BMC Oral Health

RESEARCH

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Efficacy of proinflamatory cytokines in the clinical and radiograpic outcomes of different primary molar pulpotomy agents: a comperative randomised study featuring a novel biomarker for pulpal diagnosis

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Abstract

Background While the effect of biomaterials covering the pulp tissue is considered in the success of pulpotomy treatment, the level of pulpal inflammation is still very important for treatment success. The aim of this study was to compare IL-6 and IL-8 levels, known as good indicators of pulpal inflammation, with a new biomarker, presepsin, and to evaluate the impact of biomarker levels along with the pulp capping agents used in the treatment on the one-year success of pulpotomy treatment.

Methods The study included 120 primary second molar teeth with pulpotomy indications from 75 children. To determine the pulpal inflammation status, pulpal bleeding samples were taken during treatment, and the levels of IL-6, IL-8, and presepsin were measured. During the pulpotomy treatment, MTA, NeoMTA™, and Biodentine™, and ZOE were randomly applied to groups of thirty teeth each. Patients were monitored for a period of 12 months post-treatment.

Results IL-8, IL-6, and presepsin levels were significantly higher in teeth with pathology ($p < 0.001$). Biomarker levels were found to be higher in the NeoMTA and Biodentine groups, but this did not result in a statistically significant difference. ($p > 0.05$) Following pulpotomy treatment, the most successful material groups in order were MTA, ZOE, NeoMTA™, and Biodentine™.

Conclusion Presepsin may be a usable indicator in predicting the level of inflammation. At the end of the one-year follow-up of pulpotomy treatment, more pathology was observed in the NeoMTA and Biodentine groups, where biomarker levels were higher, while no pathology was found in the MTA group, where biomarker levels were lower.

Trial registration NCT06398327/ 20,240,503.

Keywords Presepsin, Pulpotomy treatment, Pulpal inflammation, Pulpotomy agents

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Introduction

Dental caries is a disease associated with various chronic, infectious, and degenerative factors, which are more frequently observed in the children [1]. Determinants such as thinner enamel and dentin tissues, pulp horns located closer to the outer surface, and lower mineral composition of the primary teeth cause a faster lesion progression and devitalization, and thus may result in premature extraction/loss [1, 2]. In order to maintain esthetics, phonetics, and mastication, these teeth must be retained until they exfoliate [2, 3]. Due to their greater dentin permeability, pulpal inflammation develops in the primary teeth. In cases where proximal caries involve more than half of the dentin thickness, pulpotomy is regarded as the widely accepted and most successful treatment approach [4].

In the current pulpotomy procedures, the extent of the pulp's inflammatory condition is estimated by clinically assessing pulpal bleeding time, blood color, and quantity; however, these indicators may not always be accurate [5, 6]. Cytokines, which are essential predictive markers for an actual diagnosis of pulp infection, have been detected in the pulpal inflammation by many researchers. Cytokines and chemokines, which play an important role in pulpal inflammation, are determined as interleukin (IL)-lα, IL-1β, tumor necrosis factor alpha (TNF-a), IL-4, IL-6, IL-8, and IL-10 [5–7]. New studies with different molecules have been conducted for more accurate pulpal diagnosis [7]. Discovered in 2004, presepsin is a novel inflammatory biomarker with superior sensitivity and specificity in the clinical diagnosis of bacterial infections [8]. However, no studies were found in the literature indicating that presepsin may be a predictive biomarker in bacteria-induced pulpal lesions.

Besides the extent of the baseline inflammation, the type of pulpotomy agents used are important for a successful treatment [9]. Although the studies evaluating the clinical outcome of these medicaments reported that biomaterials had better outcomes in pediatric pulpotomy, none of them referred the best material [9, 10]. Medicaments with known superior clinical success rates and minimal side-effects such as Mineral trioxide aggregate (MTA) and Biodentine™ are commonly used in the pulpotomy of primary teeth [9, 11, 12]. Since biomaterials present higher rates of clinical performance in pulp treatment, new calcium silicate based materials with improved properties (e.g., superior compressive strength and sealing capability, ease of use, increased radio-opacity, faster setting time, and no dental discoloration) have continuously been developed [13].

