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ORIGINAL RESEARCH

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Evaluation of pulpal inflammation level in young permanent teeth using biomarker levels

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Abstract

Background: In today's clinic, there are no definitive diagnostic criteria for determining the level of pulp inflammation. Increased biomarker levels in pulp inflammation enable the quantitative measurement of pulp inflammation and provide objective data. Based on this, in our study, the level of pulp inflammation was measured using biomarkers, and the increased biomarker level was compared with the clinical parameters used to determine the level of pulp inflammation. In addition, biomarker levels were compared in teeth classified as reversible pulpitis (RP) and irreversible pulpitis (IRP) according to clinical diagnostic parameters. Furthermore, the effect of Presepsin, a novel biomarker used in the assessment of pulp inflammation, was demonstrated. **Methods**: The study was conducted on teeth that underwent pulpotomy and were classified as RP (n = 30) and IRP (n = 30) based on clinical parameters. During the treatment period, pain, bleeding time, severity and color findings were recorded. Bleeding samples were taken to measure the level of inflammation in pulp, and the biomarkers TNF-α (Tumor Necrosis Factor α), IL-6 (Interleukin 6), IL-1β (Interleukin 1β), IL-8 (Interleukin 8), and Presepsin were evaluated. **Results**: When clinical parameters were examined, a significant difference was found between the presence of spontaneous pain, pulp bleeding more than 3 minutes, severe bleeding, and an increase in biomarker levels. When comparing the pulp of RP and IRP teeth, biomarker levels in the coronal pulp were similar, while in the radicular pulp, biomarker levels in IRP teeth were significantly higher. Presepsin demonstrated the highest sensitivity and specificity in distinguishing IRP teeth from RP teeth. **Conclusions**: Clinically assessed pain, pulp bleeding time and severity data can be considered in the evaluation of pulp inflammation. Among the biomarkers, Presepsin, which has the highest sensitivity and specificity in distinguishing between RP and IRP teeth, can be considered an important biomarker in the evaluation of pulp inflammation.

Keywords

Dental pulp diseases; Pulpotomy; Biomarkers; Presepsin

1. Introduction

Caries in newly erupted young permanent teeth, which have not yet fully matured, can advance swiftly, resulting in extensive inflammation and necrosis of the pulp [1]. The health of young permanent teeth can be safeguarded by preserving pulp vitality, facilitating root development, attaining an optimal crown-to-root ratio, fortifying dentin structure, and averting dental fragility, thus ensuring prolonged retention of teeth in the oral cavity [2]. Consequently, whereas previously the complete removal of pulp was favored in cases of severe pulp symptoms, contemporary understanding of pulp physiology has led to the increased popularity of vital pulp treatments that seek to preserve the entire pulp or the unaffected portion of it [3, 4].

In determining essential treatment for instances with pulp exposure during caries management, the current pulp inflammation is assessed through factors including nature of pain, the extent of carious progression to the pulp, and the characteristics of pulpal hemorrhage [5]. Clinicians sometimes find it challenging to ascertain the degree of pulp inflammation from these measures, particularly in young permanent teeth [6]. It is therefore essential to assess the precision of clinical criteria in representing pulp inflammation in young permanent teeth. Pulp inflammation is currently categorized as reversible pulpitis (RP) when it is amenable to therapy based on clinical criteria, or irreversible pulpitis (IRP) when it is not, dictating the appropriate therapeutic approach [7]. Nonetheless, it is acknowledged that these indicators are not consistently dependable for assessing pulp inflammation, prompting an emphasis on the development of novel approaches for the quantitative measurement of pulp inflammation [8].

The roles of numerous biomarkers produced during pulp inflammation are believed to be distinctly identifiable, allow-

ing for the determination of highly accurate biomarkers that facilitate the quantitative assessment of pulp inflammation in clinical settings, thereby establishing a new field of study [8–10]. Prior studies have found numerous biomarkers that are significant in pulp inflammation [7, 9, 10]. In the present study, the proinflammatory cytokines TNF-α (Tumor Necrosis Factor α) IL-6 (Interleukin 6), IL-1β (Interleukin 1β), and the chemokine IL-8 (Interleukin 8), which were significant in these studies, were used to assess the degree of pulp inflammation. Moreover, Presepsin, a biomarker characterized by high sensitivity and specificity, is crucial for the early diagnosis of bacterial infections in medicine [11, 12]. It has recently been studied in primary teeth to assess pulp inflammation, yielding remarkable results [13]. This study analyzed various biomarkers and assessed their efficacy for the first time in detecting the extent of pulp inflammation in young permanent teeth.

