Original Article

Relationship Between the Remaining Dentin Thickness and Coronal Pulp Status of Decayed Primary Molars

Roula Berbari[1], Hussein Fayyad-Kazan[2], Mohamad Ezzedine[3], Mohammad Fayyad-Kazan[4], Daniel Bandon[5], Elia Sfeir[1]

[1]Department of Pediatric Dentistry, Faculty of Dental Medicine, Lebanese University, [2]Department of Biology, Laboratory of Cancer Biology and Molecular Immunology, Faculty of Sciences-1, Lebanese University, [3]Department of Biology, Faculty of Sciences, Lebanese University, Beirut, Lebanon, [4]Department of Molecular Biology, Laboratory of Neurovascular Signaling, Institute of Molecular Biology and Medicine, Free University of Brussels, Gosselies, Belgium, [5]Department of Pediatric Dentistry, University of the Mediterranean Aix-Marseille II, Marseille, France

Received : 21-07-17.
Accepted : 21-08-17.
Published : 18-09-17.

ABSTRACT

Aims and Objectives: The aim of this study was to assess the correlation between the remaining dentin thickness (RDT) in deep decayed primary molars and the inflammatory status and bacterial composition of the corresponding coronal pulp. We hypothesized that RDT could be used as a reference for clinicians in assigning the indication for pulpotomy.

Materials and Methods: Pulpotomies were conducted on the cameral pulp of 48 primary molars. Microorganisms, such as *Lactobacillus* sp., *Streptococcus* sp., and *Prevotella* sp., were identified and quantified and levels of tumor necrosis factor-alpha (TNF-α) and interlukin-6 (IL-6) were assessed. The correlation between the pre-operative RDT based on radiographic images and inflammatory-microbial profiles *in vitro* was evaluated using Spearman's rho correlation coefficient. All data analysis was performed using a statistical software program (SPSS 20.0, SPSS Inc., Chicago, IL, USA).

Results: Immunological and microbiological studies revealed elevated levels of TNF-α and IL-6 cytokines, and *Lactobacillus* sp., *Streptococcus* sp. and *Prevotella* sp. in the cameral pulp with an RDT measuring up to 1.1 mm. No significant relationship could be established between RDT, inflammatory status and microbial content of the pulps.

Conclusion: The RDT remains a key clinical factor that needs to be assessed when establishing the indication for pulpotomy. Additional parameters that can improve this therapy should be investigated in the future.

KEYWORDS: *Bacteria, cytokines, pulpotomy, remaining dentine, temporary molars*

INTRODUCTION

Like other forms of inflammatory response, pulp inflammation is a protective immune response against harmful tissue infection or injury.[1,2] Akin to other types of connective tissue, inflammatory response in dental pulp involves the recruitment of immunocompetent blood cells, including macrophages. Once macrophages become activated, they secrete pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interlukin-6 (IL-6).[1] In general, tooth pulp is a sterile tissue located in an encased nonextensible space.[3] The presence of cariogenic oral bacteria in affected dentin is the main etiology of dental pulp inflammation and infection.[3-5] Pulp reaction starts as soon as the peripheral extremities of the dentinal tubules are invaded before the pulp coming into direct contact with the microorganisms.[6] Depending on the severity of the inflammation, in deep carious lesions, immune cells can secrete pro-inflammatory cytokines (TNF-α, IL-6), and cytotoxic components to protect the pulp against disease propagation.[6-11]

Clinically, it is crucial to define the exact status of the pulpal parenchyma. However, congruity cannot always be established between the clinical diagnosis and inflammatory status of the pulpal parenchyma.

Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_267_17

Address for correspondence: Dr. Roula Berbari, Department of Pediatric Dentistry, Faculty of Dental Medicine, Lebanese University, Beirut, Lebanon.
E-mail: rouberdag@hotmail.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Berbari R, Fayyad-Kazan H, Ezzedine M, Fayyad-Kazan M, Bandon D, Sfeir E. Relationship between the remaining dentin thickness and coronal pulp status of decayed primary molars. J Int Soc Prevent Communit Dent 2017;7:272-8.

The aim of this study was to determine whether the remaining dentin thickness (RDT) between the bottom of the carious lesion and the pulp could serve as a reliable indicator for assessing the physiological status of the dental pulp in primary molars.