The aim of this study was to compare IL-6 and IL-8 levels, known as good indicators of pulpal inflammation, with a new biomarker, presepsin, and to evaluate the impact of biomarker levels along with the pulp capping agents (MTA, Biodentine™, NeoMTA™ and ZOE) used in the treatment on the one-year success of pulpotomy treatment. Accordingly, the study established two hypotheses: The new biomarker Presepsin has no effect on indicating pulp inflammation, and the level of high pulpal inflammation in pulpotomy treatment does not affect the success of the treatment according to the biomaterials used in pulpotomy.

Materials and methods

This study was conducted in accordance with the Ataturk University Faculty of Medicine Ethics Committee approval (Date: 05.2021/ Session no:04/ Decision no:66) and guidelines of the Helsinki Declaration. The primary caregivers of each participant were comprehensively briefed about the procedures before the treatment and their written consents were obtained. Initially, a total of 75 children (36 female and 39 male) presenting with at least one primary second molar indicated for pulpotomy were included in the study. The 4 pulpotomy agents (MTA, Biodentine™, NeoMTA™ and ZOE) were randomly divided into four study groups of 30 teeth and pulpotomy was applied to a total of 120 teeth. The sample size was calculated using the "G. Power-3.1.9.2" program. With a 95% confidence level ($\alpha=0.05$), a standardized effect size of 0.5495 obtained from a similar study [5], and a theoretical power of 0.80, the minimum sample size was determined to be 26.

In order to randomly distribute the study groups, the Research Randomizer (Version 4.0) computer software from http://www.randomizer.org/ was used to generate a list before initiating the pulpotomy procedure, and the pulpotomy agents were used accordingly.

Patient selection criteria

Patients aged between 4 and 9 years who:

Did not have any acute or chronic systemic diseases, were not under any treatment that would suppress the immune system, did not have allergies to the dental materials and local anesthetics to be used during treatment, maintained cooperation during dental treatments, and whose parents agreed to the treatment and participation in follow-up for 6–12 months were included in the study.

Tooth selection criteria

Clinical evaluation

Primary second molar teeth that:

Had deep dentin caries, were restorable, had pain related to stimuli, had pulp bleeding during treatment that was light in color, did not bleed excessively, and could be stopped within 5 min, had no sensitivity to percussion and palpation, no pathological mobility, no infra-occlusion, and no clinically visible abscesses and fistulas were included in this study.

Radiological evaluation

Primary second molar teeth that:

Had caries reaching the pulp but no radiolucent image in the periapical and furcation areas, had no internal or external root resorption, had physiological root resorption that had not reached two-thirds of the root length, had underlying permanent teeth, and had no calcified masses within the pulp chamber and canals were included in the study.

Protocols for treatment and sample storage

The treatment protocol was performed by a single pediatric dentist. For better visibility, a rubber-dam was placed over the tooth requiring pulpotomy following local anesthesia. The endodontic cavity was accessed by first removing the carious tissue, unroofing the pulp chamber using a diamond round bur (no:18), removing the coronal tissue using a sterile tungsten carbide round bur (no:14) mounted low-speed contra-angled handpiece, and clearing the remaining pulpal tissue with an excavator.

Before managing the pulpal hemorrhage, a sterile cotton pellet was placed over the pulp tissue for 30–45 s. in order to obtain pulpal blood samples. The specimens were collected into heparin coated tubes (Vacutainer, BD, USA) with 1 mg saline solution and stored at -20ºC (-4.0 °F) for 6 months until the day of testing. After the samples were taken, a dry sterile cotton pellet was placed in the pulp chamber. The bleeding was recorded as 1–3 min or 3–5 min, depending on the time it took to achieve hemostasis. After achieving hemostasis, 0.5% NaOCl was applied at the canal orifices for 30 s. Primary molars in which pulpal hemorrhage was controlled were randomly assigned to 4 different study groups (ProRoot MTA, Biodentine™, NeoMTA™, and ZOE, respectively) and then treated with pulpotomy. Glass ionomer cement was applied over all pulpotomy medications used in the study and pulpotomized teeth were restored with stainless steel crowns (SSCs).