The present study will assess the efficacy of clinical findings in pulpotomy treatment for young permanent teeth in predicting the degree of pulp inflammation against pulp biomarker levels. This study will compare the levels of TNF-α, IL-6, IL-1β, and IL-8, established biomarkers associated with pulp inflammation, against the effects of Presepsin, a novel biomarker for assessing pulp inflammation.

Hypotheses established for this study:

- The clinical results assessed during pulpotomy treatment of young permanent teeth (including pain characteristics, timing, severity, and pulp bleeding color) are inadequate for determining the extent of pulp inflammation.
- Clinical data indicate no disparity in inflammatory levels between the coronal and radicular pulps of teeth categorized as RP and IRP.
- Presepsin, an emerging biomarker for assessing pulp inflammation, does not differ from other markers in its reflection of pulp inflammation levels.

2. Materials and methods

Fifty-six children aged 6–12 years who were systemically healthy, not taking any medication, and had an indication for pulpotomy treatment of immature first or second molars were enrolled in the study after obtaining consent from their parents. Following approval by the Ethics Committee of Atatürk University Faculty of Medicine (Decision no.: 70/Decision date: 26 October 2023), patient enrolment for the study commenced in January 2024 and was completed in August 2024. The sample size was computed utilizing the "G*Power-3.1.9.2" software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, NRW, Germany) at a 95% confidence level, resulting in a minimum requirement of 50 teeth. The sample collection phase of the study concluded upon reaching the minimum required sample size of 60. Thus, our study was conducted on 60 teeth with the participation of 56 children.

2.1 Criteria for inclusion of teeth

- Teeth exhibiting spontaneous or stimulus-induced discomfort, where cavity cleaning results in an opening exceeding 2 mm in the pulp roof over many regions.
- In the absence of reported discomfort, caries extension into the pulp produced an orifice larger than 2 mm in the pulp roof in many regions, with inflammatory signs observed in the bleeding pulp.
- First and second molars with incomplete root development.
- Teeth exhibiting neither radiographic indications of pathological root resorption nor lesions in the furcal and periapical regions.
- Restorable teeth.
- Teeth devoid of significant pathological indicators, including mobility, edema, erythema, or fistula in the vestibular sulcus.

2.2 Criteria for exclusion of teeth

- Teeth for which direct or indirect capping is warranted during treatment, where the pulp roof is less than 2 mm exposed and the indications of pulp inflammation are quite moderate.
- Teeth exhibiting pulp necrosis following the exposure of the pulp chamber, for which regenerative endodontic therapy or apexification is indicated.
- Teeth that are excessively deteriorated to permit restoration and are more suitably removed.
- Teeth exhibiting radiographic abnormalities in the periapical or furcal regions, as well as pathological root resorption.

2.3 Pre-treatment protocol

Following the acquisition of informed consent from the parents of the patients included in our study, a detailed medical and dental history was taken. While taking the dental history, the patient's history of pain, sensitivity to hot and cold, and percussion findings were noted in detail. These findings were excluded from the study, particularly in younger patients, as they were unable to express whether they had dental sensitivity to hot or cold and gave hesitant responses during the percussion test. To ensure patients could better understand and respond when asked about their pain history, questions such as "Have you ever felt pain in your teeth?", "Do you experience pain in your teeth while eating or afterwards?", or "Do you experience prolonged pain in your teeth at school, when you come home, or at night?" were asked and the answers were recorded.

2.4 Classification of teeth

As the nature and duration of pain are currently accepted as the basic criteria for distinguishing between RP and IRP, teeth with spontaneous pain or pain in response to stimuli were included in the irreversible pulpitis group. The bleeding findings in these teeth also supported the diagnosis of IRP. However, in some teeth, despite pain in response to stimuli, the pulp bleeding findings were more favourable. Therefore, teeth with no pain or even with pain in response to stimuli but with better pulp bleeding findings were considered to have RP.

2.5 Treatment protocol

Following the administration of anaesthesia, the decay removal process commenced under a rubber dam. After removing all decay from the cavity walls, the pulp roof was carefully

cleaned. In cases where the decay caused an opening of more than 2 mm in multiple areas of the pulp roof, a decision was made to perform pulpotomy treatment. Although pulpotomy treatments were performed by three different paediatric dentists, the treatment protocol, evaluation and recording of clinical symptoms and pulp bleeding parameters were carried out under the supervision of a single academic expert in pulp treatments. As there are no clear data on pulp bleeding parameters for the assessment of pulp inflammation today, subjective data were examined. A review of the literature showed that a bleeding time of more than 3 minutes was associated with severe pulp inflammation [13–15]. Bleeding intensity was assessed as "severe" in teeth where bleeding started immediately after removing all the coronal pulp and applying a cotton tampon to the remaining radicular pulp, rapidly filling the pulp chamber, and as "normal" in teeth where bleeding started as a trickle a few seconds later. Bleeding color was assessed as "light" if it was bright red and shiny, and "dark" if it was dark red and dull. After controlling the bleeding with 2.5% NaOCl (Sodium Hypochlorite), the pulp tissue was covered with a calcium silicate-containing material, and the tooth was restored.