Materials and Methods

This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the Scientific Review Committee of the Faculty of Dental Medicine, Lebanese University Beirut, Lebanon. The parents of participants signed a consent form stipulating their acceptance of and commitment to periodic inspection visits. A standardized medical questionnaire was used to collect data on the participants' overall health.

The research was carried out into two parts, simultaneously. First, the clinical part was performed by two experienced instructors at the Department of Paediatric Dentistry in the Faculty of Dental Medicine at the Lebanese University. Second, the immunological and microbiological study of the pulp status was carried out in the research section of the Faculty of Sciences of the same university. The recruitment of patients, who came to the Department of Pediatric Dentistry for dental care, was done from May 2015 to December 2016.

Pulpotomies were conducted with respect to the following inclusion criteria: (1) asymptomatic vital deep decayed primary molars, (2) RDT ranging between 0.3 and 1.1 mm, (3) physiological root resorption not exceeding half of the root height,[12] and (4) tooth restorable by stainless steel crown. The exclusion criteria comprised of: (1) an uncooperative patient, (2) necrotic primary molars, (3) excessive bleeding during pulpotomy, (4) peri-radicular or inter-radicular radiolucency, (5) pulpal calcifications, (6) internal or external pathological resorption and (7) physiological root resorption exceeding two-thirds of the root height.[13,14]

The samples comprised of 48 temporary molars. The number chosen (n = 48) was sufficient to carry out the statistical analysis, and more importantly to determine the significance of the results. In fact, the minimum needed sample size was calculated according to the following formula for a normally-distributed sample:

$$n = \frac{z^2 \times p(1-p)}{d^2} = \frac{1.96^2 \times 0.5(0.5)}{0.15^2} = 43$$

z = z-score (1.96 for a confidence level of 95%)

p = expected proportion (estimated 50%)

d = marginal error (chosen 15% in our study)

Consequently, the sample in our study consisting of 48 primary molars can be considered statistically representative.

Pulpotomy procedure

A preoperative periapical digital X-ray was performed, and radiographic measurements of RDT were made. A nonfixative vital pulpotomy with reinforced zinc oxide eugenol (intermediate restorative material) was then conducted following the conventional pulpotomy protocol.[15,16] The procedure started with the eviction of the demineralized tissue at the peripheral cavity walls then near the pulp chamber using a round steel bur (Dentsply Maillefer ref: D0023). A thin layer of dentin covering the pulp chamber was maintained. A rubber dam was placed and disinfected to avoid any exogenous bacterial contamination.[17] Finally, the pulp ceiling was eliminated cautiously using a sterile round bur under physiological saline irrigation to avoid damage to the underlying pulp.

Preparation of pulp samples

The coronal pulp parenchyma was thoroughly extracted with a well-sharpened excavator (Zeffiro: ZFE004 #3 Batch DØ1ABA, Italy) and placed in a sterile tube containing 1 ml of reducing medium (Thioglycollate: SIGMA). Then, the samples were immediately transported to the laboratory for histological study. After completing the pulpotomy, the molar was sealed with a stainless-steel crown (3M ESPE). Postoperative periapical digital X-rays were performed under the same conditions as the preoperative ones.

Microbiological study

The tubes containing the pulp samples were shaken in a tube shaker for 30 s to disperse bacterial aggregates.

Next, 100 µl of bacterial sample was spread, in duplicate, on the following solid media: trypticase soy agar (HiMedia Laboratories, Mumbai, Maharashtra, India) supplemented with 5% sheep blood for total viable microorganism count, Rogosa Agar (HiMedia Laboratories, Mumbai, Maharashtra, India) for *Lactobacillus* sp. Count, Schaedler K-V Agar (HiMedia Laboratories, Mumbai, Maharashtra, India) for *Prevotella* sp. count and mitis salivarius agar (MSA) (HiMedia Laboratories, Mumbai, Maharashtra, India) for *Streptococcus* sp. count.[18]

The plates were incubated under anaerobic conditions for 1 week, whereas the MSA plates were incubated in an atmosphere of 5% CO_2 for 48 h.