Thawing process and assessment

Sample were thawed at room temperature and centrifuged at 4000 rpm for 15 min. The cotton pellets were then removed from the test tubes and levels of presepsin, IL-6, and IL-8 were measured using an enzyme-linked immunoassay (ELISA) [Human presepsin ELISA kit (Cat.No: E3754Hu, BT LAB, China), Human IL-6 ELISA Kit (Cat.No: E0090Hu, BT LAB, China), Human IL-8 ELISA Kit (Cat.No: E0089Hu, BT LAB, China)] test in accordance with the manufacturer's instructions. Kit measurement ranges for presepsin, IL-6, and IL-8 were 5-1000ng/L, 2-600ng/L, and 5-1000ng/L, respectively. The inter- assay CV were < 10% for all biomarkers. Briefly, the samples and standards were added to wells precoated with human presepsin, IL-6, and IL-8 antibodies. The presepsin, IL-6, and IL-8 present in the samples were bound by the antibodies coating the wells. Biotinylated human presepsin, IL-6, and IL-8 antibodies were then added to bind to the bound presepsin, IL-6, and IL-8, followed by streptavidin–horseradish peroxidase (HRP) to bind to the biotinylated presepsin, IL-6, and IL-8 antibodies. After incubation, the unbound streptavidin–HRP was washed away. A substrate solution was added, and the color developed in proportion to the amount of human presepsin, IL-6, and IL-8 in the well. The reaction was terminated by adding acidic stop solution, and absorbance was measured at 450 nm. Presepsin, IL-6, and IL-8 concentrations were determined by comparing the optical density in the sample wells with the standard curve.

Clinical & radiographic assessment method

Primary second molars were assessed clinically and radiographically at 6 and 12 months post-treatment.

Spontaneous or stimulated pain, swellings in vestibular sulcus, fistula, pathological mobility, sensitivity to percussion and palpation, and regional lymphadenopathy adjacent to the pulpotomized teeth were clinically assessed. The presence of any of these symptoms was regarded as a failed pulpotomy.

Radiolucency in the periapical or furcation areas, internal and external root resorptions, calcified root canals, and widened periodontal ligaments were radiographically evaluated. The presence of any of these symptoms was also regarded as failure.

Additionally, the presence of lesions in the periapical or furcation areas in radiographs were also accepted as failure and these teeth underwent root canal treatment or extraction. Furthermore, teeth with internal root resorption were regarded as radiographically failed and follow-ups were continued until the occurrence of any other symptoms.

Statistical analysis

Descriptive statistics (count, ratio, mean, standard deviation, minimum, and maximum variables) were used to summarize the set of data in the present study. Fisher's exact test was employed to analyze the relationship between pre-treatment clinical findings and dental pathologies developed at the 12-month post-treatment point when pathologies became more apparent. Any relationships between the mean levels of pulpal inflammatory biomarkers and dental pathologies which occurred at the 12-month post-treatment point were analyzed using the Mann-Whitney U test. The Mann-Whitney U test was also used to compare the mean levels of inflammatory biomarkers and clinical findings. The Kruskal Wallis test was used to compare the differences between the mean scores of inflammatory biomarkers by the pulpotomy agents used.

The Receiver Operating Characteristics (ROC) curve was used to determine the diagnostic performance of the IL-6, IL-8, and presepsin in serum. An area under curve (AUC) of more than 50% gives acceptable performance and the area about 100% is the best performance for the test. The optimal threshold values were determined via Youden's index [sensitivity (%) + specificity (%) – 100]. All study analyses were performed with IBM® SPSS® Statistics 25 at a significance level of 0.05.