2.6 Sample collection and ELISA test

To assess inflammation levels in the coronal and radicular pulp, the initial sample was collected at the site of the pulp chamber roof exposure, while the subsequent sample was taken from the cavity floor following the complete excision of the coronal pulp. To collect bleeding samples, sterile cotton pellets were placed on the bleeding site for 30–45 seconds and then placed in heparin tubes (4176495, Vacutainer, BD, Fraklin Lakes, NJ, USA) containing 1 mL of saline solution. Two distinct samples were collected from the same patient; hence, an identifier indicating their origin was affixed to the tube, with the coronal pulp sample designated as 1 and the radicular pulp sample as 2. The samples were preserved at −20 °C (−4.0 °F) for roughly 3 to 6 months prior to undergoing Enzyme-Linked Immunosorbent Assay (ELISA) testing.

2.7 ELISA test application protocol

The samples were solubilized at ambient temperature and centrifuged at 4000 rpm for 15 minutes. The cotton pellets were subsequently extracted from the test tubes. The IL-1β, IL-6, IL-8, TNF-α, and Presepsin levels of patients were measured on the same day to avoid inter-day variation. IL-1β, IL-6, IL-8, TNF-α, and Presepsin levels were measured using the ELISA method. The following kits were used to measure the respective levels in accordance with the manufacturer's instructions: Human IL-1β ELISA Kit (Cat No: 201-12-0144, Sunred, Shanghai, China), Human IL-6 ELISA Kit (Cat No: 201-12-0091, Sunred, Shanghai, China), Human IL-8 ELISA Kit (Cat No: 201-12-0090, Sunred, Shanghai, China), Human TNF-α ELISA Kit (Cat No: 201-12-0083, Sunred, Shanghai, China), and Human Presepsin ELISA Kit (Cat No: 201-12-5358, Sunred, Shanghai, China). The measurement ranges for IL-1β, IL-6, IL-8, TNF-α, and Presepsin were 300–9600 pg/mL, 20–640 ng/L, 10–320 ng/L, 30–960 ng/L, and 300–9600 pg/mL, respectively. For all biomarkers, Inter-Assay Coefficient of Variation (CV) was $<$10% and Intra-Assay CV was $<$8%. Samples and standards were introduced into wells pre-coated with antibodies against human IL-1β, IL-6, IL-8, TNF-α, and Presepsin. The IL-1β, IL-6, IL-8, TNF-α, and Presepsin molecules found in the samples bound to these coated antibodies. Molecules that failed to bind throughout the washing procedure were eliminated. A secondary antibody specific to IL-1β, IL-6, IL-8, TNF-α, and Presepsin, conjugated with biotin, was introduced to the wells. After an additional washing phase, peroxidase enzyme conjugated to streptavidin was introduced. The peroxidase enzyme in this complex, attached to avidin, oxidized 3,3′-5,5′-tetramethylbenzidine in the medium, resulting in a color change that correlates with the concentrations of IL-1β, IL-6, IL-8, TNF-α, and Presepsin in the samples. Subsequently, acid was introduced to each well to terminate the reaction. Absorbance values for each well were quantified using a spectrophotometer at a wavelength of 450 nm. The quantities of IL-1β, IL-6, IL-8, TNF-α, and Presepsin in each sample were determined from the absorbance-concentration graph utilizing standards created at progressively lower values.

2.8 Statistical analysis

Statistical analyses were conducted utilizing SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The normality of the variables was assessed by histogram plots, and the Shapiro-Wilk Test. Descriptive analyses were conducted utilizing mean, standard deviation, median, minimum, and maximum values. For variables without a normal distribution, the Mann-Whitney U test was used for comparisons between two groups, and the Kruskal-Wallis test was applied for comparisons among more than two groups. When significant differences were found in the Kruskal-Wallis analysis, *post-hoc* pairwise comparisons were performed with Bonferroni correction. For normally distributed variables, the Independent Samples *t*-test was used for comparisons between two groups, whereas one-way Analysis of Variance (ANOVA) followed by Tukey *post-hoc* testing was used for comparisons among more than two groups. The Receiver Operating Characteristic (ROC) curve is a graph that plots the true positive rate (sensitivity) on the vertical axis and the false positive rate (1 − specificity) on the horizontal axis for different threshold values. Each point on the ROC curve represents the sensitivity and 1 − specificity values corresponding to different threshold values. The ROC curve, which quantifies the prediction efficacy of a specific method, was employed to determine the sensitivity, specificity, and threshold value of the markers. Also, the diagnostic performance of the optimal biomarker combination was further assessed using multiple logistic regression supplemented by ROC analysis. Instances in which the *p*-value fell below 0.05 were deemed statistically significant outcomes.