After incubation, microbial counts were performed with a digital colony counter (HiMedia-LA660). Cell morphology was evaluated by Gram staining technique.

Immunological study

Each sample was collected and transferred into a single well of an IL-6/TNF-α assay plate supplied by the manufacturer (R&D System Bio-techne) and the ELISA assay was applied.[19,20]

Statistical analysis

A computer statistical software program (SPSS 20.0, SPSS Inc., Chicago, IL, USA) was used to determine the descriptive statistics (mean, standard deviation [SD]) of the different variables. The data were presented as means (±SD) and analyzed based on the results of the Kolmogorov–Smirnov and Shapiro–Wilk normality test using the nonparametric Mann–Whitney U-test and Spearman's rho correlation coefficient. The value of $P < 0.05$ (*) was considered statistically significant.

Results

We first measured the RDT in 48 primary molars. Due to the significant differences in the RDT measures, the samples were divided into two groups. Group A included 26 samples with RDT: 0.5 mm <RDT ≤1.1 mm, whereas Group B included 22 samples with RDT: 0.3 mm ≤RDT ≤0.5 mm.

In a second step, and on performing culture procedures followed by morphology identification, the cameral pulp samples were characterized by bacterial content. Three major bacterial strains, *Lactobacillus* sp., *Streptococcus* sp. and *Prevotella* sp. were identified. The abundance of these strains was not totally similar between the two groups with *Lactobacillus* sp. showing an average of 26 colony-forming unit/μg (CFU/μg) in samples of Group A versus 27 CFU/μg in samples of Group B, *Streptococcus* sp. displaying an average of 35 CFU/μg in samples of Group A versus 45 CFU/μg in samples of Group B and finally, *Prevotella* sp. exhibiting an average of 40 CFU/μg in samples of Group A versus 43 CFU/μg in samples of Group B [Figures 1 and 2].

We subsequently evaluated the levels of IL-6 and TNF-α by ELISA assay in the examined samples. These pro-inflammatory cytokines showed differential amounts between Groups A and B. Average IL-6 levels were 900 pg/ml and 1100 pg/ml in Group A and in Group B, respectively. Whereas, average TNF-α levels were 1100 pg/ml and 1250 pg/ml in samples Group A and Group B, respectively [Figures 3 and 4].

Despite the observed differential bacterial composition and cytokine levels between the two groups, these differences were not statistically significant [Table 1].

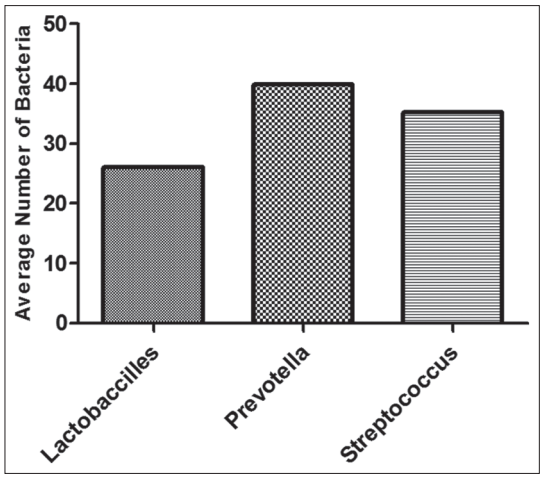

Figure 1: The three different bacteria strains identified in Group A, of which *Prevotella* is predominant (40 colony-forming unit/μg)

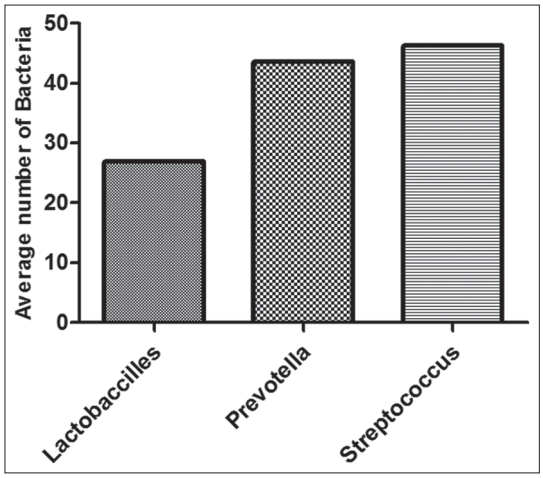

Figure 2: The three different bacteria strains identified in Group B, of which *Streptococcus* is predominant (43 colony-forming unit/μg)

