NON-RANDOMIZED COHORT STUDY

Does Achievement of Hemostasis After Pulp Exposure Provide an Accurate Assessment of Pulp Inflammation?

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Abstract: ***Purpose:*** *The purpose of this study was to determine in primary molars with carious exposures whether hemostasis at the exposure site and pulp orifice reflected inflammatory status of the pulp at the canal orifice based on cytokine levels.* ***Methods:*** *Forty mandibular primary molars with deep caries were included in the study. Teeth were divided into two groups: group A had teeth where hemostasis at the exposure site was achieved within five minutes, and group B had teeth where hemostasis at the exposure site could not be achieved within five minutes. Blood samples were harvested from the exposure sites and canal orifices. Cytokine levels for IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, and PGE_2 were measured using ELISA for all sample sites.* ***Results:*** *The IL-6 levels at the exposure sites were found to be significantly higher in group A when compared to group B, but there was no statistically significant differences in any of the cytokine levels at the canal orifices between the two groups.* ***Conclusions:*** *Controlling bleeding at the exposure site or canal orifices does not provide accurate assessment of inflammation at the canal orifice and may be misleading for diagnosing vital pulp treatment in primary teeth with a carious pulp exposure.* *(Pediatr Dent 2018;40 (1):37-42) Received October 28, 2016 | Last Revision October 31, 2017 | Accepted October 31, 2017*

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Pulpotomy is a common procedure for the treatment of deep dentin caries in primary molars with no evidence of radicular pathosis.[1] It is indicated for primary teeth with asymptomatic deep dentin caries, either mechanical or carious exposure of vital pulp, no clinical or radiographic evidence suggesting irreversible pulpitis or nonvitality, and no radiographic evidence of physiologic root resorption exceeding one-third of the root length.[2] The success of pulpotomy treatment is mainly based on a clinical diagnosis of normal pulp (symptom-free and responsive to vitality testing) and reversible pulpitis (pulp capable of healing).[1,3]

However, there is a considerable amount of literature reporting that clinical signs, sensitivity tests, and radiographic findings do not provide accurate information about pulpal status,[4-7] and no quantitative marker of primary molar pulp inflammation is currently available.[8] The status of primary molar radicular pulp tissue is diagnosed during clinical proceedings based on the color and volume of blood and the achievability of hemostasis, all of which, with the exception of hemostasis, are subjective criteria. Moreover, there is a divergence of opinion as to the site at which bleeding should be evaluated. Some researchers suggest that bleeding at the exposure site reflects pulpal pathosis and, thus, can be used to evaluate pulpal status.[2,9,10] Others disregard the exposure site, saying that only bleeding at the amputation site should be taken into consideration,[11,12] and still others claim that both sites must be evaluated in order to accurately determine the condition of radicular pulp.[13,14] This lack of clarity has also resulted in a lack of standardization and, consequently, difficulties in comparing findings among studies.

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The American Academy of Pediatric Dentistry's (**AAPD**) Guideline on Pulp Therapy recommends pulpectomy if bleeding cannot be controlled following pulp amputation after a few minutes.[1] However, some authors suggest that mechanical and carious exposure should be considered separately, since patho logical changes in pulp tissue are affected by the etiology of exposure as well as the depth of caries.[8,15,16] The presence of carious tissue around the exposure site is also said to represent a risk for bacterial contamination, pulpal inflammation, and necrosis.[2,16] The probability of the presence of bacterial contamination and pulpal inflammation make the prognosis of vital pulpotomy treatment in carious exposed primary teeth unpredictable,[2,8,17] since it is difficult to objectively measure the level of inflammation and suitability of vital pulp treatment.[15]

