

Original

## Comparative evaluation of pro-inflammatory cytokine levels in pulpotomized primary molars

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**Abstract:** The present *in vivo* study was performed to investigate the levels of the pro-inflammatory cytokines, interleukin (IL)-1 $\alpha$ , IL-6, and IL-8, in primary molars for which pulpotomy was clinically indicated, and to evaluate the success rates of three different pulpotomy agents employed for cariously (CExp) or mechanically exposed (MExp) primary molars. Forty-seven primary molars were classified as MExp or CExp according to the type of pulpal exposure. Pulp tissue was harvested and analyzed using enzyme-linked immunosorbent assay (ELISA). Subsequently, three pulpotomy agents—calcium hydroxide (CH), mineral trioxide aggregate (MTA), and formocresol (FC)—were applied randomly, and the outcome was observed radiographically for 18 months. Levels of IL-6 and IL-8 were significantly higher in CExp pulp than in MExp pulp ( $P < 0.05$ ). In the CH pulpotomy group, MExp teeth showed a higher success rate than CExp teeth. There was no significant difference in success rate between MExp and CExp teeth in both the FC and MTA groups. The levels of IL-6 and IL-8 have the potential to become indicators of pulp status and can be monitored by researchers to make the prognosis of vital pulp therapies less uncertain. As MTA and FC yielded higher rates of success than CH in CExp teeth, the choice of pulpotomy agent appears

to be important in this context.  
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Keywords: IL-1 $\alpha$ ; IL-6; IL-8; pulpotomy; primary teeth; dental pulp.

### Introduction

Vital pulp therapy is a controversial treatment option because of the unpredictable nature of the outcome (1). Hence, the factors affecting the success of vital pulp therapy are still of great concern for researchers. It is now well known that the key to successful vital pulp therapy is correct assessment of the degree of pulpal inflammation (1-3). Currently, histological examination is the gold standard for pulpal diagnosis, but this requires pulp removal, which is not feasible in a clinical situation (1).

Today, diagnosis of pulp condition is based on subjective and qualitative judgements of bleeding time, and the color and volume of blood (1). However, these parameters are often found to be inconsistent with the histological results. Since pulpal blood reflects the inflammatory state of the pulp, attention has become focused on pulpal blood analysis. The presence of cytokines in the inflamed pulp tissue has been widely reported. The degree of pulpal inflammation is usually related directly to the size and type of exposure, which may be either mechanical or carious (3,4). Evaluation of cytokine levels in samples obtained from teeth with each type of exposure can provide an indication of the degree of pulpal inflammation. The pro-inflammatory cytokines interleukin (IL)-1 $\alpha$  and  $\beta$  are released at the early stage of inflammation and are related to the acute and chronic

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phases of the inflammatory process. IL-6 and IL-8 also regulate inflammation through their pro-inflammatory activity, which includes activation of B- and T-cells and recruitment of neutrophils, respectively (5).

Researchers have also drawn attention to pulpotomy agents as a factor affecting the outcome of vital pulp treatment. The relationship between cytokines and pulpotomy agents, such as calcium hydroxide (CH), mineral trioxide aggregate (MTA), and formocresol (FC), has been evaluated in some studies (2,3).

Against this background, the primary aim of this *in vivo* study was to investigate the levels of the pro-inflammatory cytokines, interleukin IL-1 $\alpha$ , IL-6 and IL-8, in primary molars for which pulpotomy was clinically indicated. In addition, we evaluated the success rates obtained with three different pulpotomy agents when used to treat cariously or mechanically exposed primary molars.

## Materials and Methods

A total of 47 teeth of 37 healthy and cooperative children aged between 5 and 10 years (mean age 7.5 years) were included in this study. Children with one or more primary lower second molar teeth with deep occlusal carious lesions participated. The subjects did not receive any anti-inflammatory medication before or during the study. The Ethical Committee of the Medical Faculty of Ege University approved the study (reference No: 08-9/5). The purpose and clinical procedures of the study were explained to the parents, who all signed consent forms before the treatment. The clinical and radiographical inclusion criteria for the teeth were: restorable with a stainless steel crown, no spontaneous pain, no swelling or fistula, no tenderness to percussion, pulp exposure during caries removal (carious) or cavity preparation (mechanical), successful hemorrhage control within 5 min, no pathological root resorption, no widened lamina dura, no periradicular or furcal radiolucency, and physiological root resorption less than 1/3 of the total root length (6).

### Clinical procedure

The teeth were grouped according to the type of pulpal exposure: i.e. either mechanically exposed (MExp) or cariously exposed (CExp) (6).