Results

At the baseline, the present study consisted of a total of 75 children (36 female and 39 male). However, 8 patients were removed due to lack of follow-up. Ultimately, a total of 67 children (31 female and 36 male) with a mean age of 6.3±1.4 and with no difference in mean ages between gender ($p=0.74$) were included. Thus, 13 teeth were excluded from the study and 107 primary second molars in all were clinically and radiographically evaluated at the 12-month post-treatment point (26 teeth in the MTA group, 28 in Biodentine™, 27 in NeoMTA™, and 26 in ZOE). Clinical examinations of the teeth previously diagnosed with pulpotomy revealed pain in 72.9%, prolonged pulpal bleeding time in 35.5%, and increased severity of pulpal hemorrhage in 22.4%.

Results of the post-treatment Clinical & Radiographic assessments

Clinical and radiographic pathologies were observed in 6 teeth (5.6%) at the 6-month post-operative follow-up. With the addition of 4 more teeth with radiographic pathologies only, radiological failure was observed in a total of 10 teeth (9.3%) at the end of the 6th month post-op. At the end of the 12th month post-op, new clinical and radiologic pathologies were observed in 3 teeth and radiographic pathologies alone were detected in 3 more increasing the number of clinically failed teeth to 9 (8.4%) and the number of the teeth with radiographic failures to 16 (15%) in total.

At the 12-month post-op point, clinical and radiographic success rates observed in the pulpotomy material groups were as follows: 100% in MTA, 92.3% and 81.5% in NeoMTA, 82.1% and 67.9% in Biodentine, and 96.1% and 92.3% in ZOE, respectively.

Assessment of the relation between pre-treatment clinical findings and pathologies developed at 12-months post-op

While pathology was observed in 21% of the teeth that exhibited stimulus-related pain before treatment, no pathology was detected in the teeth without pain, resulting in statistical significance ($p<0.05$). The development of pathology was statistically significant in 5.8% of teeth with a normal pulpal bleeding time (1–3 min) and in 32% of teeth with a longer bleeding time (3–5 min) ($p<0.001$). The development of pathology was statistically significant in 8% of teeth with normal pulpal bleeding intensity and in 37% of teeth where the intensity increased ($p<0.001$).

Analysis of the relationship between the pre-treatment pulpal inflammatory biomarkers and dental pathologies developed at 12 months post-op

Statistical analysis revealed a significant difference between the levels of IL-8, IL-6, and presepsin, and dental pathologies at the 12th month post-op which were associated with the higher mean scores of these inflammatory biomarkers in the teeth identified with pathology than the teeth without pathology ($p<0.001$) (Table 1).

Assessment of the relationship between the pre-treatment clinical findings and scores of pulpal inflammatory biomarkers

When the relationship between pre-treatment pain, time and intensity of the pulpal bleeding, and the IL-6, IL-8, and presepsin scores was examined, elevated serum levels of IL-6, IL-8, and presepsin seen in the primary second molars with prolonged bleeding time (3–5 min) and with the increased severity of pulpal hemorrhage were found to be statistically significant ($p<0.001$) (Table 2).

Assessment of the biomarker threshold values

Optimal threshold values (cut-offs) for biomarkers were determined with sensitivity and specificity, which were calculated using a ROC curve. Among all obtained optimum cut-off values for inflammatory parameters, scores

Table 1 Comparison of the mean scores of pulpal inflammatory biomarkers with the presence of pathology

	Mean scores of inflammatory biomarkers ±SD (ng/ml)	Presence of pathology	*n*	Mean scores of biomarkers regarding the presence of pathology ±SD (ng/ml)	*p*
IL-8	190.05±112.52	No	91	172.28±96.14	<0.001*
		Yes	16	291.12±145.60	
IL-6	210.58±162.94	No	91	184.17±119.72	<0.001*
		Yes	16	360.76±270.81	
Presepsin	304.02±146.17	No	91	281.83±136.85	<0.001*
		Yes	16	430.19±136.54	

*$p<0.05$, SD: Standard Deviation

Table 2 Comparison of the relationship between the pre-treatment clinical findings and scores of pulpal inflammatory biomarker