For teeth with carious exposures, it is assumed that the inflammation is confined to the coronal pulp; hence, these teeth are considered to be suitable for vital pulpotomy treatment.[1,2,18] However, studies report contradictory results regarding the condition of the pulp in teeth with deep caries lesions. According to Kassa et al.,[19] the coronal pulp was inflamed long before the caries reached the pulp in primary molars with interproximal carious lesions greater than 50 percent of the dentin thickness, and carious exposure was not necessary to create coronal pulpal inflammation. On the other side, Asgary and Ehsani[20] reported that, in permanent molars with irreversible pulpitis and (in some cases) apical rarefaction, pulpotomy had better outcomes than root canal therapy at two years; this implies that acceptable clinical and radiographic outcomes can be achieved with pulpotomy in teeth with extensive irreversible pulpal inflammation. Waterhouse et al.[3] reported that there was no significant difference between inflamed and non-inflamed pulps regarding time required for hemostasis. These papers stake out opposite ends of a continuum of pulpal inflammation and suggest that the degree of pulpal inflammation status that can lead to a successful pulpotomy may be broader than the current conventional parameters.

The possibility has been raised of diagnosing pulpal pathological status using the cytokines that are released during

the inflammation process in healthy as well as infected tooth pulp.[21-23] Cytokines are small polypeptides secreted by leucocytes and other inflammatory cells[24] and are known to play important roles in the intensity and duration of the immune response.[25] Pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IL-8, TNF-α) are involved in the regulation and development of inflammation, while anti-inflammatory cytokines (IL-4, IL-10, and IL-13) play roles in its suppression. Cytokines are also involved in increasing pulp pressure and providing pulpal hemostasis.[9,26] Studies have emphasized that infected teeth have higher levels of cytokines than healthy teeth,[3,21,26-28] making it possible to use these inflammatory markers to diagnose the pathological condition of dental pulp tissue.[9]

The purpose of this study was to investigate whether hemostasis at the exposure site reflected the level of inflammation at canal orifices of primary teeth with carious pulp exposures. For measuring the level of inflammation, cytokine levels at the exposure sites and canal orifices were used. The null hypothesis was that cytokine levels at the exposure site were not different from cytokine levels at the canal orifices.

Methods

Ethical approval. The protocol for this study was approved by the Ethical Committee for Clinical Research, Kirikkale University, Kirikkale, Turkey. Informed consent was obtained from all individual participants included in the study. The purpose and clinical procedures of the study were explained to the patients' parents, and written consents of parents and assents of patients were obtained.

Patient selection. The study included 38 five- to nine-year-old children who were recruited through Kirikkale University's Department of Pediatric Dentistry between April 2014 and March 2015. Medical histories of all patients were non-contributory, and patients had not used any anti-inflammatory medication. Primary molar teeth of these children were included based on the following clinical and radiological criteria: (1) no clinical symptoms or evidence of pulp degeneration (e.g., pain on percussion, history of swelling, or sinus tract); (2) no spontaneous pain; (3) no radiographic signs of internal/external resorption, widened periodontal ligament space, or furcal/periapical radiolucency; (4) physiological root resorption of no more than one-third the root length; and (5) a deep caries lesion with a likelihood of pulp exposure during caries removal.

The study was planned to be conducted with two groups; in order to detect differences with an 80 percent power at a two-sided five percent significance level, 20 teeth were planned to be included in each group.

Following administration of local anesthesia using a solution that did not contain a vasoconstrictor (Safecaine three percent; Vem Ilac, Istanbul, Turkey), teeth were isolated with a rubber dam, a low-speed round bur was used to remove carious tooth structure, and teeth with carious pulp exposures during caries removal were included in the study. Primary molars with the presence of exudate, purulence, or necrotic pulp tissue around the exposure site were excluded.

To assess inflammatory markers at the exposure site, a blood sample was collected by placing a sterile cotton pellet on the site for 45 seconds. This was removed, and a damp cotton pellet was then placed on the site with slight pressure for five minutes to obtain hemostasis. Primary molars in which hemostasis at the exposure site was achieved in five minutes were included in group A, and primary molars in which hemostasis at the exposure site could not be achieved in five minutes were included in group B (Figure). For all teeth, access to the pulp chamber was created, and the coronal pulp was removed with a spoon excavator. To assess inflammatory markers at the canal orifices, a second blood sample was collected by a sterile cotton pellet, which was placed in the pulp chamber adjacent to all canal orifices for 45 seconds. This was removed, and a damp cotton pellet was placed at the orifice using slight pressure to control hemorrhaging. Teeth for which hemostasis at the canal orifice could not be achieved in five minutes were excluded from the study. Primary molars included in the study were treated with vital pulpotomy with a calcium-silicate-based material (Biodentine, Septodont, Saint-Maur-des-fossés, France) and restored with stainless steel crowns.