Unless a rubber dam was necessary due to lack of patient cooperation, cotton rolls were used for isolation of the teeth from saliva. Pulpotomies were performed under local anesthesia using 2% lidocaine (1:000,000). The type of pulpal exposure and the amount of bleeding at the exposure site were considered. The roof of the pulp

**Table 1** Distribution of sample size according to the pulpotomy agent employed and type of exposure

	MExp	CExp	Total
CH	9	8	17
MTA	6	9	15
FC	8	7	15
Total (n)	23	24	47

CH: Calcium hydroxide, MTA: Mineral trioxide aggregate, FC: Formocresol, MExp: Mechanically exposed, CExp: Cariously exposed

chamber was weakened with a high-speed handpiece and cracked with a sharp spoon excavator to avoid damaging the coronal pulp tissue.

### Pulp tissue sampling

The coronal pulp tissue was gently harvested from the coronal pulp chamber cavity with a spoon excavator. All pulp samples were placed in 0.5 mL phosphate-buffered saline (PBS) in pre-weighed Eppendorf tubes. The samples were weighed again and their weights were noted before they were stored at -80°C until the ELISA procedure.

### ELISA procedure

The pulp samples were analyzed for cytokine levels by enzyme-linked immunosorbent assay (ELISA). Frozen pulp samples were thawed for 15 min, and the tissue was crushed with a glass rod in an Eppendorf tube to elute the cytokines from the pulp tissue (7,8). Concentrations of IL-1 $\alpha$ , IL-6, and IL-8 were measured using an Invitrogen Immunoassay Kit (#KAC1192/KAC1191, #KHC0061, and #KHC0081, Invitrogen Corporation, Grand Island, NY, USA), employing a double-antibody sandwich technique. Procedures were performed according to the instructions supplied with the kit. The minimum detection limits of IL-1 $\alpha$ , IL-6, and IL-8 were 1 pg/mL, <2 pg/mL and <5.0 pg/mL respectively. The optical density of each sample relative to standards and known cytokine concentrations was expressed as pg/mL.

### Calculation of cytokine concentrations

The amount of each cytokine (pg) per unit mass of each pulp tissue specimen (mg) was determined as (8,9):

a) Pulp weight (mg) / 0.5 mL PBS = pulp concentration (mg/mL)

b) Cytokine concentration (pg/mL) / pulp concentration (mg/mL) = cytokine amount per unit pulp mass (pg/mg)

### Pulpotomy procedure

After controlling any hemorrhage with sterile pledgets of



**Fig. 1** An example of failure due to internal root resorption.



**Fig. 2** An example of successful treatment at 18 months.



**Fig. 3** An example of failure due to external root resorption.



**Fig. 4** Another example of successful treatment at 18 months.

dry cotton, three different pulpotomy agents, CH, MTA, and FC, were applied randomly. The distribution of sample size in the groups is shown in Table 1. In the CH group, the pulp chamber was filled with glass ionomer cement (GIC) (Ketac™ Molar Easymix, 3M ESPE Dental Products, Seefeld, Germany) after application of the CH (Kalsin, Aktu Inc., İzmir, Turkey). In the MTA group, MTA paste (Proroot MTA, Dentsply-Maillefer, Ballaigues, Switzerland) was prepared by mixing the powder with sterile water at a ratio of 3:1. The tooth was then covered with a moistened pellet and a temporary paste plug was applied before the GIC during the second session. In the FC group, the teeth were covered with ZOE paste (Alganol, Associated Dental Products Ltd., Kemdent Works, Wiltshire, UK) after application of 1:5-diluted FC (Buckley's Formo Cresol, Sultan Healthcare Inc., Englewood, NJ, USA) for 5 min. In the final session, all teeth ( $n = 47$ ) were restored with stainless steel crowns (3M ESPE Dental Products).

#### Follow-up

The teeth were evaluated after 3, 6, 12, and 18 months by a qualified examiner who was blinded to the treatments. The intraexaminer kappa value was 1. Treatment of a tooth was considered to have failed if one of the

following findings was present: history of pain, swelling, fistula, tenderness to percussion/palpation, a widened lamina dura, periradicular/furcal radiolucency, internal/external root resorption, or tooth mobility. Examples of radiographs in cases of failure and success are shown in Figs. 1-4.

