similar in coronal and radicular pulp in 40 cases; however,

four cases revealed severe acute inflammatory changes in coronal pulp and mild acute inflammation in the radicular pulp as observed in previous study.^[13] In this study, for the first time, we are reporting that histological type of inflammation (acute vs. chronic) depends on the presentation of the symptoms. All cases showing acute inflammatory changes presented to the clinic within 2-3 days. Children presenting after 1 week of symptoms had chronic inflammatory changes noted in the pulp tissues. Hence, there is a strong correlation between the history of spontaneous pain for 10 days and chronic inflammatory changes in the dental pulp noted in all tooth samples, which had a history of irreversible pulpitis in the present study. However, validity of pain history and symptoms was poorly correlated with histopathological findings in previous studies.^[1,14]

All teeth selected in this study had symptoms of pain suggestive of irreversible pulpitis, intraoral examination revealed healthy gingiva surrounding the tooth whilst teeth being in the range of normal physiological mobility. Therefore, the children participating in the present study clinically appeared to suffer from acute to chronic pulpitis depending on the duration of the pain. The absence of mobility and swelling of the gums ruled out non-vital teeth in this study.^[4] Presence of inter-radicular rarefaction in all the radiographs examined indicates inflammation of the coronal pulp, which is tracking down into the furcation area due to the presence of accessory root canals or area of macroscopic root resorption.^[15]

Radiographic assessment of dental caries extensions in occlusal and proximal lesions were between 80% and 100% within the total dentin thickness, which showed acute inflammatory changes in 10 specimens while the rest revealed chronic changes in the pulp tissues histologically. The findings are in agreement with previous reports comparing pulp response to caries depth.^[3] RDTs were between 0.25 mm and 1 mm in 24 specimens while in the rest dental caries had reached pulp. RDT was used as a predictive measure of the likely pulpal reaction.^[16] We observed that RDT in the range noted in the present work were associated with inflammatory changes in the pulp.

Correlating clinical caries depth of 1-5 mm with histological status of the dental pulp tissues revealed acute to chronic inflammatory changes.^[17] It should

be noted that actual caries depth as determined by radiograph was $\geq 80\%$ in clinically shallow occlusal and proximal lesions showing pulp response in the present study. Hence, clinical depth of the cavity could not be a reliable indicator in selecting the right treatment for the tooth; it should be augmented with radiographic and clinical findings. It is interesting to note that proximal break down of 1 mm in two cases were associated with acute inflammation, which could be attributed to rapid progression of caries in a short time.^[18]

Correlating histological findings with clinical parameters in the present study justified the authors' choices of treatment (single visit pulpectomy). It could be hypothesized from the present results that a primary molar with dental caries depth less than 80%, RDT more than 1 mm, no radiographic rarefactions in the periapical or interradicular regions, absence of pain, firm tooth with healthy surrounding gingiva could be effectively treated with stepwise excavation method.^[19]

CONCLUSION

Primary second molars with more than two-third caries involvement with symptoms of pain histologically showed inflammation of both coronal and radicular pulp tissues in all cases. Considering clinical and radiographic parameters, single visit pulpectomy was considered to be the optimal treatment choice for the selected primary second molars in this study.

ACKNOWLEDGMENTS

We acknowledge the support of Sharjah University Grant, Research Project No. UOS 111008.

REFERENCES

1. Schröder U. Agreement between clinical and histologic findings in chronic coronal pulpitis in primary teeth. *Scand J Dent Res* 1977;85:583-7.
2. Di Nicolo R, Guedes-Pinto AC, Carvalho YR. Histopathology of the pulp of primary molars with active and arrested dental caries. *J Clin Pediatr Dent* 2000;25:47-9.
3. Kassa D, Day P, High A, Duggal M. Histological comparison of pulpal inflammation in primary teeth with occlusal or proximal caries. *Int J Paediatr Dent* 2009;19:26-33.

4. Rodd HD, Waterhouse PJ, Fuks AB, Fayle SA, Moffat MA, British Society of Paediatric Dentistry. Pulp therapy for primary molars. *Int J Paediatr Dent* 2006;16 Suppl 1:15-23.
5. Silva AC, Faria MR, Fontes A, Campos MS, Cavalcanti BN. Interleukin-1 beta and interleukin-8 in healthy and inflamed dental pulps. *J Appl Oral Sci* 2009;17:527-32.
6. Grogg KL, Lohse CM, Pankratz VS, Halling KC, Smyrk TC. Lymphocyte-rich gastric cancer: Associations with Epstein-Barr virus, microsatellite instability, histology, and survival. *Mod Pathol* 2003;16:641-51.
7. Monteiro J, Day P, Duggal M, Morgan C, Rodd H. Pulpal status of human primary teeth with physiological root resorption. *Int J Paediatr Dent* 2009;19:16-25.
8. Estrela C, Guedes OA, Silva JA, Leles CR, Estrela CR, Pécora JD. Diagnostic and clinical factors associated with pulpal and periapical pain. *Braz Dent J* 2011;22:306-11.
9. Fuks AB. Vital pulp therapy with new materials for primary teeth: New directions and treatment perspectives. *Pediatr Dent* 2008;30:211-9.
10. Mello-Moura AC, Bonini GA, Zardetto CG, Rodrigues CR, Wanderley MT. Pulp calcification in traumatized primary teeth: Prevalence and associated factors. *J Clin Pediatr Dent* 2011;35:383-7.
11. Lad N, Hosey MT, Hunter KD. Localized idiopathic internal resorption in the primary dentition. *J Clin Pediatr Dent* 2010;34:339-41.
12. Bjørndal L. The caries process and its effect on the pulp: The science is changing and so is our understanding. *Pediatr Dent* 2008;30:192-6.
13. Raslan N, Wetzel WE. Exposed human pulp caused by trauma and/or caries in primary dentition: A histological evaluation. *Dent Traumatol* 2006;22:145-53.
14. Michaelson PL, Holland GR. Is pulpitis painful? *Int Endod J* 2002;35:829-32.
15. Winter GB. Abscess formation in connexion with deciduous molar teeth. *Arch Oral Biol* 1962;7:373-9.
16. Murray PE, Smith AJ, Garcia-Godoy F, Lumley PJ. Comparison of operative procedure variables on pulpal viability in an *ex vivo* model. *Int Endod J* 2008;41:389-400.
17. Bjørndal L, Mjör IA. Pulp-dentin biology in restorative dentistry. Part 4: Dental caries – Characteristics of lesions and pulpal reactions. *Quintessence Int* 2001;32:717-36.
18. Vanderas AP, Manetas C, Koulatzidou M, Papagiannoulis L. Progression of proximal caries in the mixed dentition: A 4-year prospective study. *Pediatr Dent* 2003;25:229-34.
19. Bjørndal L. Indirect pulp therapy and stepwise excavation. *Pediatr Dent* 2008;30:225-9.

How to cite this article: Gopinath VK, Anwar K. Histological evaluation of pulp tissue from second primary molars correlated with clinical and radiographic caries findings. *Dent Res J* 2014;11:199-203.

Source of Support: Nil. **Conflict of Interest:** None declared.