	Pain				Bleeding time				Bleeding Intensity			
		n	Mean biomarker scores ±SD (ng/ml)	*p*		*n*	Mean biomarker scores ±SD (ng/ml)	*p*		*n*	Mean biomarker scores ±SD (ng/ml)	*p*
IL-8	No	29	77.18 ± 7.94	< 0.001*	Normal (1-3min)	69	156.00 ± 83.40	< 0.001*	Normal	83	158.90 ± 84.26	< 0.001*
					Prolonged (3-5min)	38	251.89 ± 131.89		Increased	24	297.78 ± 132.00	
	Yes	78	232.02 ± 104.03									
IL-6	No	29	77.76 ± 12.77	< 0.001*	Normal (1-3min)	69	162.68 ± 102.65	< 0.001*	Normal	83	166.14 ± 105.28	< 0.001*
					Prolonged (3-5min)	38	297.55 ± 211.31		Increased	24	364.27 ± 225.98	
	Yes	78	259.96 ± 165.46									
Presepsin	No	29	131.26 ± 31.95	< 0.001*	Normal (1-3min)	69	256.45 ± 123.79	< 0.001*	Normal	83	265.64 ± 125.54	< 0.001*
					Prolonged (3-5min)	38	390.38 ± 145.49		Increased	24	436.73 ± 136.32	
	Yes	78	368.25 ± 116.71									

*$p<0.05$, SD: Standard Deviation

of presepsin, IL-6, and IL-8, respectively, demonstrated the highest accuracy on predicting the presence of dental pathological conditions (Table 3).

Assessment of the relationship between biomarker levels and pulpotomy agents

Assessment of the mean biomarker values by the pulpotomy materials used illustrated that IL-8, IL-6, and presepsin scores were equal to or higher than the cut-off values in the Neo MTA™ and Biodentine™ groups. However, analysis showed no significant difference between the levels of IL-8, IL-6, and presepsin ($p>0.05$) (Table 4).

Predictive modelling for Post-treatment pathologies using Pre-treatment Clinical findings and Inflammatory Biomarker scores

Modelling the effects of all clinical findings and biomarker scores was based on the no-pathology group. It was shown that the teeth with prolonged bleeding times had a 3.951 times higher chance of developing post-op pathologies while the teeth with higher presepsin scores had a 4.978 times elevated likelihood of experiencing pathologies. Among all the variables, presepsin levels and bleeding time significantly affected the presence of post-op pathologies (Table 5).

Discussion

Pulpotomy is one of the major treatment modalities in pediatric dentistry. Its prognosis is based on the extent of pulpal inflammation and the agents employed [9]. Inflammatory status of the pulp tissue is clinically evaluated using subjective parameters such as presence and type of pain, sensitivity to percussion and palpation, color, time, and the severity of the pulpal bleeding. However, there is lack of evidence that these prognostic factors definitively indicate pulpal inflammation [6, 14]. Furthermore, currently there is neither a method for objectively assessing the status of pulpal inflammation nor an ideal pulpotomy material [9, 10]. The present study examined the effects of biomarkers in the early inflammatory stages of pulpotomy-indicated primary second molars, as well the impact of legacy and modern pulpotomy agents on the prognosis.

In this study, presence of pain observed in all teeth, which developed radiographic pathology at the end of a 12-month post-op follow-up, suggesting that pain was a significant determinant in the development of dental pathologies. Although clinical pain and its severity are still considered important prognostic factors in assessing the severity of pulpal inflammation, pain has not been observed in cases of irreversible pulp inflammation defined as "painless pulpitis". Painless pulpitis progresses with the completion of maturation in older ages since young primary/permanent teeth are more densely

Table 3 Determining threshold values for the biomarkers, measured in the primary second molars with pathology