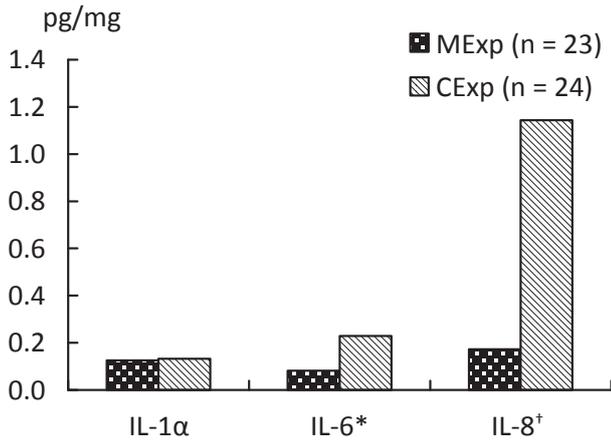
#### Statistical analysis

Differences in cytokine levels among the groups were analyzed non-parametrically by chi-squared test and Fisher's exact test, whereas differences between the success rates of the pulpotomy agents were assessed by Mann-Whitney *U* Test using the SPSS 19.0 software package. The level of significance was 5% ( $P < 0.05$ ).

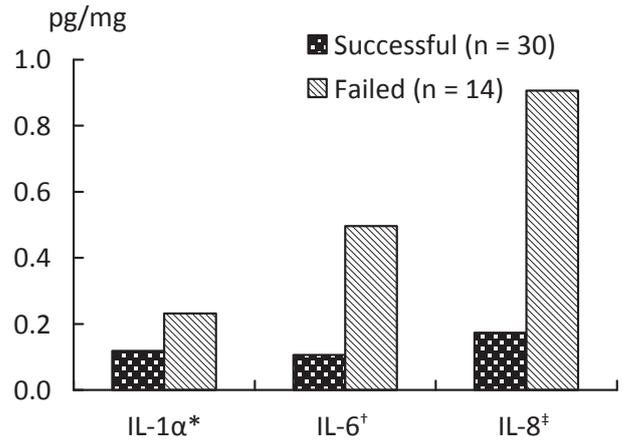
#### Results

IL-1 $\alpha$ , IL-6, and IL-8 were detected in all of the harvested pulp samples ( $n = 47$ ) using ELISA. Three patients were lost to follow-up. Therefore determination of treatment outcomes was based on 44 teeth that were followed up radiographically for 18 months.

The cytokine levels in the MExp and CExp groups were compared to determine differences in the biological status of the pulp (Fig. 5). Levels of IL-6 and IL-8 were higher in the CExp group. However, there was no statisti-



**Fig. 5** Cytokine levels in the MExp and CExp groups. The levels of IL-6 and IL-8 were significantly higher in the CExp group (\* $P = 0.011$ , † $P = 0.025$ ).



**Fig. 6** Cytokine levels in teeth for which treatment was successful or failed. The levels of all cytokines were significantly higher in the failure group (\* $P = 0.031$ , † $P = 0.020$ , ‡ $P = 0.09$ ).

**Table 2** Distribution of radiographic success rates for the materials at 18 months of follow-up

	Successful <i>n</i> (%)	Failed <i>n</i> (%)
CH	7 <sup>a</sup> (41.2)	10 (58.8)
FC	11 <sup>b</sup> (78.6)	3 (21.4)
MTA	12 <sup>b</sup> (92.3)	1 (7.7)
<i>P</i> value	$P = 0.005^*$	

\* a, b: significant difference ( $P < 0.05$ )

**Table 3** Effect of the type of pulp exposure on the success rates of the three pulpotomy agents

		Successful <i>n</i> (%)	Failed <i>n</i> (%)	<i>P</i> value
CH	MExp	6 <sup>a</sup> (66.7)	3 (33.3)	$P = 0.036^*$
	CExp	1 <sup>b</sup> (12.5)	7 (87.5)	
FC	MExp	7 (87.5)	1 (12.5)	$P = 0.385$
	CExp	4 (66.7)	2 (33.3)	
MTA	MExp	4 (80.0)	1 (20.0)	$P = 0.385$
	CExp	8 (100)	-	

\* a, b: significant difference ( $P < 0.05$ )

cally significant difference in IL-1α levels between the groups.

We placed 14 and 30 teeth into groups in which the treatment had failed and had been successful, respectively, in order to compare the cytokine levels. IL-1α, IL-6, and IL-8 levels in the failure group were significantly higher than those in the successful group (Fig. 6).

The distribution of the radiographically determined success rates for the pulpotomy agents is presented in Table 2. The success rate for CH was significantly lower than that for FC or MTA ( $P = 0.000$  and  $P = 0.005$ ). There was no statistically significant difference in the success rate between FC and MTA.