All blood samples were immediately eluted with 0.08 ml phosphate-buffered saline (PBS pH equals 7.2) and stored at -80 degrees Celsius until analysis.

ELISA. Pulpal blood samples were analyzed for IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, and PGE_2 levels (pg/ml) using Enzyme Linked-Immuno-Sorbent Assays (**ELISA**). After thawing, the blood samples were centrifuged at 1,500 g for 10 minutes at four degrees Celsius, and the cotton pellets were removed. Cytokine concentrations were measured using an Invitrogen Immunoassay Kit (DIA Source ImmunoAssays, Nivelles, Belgium) with a double-sandwich technique.

Statistical analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 17.0 software (SPSS Inc., Chicago, Ill., USA). Statistical differences between groups in the levels of inflammatory markers at the exposure sites and canal orifices were identified using the Mann-Whitney test. Correlations between the levels of inflammatory

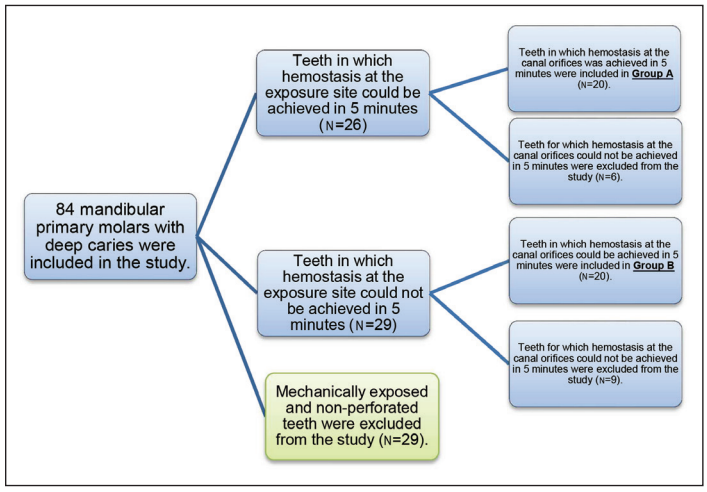

Figure. Allocation of teeth according to hemostasis at the perforation site and canal orifices.

Table 1. RULES FOR INTERPRETING THE SIZE OF SPEARMEN'S CORRELATION COEFFICIENT[29]

Size of correlation	Interpretation
0.90 to 1.00 (-0.90 to -1.00)	Very high positive (negative) correlation
0.70 to 0.90 (-0.70 to -0.90)	High positive (negative) correlation
0.50 to 0.70 (-0.50 to -0.70)	Moderate positive (negative) correlation
0.30 to 0.50 (-0.30 to -0.50)	Low positive (negative) correlation
0.00 to 0.30 (0.00 to -0.30)	Negligible correlation

markers at the exposure sites and at the canal orifices for both groups were identified using Spearman's correlation tests. A *P*-value of less than 0.05 was considered statistically significant. The r values were interpreted according to Table 1.[29]

Results

The study was conducted with 20 teeth per group, for a total of 40 primary mandibular molar teeth in 38 children (27 boys, 11 girls) aged five to nine years (mean age equals 6.7 years).

Table 2. CORRELATION BETWEEN LEVELS OF CYTOKINES AT THE EXPOSURE SITES AND CANAL ORIFICES IN GROUP A

	Correlation coefficient (r)	*P*-value
IL-1β	0.278	0.235
IL-2	0.477	0.033
IL-6	0.182	0.442
IL-8	-0.217	0.359
IL-10	-0.261	0.267
TNF-α	0.721	0.000*
PGE_2	0.374	0.104

Table 3. CORRELATION BETWEEN LEVELS OF CYTOKINES AT THE EXPOSURE SITES AND CANAL ORIFICES IN GROUP B

	Correlation coefficient (r)	*P*-value
IL-1β	0.035	0.885
IL-2	0.677*	0.001*
IL-6	0.389	0.090
IL-8	0.249	0.290
IL-10	0.660*	0.002*
TNF-α	-0.078	0.743
PGE2	-0.084	0.724

IL-6 levels at the exposure sites were significantly higher in group A (where hemostasis was controlled) when compared to group B (*P*=0.029; Table 2). There were no statistically significant differences in the levels of any other of the cytokines at the exposure sites (*P*>0.05; Table 2).