	Area under the curve	95% Confidence interval		*p*	Sensitivity	1-Specifity	Threshold value
		Upper bound	Lower bound				
IL-8	0.775	0.670	0.881	0.000*	0.750	0.308	202.825
IL-6	0.783	0.681	0.885	0.000*	0.750	0.286	217.0
Presepsin	0.791	0.695	0.887	0.000*	0.813	0.330	329.435

Table 4 Mean biomarker scores by the pulpotomy materials

	Material	*n*	Mean biomarker scores ±SD (ng/ml)	*p*
IL-8	MTA	26	156.01±88.48	0.112
	NeoMTA™	27	215.72±138.12	
	Biodentine™	28	214.96±116.86	
	ZOE	26	170.61±90.87	
IL-6	MTA	26	166.46±112.64	0.096
	NeoMTA™	27	232.10±179.78	
	Biodentine™	28	260.01±212.30	
	ZOE	26	179.11±106.33	
Presepsin	MTA	26	266.82±130.97	0.258
	NeoMTA™	27	323.86±165.71	
	Biodentine™	28	342.97±143.50	
	ZOE	26	278.66±135.67	

*$p<0.05$, SD: Standard Deviation

innervated [15]. Thus, presence of clinical pain symptoms, observed in the present study was considered to be a major predictor for the inflammatory condition of the pulp.

Presence of pain was reported as a symptom that might not always be detected at several inflammatory stages from the early involvements to more severe irreversible pulpal inflammation, while pulpal bleeding time and intensity were remarked as more reliable predictors for the clinical diagnosis of pulpal inflammation [14]. The AAPD Guideline on Pulp Therapy for Primary and Immature Permanent Teeth (2016) reported that inflammatory status of the pulp could be detected by assessing the hemorrhage and could require pulpectomy if the hemostasis could not be managed with a wet cotton pellet in a couple of minutes, considering progressed inflammatory condition [16].

In the present study, prolonged bleeding time (3–5 min) was previously observed during pulpotomy in 75% of the primary second molars, which developed postoperative pathology at the end of 12th month, whereas severe hemorrhage had been detected in 56% of the investigated teeth, constituting a statistical difference between the study group presenting normal pulpotomy symptoms [and which other group].

Today, bleeding time and intensity are regarded as symptoms which can reliably predict inflammatory status of the pulp, while prolonged and severe hemorrhage indicate irreversible pulpitis, thus pulpal tissue [14, 17]. Similar to the findings in the present study, Matsou et al. reported a clinical success rate of 88.6% in the presence of moderate pulpal bleeding and rapid hemostasis and 55.5% with severe and prolonged pulpal hemorrhage demonstrating that bleeding time and intensity had a significant impact on the prognosis [17].

When cariogenic bacteria and their pathogenic by-products access the dental pulp, odontoblasts secrete several receptor molecules by producing various cytokines and antimicrobial peptides and stimulating other immunocompetent cells, which then imitate a strong immune response in the dental pulp involving various cytokines and chemokines [18]. The IL-6 and IL-8 biomarkers are reported to play a major role in this pro-inflammatory response [19]. Presepsin is a novel biomarker represented in 2004 as the sCD14 subtype with superior sensitivity and specificity in bacterial infections; however, no scientific study has been released to date which evaluates its role in pulpal inflammation [8, 20].

Since pulpal inflammations are bacteria associated, presepsin is thought to be a new diagnostic biomarker in early stage pulpal inflammations. Histological analysis

Table 5 Predictive binary logistic regression modelling for the post-treatment pathologies using pre-treatment clinical findings and biomarker scores

						95% Confidence Level	
Variables	**β**	**S.H.**	**Wald**	***p***	**O.*R.***	**Lower Bound**	**Upper Bound**
Bleeding time (Prolonged)	1.374	0.678	4.109	0.043*	3.951	1.047	14.916
Presepsin (Elevated)	1.605	0.733	4.801	0.028*	4.978	1.184	20.923
Constant	-3.398	0.658	26.626	0.000*			

*$p<0.05$ S.E.M=Standard Error of Mean O.R.= Odds Ratio and Ref.: Reference

Hosmer - Lemeshow; $X^2= 1.101$ and $p=0.577$

Cox and Snell $R^2= 0.153$ and Nagelkerke $R^2= 0.269$

Correct classification rate=0.850

had previously been regarded as the gold standard for the diagnosis of pulpal inflammation; however, this type of analysis is not clinically convenient [21]. For this reason, in the present study, IL-8 and IL-6 levels were measured using ELISA, which is considered the most prevalent immunodiagnostic technique today [22].