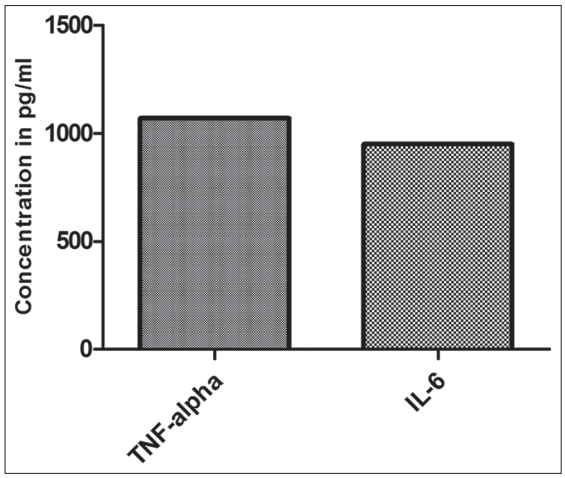

Figure 3: Average interlukin-6 and tumor necrosis factor-α cytokine levels in Group A

Discussion

In deep decayed temporary molars, it is impossible to accurately assess the pulpal inflammatory status and its severity, both of which are key factors for assessing prognosis in primary teeth.[21]

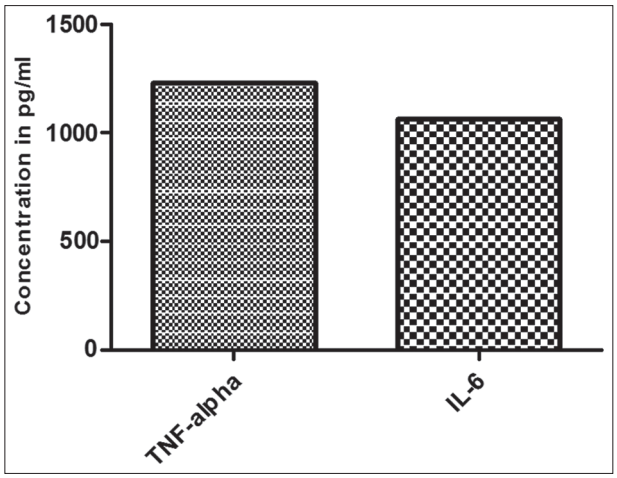

Figure 4: Average interlukin-6 and tumor necrosis factor-α cytokine levels in Group B

Table 1: Correlation between Group A (0.5 mm<remaining dentin thickness ≤1.1 mm) versus Group B (0.3 mm ≤ remaining dentin thickness ≤0.5 mm), bacterial composition and cytokine levels in both groups using the nonparametric Mann-Whitney U-test and Spearman's rho correlation coefficient

	Mann-Whitney U-test (significance)
Lactobacillus spp. (Group A vs. Group B)	*P*=0.69 (no significant difference)
Prevotella spp. (Group A vs. Group B)	*P*=0.736 (no significant difference)
Streptococcus spp. (Group A vs. Group B)	*P*=0.197 (no significant difference)
IL-6 levels (Group A vs. Group B)	*P*=0.679 (no significant difference)
TNF-α levels (Group A vs. Group B)	*P*=0.733 (no significant difference)

P<0.05 is considered significant. TNF-α=Tumor necrosis factor-alpha, IL-6=Interlukin-6

There is growing consensus that RDT may be the most predictive measure of pulpal reactions.[22] According to Yu and Abbott,[23] a distance between caries and pulp of more 1.1 mm, is associated with negligible pulp inflammation. However, when that distance is <0.5 mm, there is a significant increase in the extent of inflammation. The pulp becomes acutely inflamed only when the reparative dentine is invaded by irritants such as microorganisms or their by-products.[24] The lesion depth and duration of hemostasis remain the accepted parameters for endodontic procedures on temporary molars.[24,25]