There were no statistically significant differences in the levels of any of the cytokines measured at the canal orifices (*P*>0.05; Table 3).

Correlation between marker levels at exposure sites and canal orifices in groups A and B. The degrees of correlation between inflammatory marker levels at the exposure site and inflammatory marker levels at the canal orifices are shown in Table 2 (group B) and Table 4 (group A).

In both groups A and B, negligible or low positive correlation was found for IL-1β (r equals 0.278, r equals 0.035) and IL-6 (r equals 0.182, r equals 0.389) levels. IL-2 levels showed low positive correlation in group A (r equals 0.477) and moderate positive correlation in group B (r equals 0.677). IL-8 levels showed negligible correlation in group A (r equals -0.217) and in group B (r equals 0.249). IL-10 levels showed negligible correlation in group A (r equals -0.261) and moderate positive correlation in group B (r equals 0.660). TNF-α levels showed moderate positive correlation in group A (r equals 0.642) and negligible correlation in group B (r equals -0.078). PGE_2 levels showed low positive correlation in group A (r equals 0.374) and negligible correlation in group B (r equals -0.084).

When teeth where hemostasis could be achieved (N equals 26) and could not be achieved (N equals 29) in five minutes (Figure) at the exposure sites were compared in terms of achievement of hemostasis at canal orifices, no significant differences were found between the two groups (chi-square equals 0.438, *P*=0.51).

Discussion

When deciding whether pulpotomy or direct pulp capping is indicated, the extent of radicular pulp inflammation is particularly hard to determine.[2,8,17] During clinical procedures, the only operative criterion available to determine the treatability

Table 4. MEAN AND MINIMUM TO MAXIMUM (RANGE IN PARENTHESES) LEVELS OF CYTOKINES AT THE EXPOSURE SITES

	IL-1β (pg/ml)	IL-2 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	PGE_2 (pg/ml)
Group A	0.363 (0.048-1.546)	0.213 (0.061-0.438)	0.354 (0.117-1.144)	0.485 (0.200-0.957)	0.721 (0.151-4.188)	0.225 (0.120- 0.351)	1.482 (0.025-4.229)
Group B	0.382 (0.120-1.334)	0.167 (0.061-0.424)	0.264 (0.025-1.162) *	0.686 (0.217-2.251)	0.740 (0.048-2.216)	0.406 (0.101- 2.219)	1.073 (0.458-2.399)

* Significant difference (*P*=0.029).

Table 5. MEAN AND MINIMUM TO MAXIMUM (RANGE IN PARENTHESES) LEVELS OF CYTOKINES AT THE CANAL ORIFICES

	IL-1β (pg/ml)	IL-2 (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)	PGE_2 (pg/ml)
Group A	0.428 (0.027-1.067)	0.210 (0.061-0.460)	0.350 (0.104-0.720)	0.770 (0.222-2.810)	0.630 (0.144-1.363)	0.223 (0.107- 0.475)	1.267 (0.253-4.761)
Group B	0.579 (0.140-1.724)	0.198 (0.054-0.562)	0.263 (0.033-0.822)	0.538 (0.200-1.600)	0.893 (0.202-1.760)	0.225 (0.108- 0.629)	0.910 (0.198-2.667)

of these teeth is hemostasis,[8] which is a criterion that has not previously been shown to decisively determine the level of inflammation in radicular pulp tissue. Moreover, there is no agreement as to whether hemostasis should be evaluated at the exposure site or at the canal orifice.[2,9-12,30] In an attempt to develop a more objective and accurate criterion for vital pulp treatment, this study aimed to evaluate whether or not hemostasis at the exposure site reflected the inflammatory level as determined by cytokine levels of pulp tissue at the canal orifices of primary teeth with carious pulp exposure. Since only very small amounts of blood can be collected in primary teeth, especially at the exposure site, an anesthetic solution that did not contain a vaso-constrictor was used for local anesthesia to improve collection of enough amounts of blood sample for analysis.