Evaluation of IL-8, IL-6, and presepsin biomarker levels revealed a statistically significant difference between the healthy teeth and the teeth which experienced pathologies at 12-months post-op. The significant relationship between the elevated levels of IL-8, IL-6, and presepsin and pathology demonstrates that these biomarkers increase with the progression of pulpal inflammation. In previous research assessing pro-inflammatory cytokines, significantly higher IL-8 and IL-6 levels were observed in the cases of advanced pulpitis with considerable inflammation, validating the results of the current study [5–7, 19].

Zou et al. reported that presepsin levels increased in both gram-positive and gram-negative infections [8]. The stronger relationship between the occurrence of pathology and presepsin than IL-6 and IL-8 observed in the present study revealed that presepsin might be a crucial diagnostic biomarker for the gram-positive bacteria-associated pulpal inflammations.

Regarding the limitations of the current standard diagnostic techniques for dental pulp inflammation, molecular testing methods are considered to be promising in the future [19]. Although there is no quantitative bed-side testing modality for identifying the inflammatory status of the dental pulp at the present time, in periodontics, matrix metalloproteinase-8 (MMP-8) was measured for predictive and diagnostic purposes by using chairside kits [23].

Presepsin, which was also assessed in the present study, can be measured in intensive care units (ICUs) and emergency departments (ED) with rapid blood diagnostic test kits in 17 min in cases of serious clinical situations [20]. However, the current high prices of these kits are limiting their widespread use. Given the material, time, and workforce losses, the outcome of a failed pulpotomy due to laborious and inaccurate pretreatment diagnostic assessment of pulp inflammation would also be expensive. If the cost of medical point-of-care testing (POCT) kits could be reduced and adapted into dentistry, presepsin levels might be measured in the preoperatively sampled dental pulp blood using chairside testing kits. Thus, dental treatments could be improved dramatically by enabling an accurate prediction of the pulpal condition.

Another parameter impacting post-op prognosis in the primary teeth to be treated with pulpotomy is material and method to be used [9]. Previous meta analyses and reviews evaluating the clinical success rates of pulpotomy agents reported that as a choice of pulpotomy medication, biomaterials had a more successful bearing on the outcome of the treatment; however, the suitable material has yet to be proven [9, 10, 24].

Today, on the basis of researching the most appropriate agent, biomaterials such as MTA and Biodentine™ are regarded as the most prominent pulpotomy medicaments [9, 24]. The 12-month post-op clinical/radiographic success rates of the materials used in this study were as follows:100% / 100% in the MTA group, 92.3% / 81.5% in NeoMTA, 82.1% / 67.9% in Biodentine™, and finally 96.1% / 92.3% in ZOE group, respectively.

The absence of clinical and radiographic pathologies in the MTA group at the end of the 12-month post-op period demonstrated MTA as the suboptimal pulpotomy medication. Recent meta analyses suggesting MTA as the ideal pulpotomy medication validated the results of the current research [9, 24]. When the success rates of other pulpotomy agents were assessed within the present study, it was observed that the clinical and radiographic success rates of Biodentine™ [12, 25, 26] and NeoMTA [27, 28] fell behind on the ratios published in the literature.

In view of the previous research, it was thought that the inconsistency of the present research results was based on improper clinical diagnosis of pulp inflammation or that it originated from the inflammatory conditions clinically yet to be unveiled despite the existence inflammation [5, 14, 29]. At the end of 12-month post-op period, the ZOE group yielded an overachieving clinical and radiographic success rate. Consistent with the present study, previous studies reported that ZOE pulpotomies might have more successful clinical outcomes when performed under appropriate conditions and concerning normal pulpal inflammation symptoms [29, 30].