Table 3 represents the success rates of the three pulpotomy agents according to the type of pulp exposure. In the CH group there was a significant difference between the success rates for MExp and CExp teeth at the end of the 18-month observation period. No significant difference was detected between the success rates of MExp and CExp in both the FC and MTA groups.

## Discussion

This study examined the biological status of pulp as a factor affecting the outcome of pulpotomy treatment for primary teeth. In addition, the success of pulpotomy treatment was investigated according to the type of pulp exposure. Pulpal inflammation increases in parallel with progression of carious lesions. Carious exposure is usually accompanied by pulp inflammation. During the treatment of a deep carious lesion, a clinician must decide whether the pulpal inflammation is reversible or irreversible based on subjective findings (4), and therefore objective analysis of pulpal cytokine levels can provide useful information about pulpal pathology. Several cytokines have been suggested to be indicators of inflammation (8,10-11). In the present study, comparison of the cytokine levels in mechanically and cariously exposed pulp was able to provide a clearer picture of pulp status at the time of treatment. We found that the levels of IL-6 and IL-8 were higher in CExp pulp than in MExp pulp, thus supporting previous studies that have demonstrated these cytokines to be associated with pulpal pathogenesis

(10,12-14). One of those studies demonstrated a strong relationship between IL-8 expression and the presence of inflammatory cells (10), and Zehnder et al. detected a positive correlation between IL-6 and IL-8 in inflamed pulp (13). Our results validate the initiation of pulpal inflammation in teeth affected by caries at this stage. However, we found no significant increase of the IL-1 $\alpha$  level in CExp teeth relative to Mexp teeth. Zehnder et al. found no significant difference in IL-1 $\alpha$  levels between healthy and symptomatic teeth (13). Since the precursor of IL-1 $\alpha$  is present in resting non-hematopoietic cells and is released when tissue damage occurs, its presence in dental pulp probably indicates irreversible inflammation (15). Further investigation is needed to validate this possibility.

Another finding of our study was that the levels of IL-1 $\alpha$ , IL-6, and IL-8 were increased in the failure group. In addition to IL-6 and IL-8, IL-1 $\alpha$  is also associated with pulpal pathogenesis in view of its pro-inflammatory activity (16-18). In a study by Waterhouse et al. of prostaglandins, which are biologically active lipids that also mediate pulp inflammation (4), a correlation between the level of PGE<sub>2</sub> and treatment failure was also noted, similarly to our present findings. High levels of cytokines can thus be an indicator of preoperative pulpal inflammation, which is one of the causes of treatment failure.

We found that CExp teeth showed lower success rates than MExp teeth, although the difference was not significant. However, there was a significant difference in the success rates when the data were analyzed according to the pulpotomy agent employed. When CH was applied to CExp teeth, it yielded lower success rates in comparison to MExp teeth. Similarly, using CH as a pulpotomy agent, Sonmez et al. reported a significantly lower treatment success rate for CExp pulp relative to Mexp pulp (19). These results reflect the fact that pulpal exposure due to caries carries an increased risk of inflammation spreading deeper into the pulp.

MTA yielded the highest success rate (92.3%) among the three pulpotomy agents, but the outcome did not differ significantly between MTA and FC. In the FC group, failure was observed in two cases of internal and one case of external root resorption, whereas in the MTA group failure occurred in one case of internal root resorption. Although Ainehchei et al. found a significantly higher incidence of root resorption in a group treated with FC relative to MTA (20), there was no significant difference in outcome between the MTA and FC groups in our present study. On the basis of radiographic criteria, the CH group showed the lowest success rate (41.2%). These results confirm the findings of previous studies indicating

that MTA is the most appropriate pulpotomy agent for use in primary teeth (20-22).

Remarkably, when MTA or FC was applied, the difference in outcome between MExp and CExp teeth was not significant. This indicates that pulpal status is more crucial when CH is used as a pulpotomy agent in comparison with MTA or FC. The fixative properties of FC facilitate asymptomatic stabilization of pulp tissue (23), whereas the outstanding feature of MTA is its superior sealing ability, providing an appropriate environment for the healing process (24).

Within the limitations of our study, it can be concluded that the levels of IL-6 and IL-8 can be used as a potential indicator of pulp status and can be monitored to improve the reliability of prognosis of vital pulp therapy. On the other hand, it appears that the choice of pulpotomy agent for treatment has an important bearing on outcome. In this study, pulpal status did not affect the success rates obtained with MTA and FC, unlike the situation for CH.

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