Today, it is possible to measure multiple markers from small amounts of samples.[31] ELISA is one of the most commonly used immunological methods for measuring antigen-antibody reactions[32] and is reported to be more precise and sensitive than other methods.[32] Therefore, the present study used ELISA, the gold standard among immunological detection methods,[31] to detect inflammatory marker levels from blood samples.

Several cytokines produced and released by dental pulp cells play an important role in the pathogenesis of pulpitis.[28] An increase in IL-1β can stimulate acute inflammation[33]; IL-2, IL-8 and PGE_2, an arachidonic acid metabolite, dramatically increase during irreversible pulpitis[9,26,34]; IL-6 is associated with edema[35] and pulpal tissue destruction;[36] and TNF-α is related to early host response and symptoms of pulpitis.[22,37] IL-10, on the other hand, plays a role in suppressing inflammation.[38] Considering that pro- and anti-inflammatory cytokines are able to determine pulpal inflammatory level,[9,26] these markers were used in the present study as objective indicators of inflammation.

With the exception of IL-6, no significant differences were found in the levels of inflammatory markers at the exposure sites between group A and group B, indicating that inflammatory levels of coronal pulp did not vary according to the ability to achieve hemostasis in five minutes at the exposure site.

A pro-inflammatory cytokine produced by mononuclear phagocytes, fibroblasts, and other cells in response to the activity of other pro-inflammatory cytokines,[39] IL-6 causes up-regulation of adhesion molecules and induces angiogenesis, leading to an increase in vascular permeability and inflammatory edema.[35] Wisithphrom and Windsor[36] reported that increases in IL-6 levels increase pulpal destruction by mediating the cells responsible for collagen degradation. Both Barkhordar et al.[21] and Zehnder et al.[23] reported significant increases in the IL-6 levels of inflamed permanent tooth pulp tissue. On the contrary, Elsalhy et al.[26] reported no statistically significant differences in the IL-6 levels of permanent teeth with caries exposure indicated for direct pulp capping and permanent teeth with irreversible pulpitis. However, the samples used in that study were harvested from the exposure sites only, which, given that inflammatory status of coronal and radicular pulp may vary, particularly in cariously exposed teeth, could explain the difference in findings between that study and the present study.

Ozdemir et al.[40] investigated IL-1α, IL-6, and IL-8 levels in cariously and mechanically exposed primary molars for which pulpotomy was indicated. They reported IL-6 and IL-8 levels to be significantly higher in cariously exposed primary molar pulp when compared to mechanically exposed primary molar pulp, and they suggested that IL-6 and IL-8 levels had potential as indicators of pulp status that could improve the accuracy of prognoses in vital pulp therapy. In the present study, IL-6, which is associated with edema and inflammation, was found to be significantly higher in teeth in which hemostasis at the exposure site could be achieved (group A) when compared to teeth for which hemostasis at the exposure site could not be achieved (group B).

According to some authors, chronic inflammation exists in the coronal pulp of primary teeth with deep caries, because the structural characteristics of primary teeth provoke the initiation of an inflammatory response before the caries lesion reaches the pulp.[3,12,41] If this is indeed the case, when the caries lesion does reach the pulp, bacterial invasion may engender an acute response. Thus, the ability to achieve hemostasis in teeth with higher IL-6 levels could be explained by the chronic inflammation of the coronal pulp in these teeth. The higher PGE_2 levels in these teeth, although not statistically significant, supports this view, considering that high PGE_2 levels have been reported to be associated with chronic inflammation.[3] On the other hand, concurrent increases in both IL-6 and IL-8 have been observed in the late phase of inflammation.[23] Since the present study found no significant difference in the IL-8 levels of groups A and B, the increase in IL-6 alone cannot be considered a strong indicator of late-phase inflammation.