In this study, two groups of pulp samples with RDT measures of more or <0.5 mm were characterized by their pro-inflammatory cytokines (IL-6 and TNF-α) levels and bacterial composition. The Group B (0.3 mm ≤RDT ≤0.5 mm) demonstrated higher values of cytokines and bacterial composition than Group A (0.5 mm <RDT ≤1.1 mm) for all above-mentioned factors. However, our results revealed no statistically significant differences, thus a relationship between the intensity of the inflammation and proximity of the lesion could not be established. Interestingly, our results showed that a carious cavity with an RDT <0.5 mm, and therefore close to the pulp, did not always imply a more exacerbated inflammatory reaction than a pulp facing a carious cavity with an RDT >0.5 mm (e.g., samples 27 and 14) [Table 2]. In addition, for the same RDT values obtained in some samples, the inflammatory responses could be different (e.g., samples 8 and 9) [Table 2].

As Martin *et al*. noted, although it is well recognized that bacteria and their products play a key role in tooth decay and pulpal inflammation, the pulp responses differ from individual to individual.[26]

Regardless of the decay depth, other factors could explain the diversity of the results such as the age of the pulpal parenchyma. It is widely reported that, even at early stage III, the pulp has numerous vascular anastomoses to ensure favorable pulpal vascularization and sufficient defence potential. Therefore, the high IL-6 and TNF-α cytokine levels in all of the tested pulp samples were a result of the entire pulpal cameral parenchyma excavated in the samples and not only the pulpal part facing the caries area. Thus, *in vitro*, inflammation markers and bacteria were spread throughout the sample, whereas *in vivo* they remained confined to the lesion. This explains why the pulp vitality is preserved longer before the development of pulpitis or necrosis. In contrast, some authors have assumed that pulpal defences are greatly reduced in stage III. Thus, practitioners still need to evaluate the pulpal condition according to each clinical situation based on their clinical experience.[27-29]

The nature and appearance of the residual dentin are factors that some practitioners do not consider as significant favoring the depth of the lesion. In this study, and in agreement with other studies, the nature of the dentin and especially the reactionary dentin or sclerotic dentin represented an effective physical barrier for the propagation of microorganisms and their toxins toward the pulp, independent of the RDT. The pulp response is also related to the thickness and degree of calcification of the remaining dentine because dentine permeability can be reduced by dentinal sclerosis and reparative dentine formation.[23] Scrutiny observation of the lesion can serve as a valuable guide in evaluating the pulp inflammatory state and consequently in establishing the appropriate endodontic treatment.[30]

Table 2: The bacterial composition and cytokine levels (pg/ml) for each sample with the corresponding remaining dentin thickness measured in mm

Sample number	*Lactobacillus* spp. (CFU/μg)	*Prevotella* spp. (CFU/μg)	*Streptococcus* spp. (CFU/μg)	TNF-α (pg/ml)	IL-6 (pg/ml)	RDT (mm)
1	++	++	+	1580	1420	0.7
2	0	0	12	40	32	0.8
3	+	+++	+++	2250	1880	1.1
4	7	16	16	520	450	0.3
5	0	0	0	16	11	0.6
6	++	+++	++++	2840	1980	0.4
7	0	0	1	19	14	0.3
8	0	0	1	17	15	0.4
9	++++	++++	++++	3840	2940	0.4
10	0	3	1	35	29	0.6
11	0	0	2	22	23	0.5
12	1	0	0	17	18	0.4
13	++	+	+	1220	890	0.4
14	0	1	3	95	55	0.4
15	0	7	4	160	190	0.4
16	0	0	0	14	12	0.4
17	++	++++	++++	2920	2100	0.5
18	3	8	0	145	189	0.9
19	0	++	++++	1890	1750	0.5
20	0	0	0	13	14	0.5
21	0	++	++	1100	795	0.7
22	0	3	0	25	19	1
23	++++	++++	++++	3420	2850	0.9
24	0	0	0	14	12	0.7
25	0	0	2	22	18	0.8
26	0	++	++	1100	1524	0.5
27	+++	+++	+++	2980	3620	0.9
28	0	0	0	21	20	1.1
29	+++	+++	+++	3950	2970	0.7
30	++++	++++	++++	3570	3100	0.8
31	++	++	+	1720	1290	0.4
32	0	1	0	22	19	0.6
33	0	+++	+++	2200	2420	0.4
34	0	0	0	19	17	0.7
35	0	++	++	1240	1320	1
36	++++	++++	++++	2970	3460	0.4
37	+	+++	+++	2650	1795	0.9
38	+++	+++	+++	3790	2890	0.5
39	5	++	++	1090	890	0.5
40	3	5	3	110	85	0.4
41	5	++	+	790	820	0.6
42	5	+	+	530	480	0.4
43	5	+	+	550	495	0.7
44	0	+	+	462	520	1.0
45	3	3	0	55	41	0.8
46	++++	++++	++	2890	2690	0.9
47	0	0	0	17	19	0.6
48	0	0	0	20	22	0.8