It has been revealed that higher incidence of treatment failure rates in the Biodentine™ [12, 25, 26] and NeoMTA [27, 28] groups with outstanding baseline expectancy of success was associated with the mean scores of inflammatory biomarkers detected above the thresholds from the ROC curve analysis, indicating that primary second molars in these material groups had higher inflammation levels than the others. While there is no subjective data, such as statistics on cytokine levels for determining the pre-treatment inflammatory condition of the dental pulp, in almost none of the studies comparing the outcomes of pulpotomy agents, it is thought that the present study will address the gap in the literature and lead future studies.

Statistical insignificance of elevated cytokine levels detected in the less successful Biodentine™ and NeoMTA groups, although they were higher than the threshold values, was associated with the limited study sample size due to the material costs and difficulty of patient follow-ups.

Furthermore, higher failure rates in the Biodentine™ and NeoMTA groups seem to be caused by the incidental

incorporation of the primary second molars with severe inflammation since they were randomly distributed to the study groups. Thus, elevated rates of failure observed in these medication groups cannot be said to be directly associated with the pulpotomy agents alone. Literature review showed that there were several studies comparing only pulpotomy medications or cytokine levels; however, these two parameters are essential for the clinical outcome of pulpotomy and should not be compared separately. Thus, in order to minimize randomization errors, further research similar to the present study but with larger sample sizes is needed.

Incorrect assessment of the pulpal inflammatory condition results in unsuccessful outcome of pulpotomy. Assessment of the clinical findings and symptoms still remains significant for determining pulpal inflammation; however, molecular diagnostics provide more reliable and accurate results. In this study, the inflammatory status of the pulp was evaluated using molecular test techniques by measuring the levels of IL-6, IL-8, and presepsin, all of which were observed to increase in pulpal inflammation. Presepsin has been found to be a significantly more prominent biomarker than either IL-6 or IL-8. The results of the present study indicate that the hypothesis "The new biomarker presepsin has no effect on indicating pulp inflammation" is to be rejected, as it was demonstrated that presepsin is an important biomarker for reflecting pulp inflammation. Furthermore, after one year of treatment follow-up, increased pathology was observed in the NeoMTA and Biodentine groups, where biomarker levels were higher, while no pathology was observed in the MTA group, where biomarker levels were lower. This indicates that the level of pulp inflammation is significant for treatment success, leading to the rejection of the hypothesis "The level of high pulpal inflammation in pulpotomy treatment does not affect the success of the treatment according to the biomaterials used in pulpotomy.".

Conclusion

The present study illustrated that presepsin is a highly successful biomarker for identifying the inflammatory condition of the pulp. Based on the results of this study, development and production of affordable chairside presepsin testing kits for dentistry will provide a rapid and reliable assessment approach for pulpal inflammation and thus, assist in improving the accuracy of diagnosis. Accordingly, pulpotomies will be more successful and subsequent treatments will be prevented, which in turn will improve patient comfort and reduce exorbitant treatment costs. However, additional research is required to develop promising molecular diagnostic techniques for convenient and reliable identification of pulpal inflammation.

Author contributions
Conceptualization: [A. B, S.S.D],;Methodology: [S. S. D, E. L],; Formal analysis and investigation: [A.B, E.L],; Writing - original draft preparation: [A. B, S. S. D, E, L] Writing - review and editing: [A. B],; Funding acquisition: [A. B, S. S. D, E. L], Resources: [A. B, E. L],; Supervision: [S. S. D, E. L]

Funding
Our study has been financed by the Unit of Scientific Research Projects.

Data availability
This manuscript does not report data generation or analysis.

Declarations

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of Ataturk (Date: 05.2021/ Session no:04/ Decision no:66).

Consent to participate
Informed consent was obtained from the parents of all children included in the study.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

Received: 18 July 2024 / Accepted: 26 September 2024
Published online: 15 October 2024

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