According to the results of the present study, there were no significant differences in the levels of cytokines at the canal orifices between groups A and B. Also, in general, none of the cytokine levels at the exposure sites and at the canal orifices showed good correlation in both groups ($r<0.50$; Tables 4 and 5). These results indicate that there is no direct relationship between bleeding at the exposure site and the pathological status of pulp tissue at the canal orifices and that the degree of inflammation in coronal pulp does not precisely reflect the degree of inflammation and pathological condition of pulp tissue at the canal orifices in carious exposed primary teeth.

The main motive of the present study was to question whether the hemostasis at the exposure site reflected pathological condition of the root pulp. Although only hemostasis at the canal orifices is used in the standard pulpotomy procedure, some researchers argue that hemostasis at the exposure site should also be used when deciding a pulpotomy indication.[2,9,10] The results in the present study refute this assertion. Also, a recent systematic review suggested that direct pulp capping in primary teeth has similar success rates with mineral trioxide aggregate and formocresol pulpotomy.[42]

However, the findings of the present study indicate that achievement of hemostasis at the exposure site does not definitively evidence a healthy root pulp. Since bleeding at the exposure site cannot be said to provide accurate information about the pathological status of pulp tissue at the canal orifices, using it as a criterion for diagnosis during vital pulp amputation and direct pulp capping can be misleading. Furthermore, according to the results of the present study, there was no statistically significant relationship between the achievement of hemostasis at the exposure site and canal orifices, and this enhances the previous argument. Another interesting finding of this study is that, during allocation, hemostasis could be achieved at the exposure site in six teeth; however, it could not be achieved at the canal orifices. Considering that exposure site is closer to the carious lesion, presence of inflammation would be expected at the coronal pulp before the radicular pulp. When the aforementioned findings and the results of previous studies[3,9] are interpreted together, lack of a direct link between hemostasis and inflammatory status of the pulp can be suggested; this is

opposed to the current commonly accepted concept of using hemostasis as an indicator of pulpal status.

According to the AAPD's present guideline on pulp therapy, not controlling hemorrhage in the radicular pulp with a damp cotton pellet applied for several minutes is an indication of pulpectomy treatment.[1] However, this study's findings suggest that consideration of hemostasis can be misleading during diagnosis. Future studies investigating the relationship between inflammation and hemostasis in healthy and inflamed pulps can shed new light on the subject.

Despite its small size, dental pulp tissue responds compartmentally to bacterial irritation, with the area closest to the irritation being greatly affected, while the area furthest is only minimally affected or even unaffected by inflammation.[4,25,43] Thus, it can be suggested that primary teeth with carious pulp exposures in which hemostasis cannot be achieved at the exposure site can have healthy radicular pulps, and these teeth can be suitable for vital pulpotomy. Unless there is necrosis or exudate present at the exposure site, whether or not hemostasis can be achieved at the exposure site is unimportant, as long as hemostasis can be achieved at the canal orifices. However, further studies are needed to clarify in greater detail the treatability of primary teeth with carious pulp exposure based on an evaluation of inflammatory status of pulp tissue at the canal orifices.

Since the pulp chambers of primary teeth are too small and controlling bleeding at each orifice separately and isolating them from each other was impossible, collection of samples from each orifice separately was not possible. Thus, the blood samples in the present study were collected from all canal orifices together by placing the cotton pellet in the pulp chamber. As a result of this method, the cytokine levels that are being reported were from pulp tissue at all canal orifices, and there was no chance to see inflammation separately in each canal orifice. This is a limitation in the present study; however, if collecting separate samples could be possible, this would bring additional limitations, such as being able to collect enough blood sample for analysis and perfect isolation of each pulp orifice and blood sample from others during blood collection.

Conclusions

Based on this study's findings, the following conclusions can be made:

1. Achievement of hemostasis at the exposure site does not provide accurate assessment of inflammatory status of pulp tissue at the canal orifices; thus, it cannot be used as a criterion in pulpotomy.
2. No direct link between achievement of hemostasis and inflammatory status of the dental pulp seems to exist.

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