+=20-40 CFU/μg, ++=40-60 CFU/μg, +++=60-80 CFU/μg, and ++++=Over 80 CFU/μg. RDT=Remaining dentin thickness, TNF-α=Tumor necrosis factor-alpha, IL-6=Interlukin-6

Kassa *et al*.[31] found that primary teeth with proximal carious lesions extending >50% through the dentine thickness appear to have more extensive inflammatory pulpal changes than teeth with occlusal caries of a

similar depth. The proximal carious lesions in this study also constituted the major part of the samples (91.6%).

In a recent systemic review of assessing inflammatory cytokines in normal and irreversibly inflamed dental pulps, Hirsch *et al*.[20] highlighted the difficulties in conducting accurate diagnoses of the pulp status. Moreover, when dealing with temporary teeth, it is difficult to define the criteria of reversible inflammatory situations of the pulp, and when pain occurs, it means that the pulpitis is turning into pulp necrosis.

The hypothesis that we set out to study is of major clinical importance. This can be of paramount value, particularly, if we can establish a relation between clinical and radiological observation on one hand and microbial and inflammatory status of pulp tissue on the other hand. The methodological protocol we have followed meets very strict criteria. Nevertheless, it did not allow us to show a significant relationship between the studied factors.

Despite the limitations of this study, especially the difficulties encountered during radiological interpretation and also in the sample size, our observations indicate that RDT, by itself, cannot explain the immuno-bacteriological status of the pulp. Therefore, when all the aforementioned criteria are taken into account, the RDT may serve as an additional indicator in pulpotomies. We concur with Hirsch *et al*.[20] that the only way to unequivocally assess the status of the pulp is to conduct an extemporaneous pulpal blood test, which should allow verification of the indication of the pulpotomy.

However, the standing challenge is to decide whether to maintain the vitality of the pulp in the case of deep cavities. The clinical experience of the operator remains a key to the success of such treatments.

Future research that takes into account a larger sample, more efficient means in radiological reading and accurate analysis of the nature of the remaining dentin, are required to provide a satisfactory response to our hypothesis.

Conclusion

Other factors than RDT need to be considered to properly diagnose the pulp status of primary teeth. Combined clinical and radiographic examinations are essential to establish a correct diagnosis and the most appropriate treatment for each clinical situation. Pediatric dentists should be familiar with the current trends in the field of children's dentistry.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. Chem Rev 2011;111:5922-43.
2. Prescott SL. Early-life environmental determinants of allergic diseases and the wider pandemic of inflammatory noncommunicable diseases. J Allergy Clin Immunol 2013;131:23-30.
3. Love RM, Jenkinson HF. Invasion of dentinal tubules by oral bacteria. Crit Rev Oral Biol Med 2002;13:171-83.
4. Haniastuti T. Potential role of odontoblasts in the innate immune response of the dental pulp. Dent J 2008;41:142-6.
5. Renard E, Gaudin A, Bienvenu G, Amiaud J, Farges JC, Cuturi MC, *et al.* Immune cells and molecular networks in experimentally induced pulpitis. J Dent Res 2016;95:196-205.
6. Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, *et al.* Dental pulp defence and repair mechanisms in dental caries. Mediators Inflamm 2015;2015:230251.
7. Nakanishi T, Matsuo T, Ebisu S. Quantitative analysis of immunoglobulins and inflammatory factors in human pulpal blood from exposed pulps. J Endod 1995;21:131-6.
8. Pezelj-Ribaric S, Anic I, Brekalo I, Miletic I, Hasan M, Simunovic-Soskic M, *et al.* Detection of tumor necrosis factor alpha in normal and inflamed human dental pulps. Arch Med Res 2002;33:482-4.
9. Le Y, Zhou Y, Iribarren P, Wang J. Chemokines and chemokine receptors: Their manifold roles in homeostasis and disease. Cell Mol Immunol 2004;1:95-104.
10. Ogawa Y, Calhoun WJ. The role of leukotrienes in airway inflammation. J Allergy Clin Immunol 2006;118:789-98.
11. Elsalhy M, Azizieh F, Raghupathy R. Cytokines as diagnostic markers of pulpal inflammation. Int Endod J 2013;46:573-80.
12. Rodd HD, Waterhouse PJ, Fuks AB, Fayle SA, Moffat MA, British Society of Paediatric Dentistry. *et al.* Pulp therapy for primary molars. Int J Paediatr Dent 2006;16 Suppl 1:15-23.
13. Naulin-Ifi C. Traitements endodontiques des dents temporaires. Real Clin 2001;12:73-82.
14. Duggal MS. Pulp therapy for primary teeth. In: Restorative Techniques in Paediatric Dentistry. 2nd ed. London: M. Dunitz; 2002. p. 45-74.
15. Hui-Derksen EK, Chen CF, Majewski R, Tootla RG, Boynton JR. Retrospective record review: Reinforced zinc oxide-eugenol pulpotomy: A retrospective study. Pediatr Dent 2013;35:43-6.
16. Gonzalez-Lara A, Ruiz-Rodriguez MS, Pierdant-Perez M, Garrocho-Rangel JA, Pozos-Guillen AJ. Zinc oxide-eugenol pulpotomy in primary teeth: A 24-month follow-up. J Clin Pediatr Dent 2016;40:107-12.
17. Lula EC, Monteiro-Neto V, Alves CM, Ribeiro CC. Microbiological analysis after complete or partial removal of carious dentin in primary teeth: A randomized clinical trial. Caries Res 2009;43:354-8.
18. Singhal DK, Acharya S, Thakur AS. Microbiological analysis after complete or partial removal of carious dentin using two different techniques in primary teeth: A randomized clinical trial. Dent Res J (Isfahan) 2016;13:30-7.
19. Leng SX, McElhaney JE, Walston JD, Xie D, Fedarko NS,

Kuchel GA, *et al*. ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. J Gerontol A Biol Sci Med Sci 2008;63:879-84.
20. Hirsch V, Wolgin M, Mitronin AV, Kielbassa AM. Inflammatory cytokines in normal and irreversibly inflamed pulps: A systematic review. Arch Oral Biol 2017;82:38-46.
21. Çelik BN, Sarı Ş. Carious exposure versus mechanical exposure for MTA pulpotomy in primary teeth. Biomed Res Int 2016;2016:2753429.
22. 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.
23. Yu C, Abbott PV. A clinical classification of the status of the pulp and the root canal system. Aust Dent J 2007;52:S17-31.
24. Agamy HA, Bakry NS, Mounir MM, Avery DR. Comparison of mineral trioxide aggregate and formocresol as pulp-capping agents in pulpotomized primary teeth. Pediatr Dent 2004;26:302-9.
25. Vieira-Andrade RG, Drumond CL, Alves LP, Marques LS, Ramos-Jorge ML. Inflammatory root resorption in primary molars: Prevalence and associated factors. Braz Oral Res 2012;26:335-40.
26. Martin FE, Nadkarni MA, Jacques NA, Hunter N. Quantitative microbiological study of human carious dentine by culture and real-time PCR: Association of anaerobes with histopathological changes in chronic pulpitis. J Clin Microbiol 2002;40:1698-704.
27. Simşek S, Durutürk L. A flow cytometric analysis of the biodefensive response of deciduous tooth pulp to carious stimuli during physiological root resorption. Arch Oral Biol 2005;50:461-8.
28. Bolan M, Rocha MJ. Histopathologic study of physiological and pathological resorptions in human primary teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:680-5.
29. 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.
30. Stanley HR, Pereira JC, Spiegel E, Broom C, Schultz M. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. J Oral Pathol 1983;12:257-89.
31. 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.