3. Results

Upon assessment of dental pain, 12 teeth were identified as pain-free, 24 teeth exhibited pain upon stimulation, and 24 teeth experienced spontaneous pain. Levels of IL-1β, Pre-

sepsin, IL-6, IL-8, and TNF-α were minimal in individuals without pain and maximal in those experiencing spontaneous dental pain. The biomarker level in teeth exhibiting spontaneous pain was statistically significant when compared to teeth without pain and those with pain caused by stimulation ($p < 0.05$). Nonetheless, while the biomarker levels in teeth experiencing pain due to stimulation were elevated compared to those without discomfort, the difference was not statistically significant (Table 1).

As bleeding time and the severity of radicular pulp increase, levels of IL-1β, Presepsin, IL-6, IL-8, and TNF-α rise dramatically ($p < 0.05$). No significant differences were noted in the levels of IL-1β, IL-6, IL-8, and TNF-α as the color of the blood deepened; however, a substantial increase in Presepsin levels was reported (Table 2).

The concentrations of IL-1β, Presepsin, IL-6, IL-8, and TNF-α in the coronal pulp samples of the RP group were markedly elevated compared to those in the radicular pulp samples. In the IRP group, the concentrations of IL-1β, Presepsin, IL-6, IL-8, and TNF-α in the radicular pulp samples were markedly elevated compared to those in the coronal pulp samples (Table 3).

The concentrations of IL-1β, Presepsin, IL-6, IL-8, and TNF-α in hemorrhagic samples obtained from the coronal and radicular pulps of RP and IRP teeth were analyzed between the two cohorts. While no differences were found between the two groups in the coronal pulp samples, significant differences were found in the radicular pulp, with high levels of IL-1β, Presepsin, IL-6, IL-8, and TNF-α in the IRP group (Table 4).

Presepsin exhibited the greatest sensitivity and specificity in differentiating IRP teeth from RP teeth. Utilizing a cut-off value of 2061.14 for Presepsin yielded a sensitivity of 96% and a specificity of 89%. Utilizing a cut-off value of 121.14 for IL-6 yielded a sensitivity of 95% and a specificity of 86%. Utilizing a cut-off value of 187.67 for TNF-α yielded a sensitivity of 93% and a specificity of 84%. Utilizing a cut-off value of 1525.35 for IL-1β yielded a sensitivity of 88% and a specificity of 83%. The cut-off value for IL-8 was established at 134.08, yielding a sensitivity of 87% and a specificity of 80% (Fig. 1).

The combined analysis of biomarkers revealed the highest diagnostic efficacy in the combination Presepsin + IL-8 (AUC (Area Under the Curve) = 0.949), followed by the combination Presepsin + IL-6 (AUC = 0.944) and Presepsin + IL-1β (AUC = 0.941). The lowest discriminatory potential was reported for Presepsin + TNF-α (AUC = 0.938) (Fig. 2).

4. Discussion

Currently, there is no evidence regarding which clinical parameter is more reliable when determining the level of pulp inflammation. Progress in dentistry suggests that upcoming technology or techniques will facilitate the chairside measurement of elevated biomarkers in pulp inflammation, hence permitting the assessment of inflammation levels and allowing for the implementation of more precise treatments. To assess elevated biomarkers of pulp inflammation at the patient's chairside, it is essential to first examine the reliability, validity, and evidential strength in representing pulp inflammation. The present study assessed the precision of clinical measures utilized for diagnosing pulp inflammation and the impact of Presepsin, a novel biomarker recognized for its significant role in assessing pulp inflammation. Consequently, it is anticipated that this study will substantially enhance the literature, as it extensively assessed the degree of pulp inflammation through multiple

TABLE 1. Comparison of IL-1β, Presepsin, IL-6, IL-8, and TNF-α concentrations in patients categorized by pain characteristics.

	Biomarker levels in teeth without pain (n = 12)	Biomarker levels in the teeth where pain began after eating (n = 24)	Biomarker level in teeth with spontaneous pain (n = 24)	*p*
IL-1β*	1070.00 ± 164.31	1333.33 ± 632.04	1779.30 ± 424.69	0.290[a] <0.001[b] 0.007[c]
Presepsin*	1154.62 ± 433.36	1776.77 ± 1047.90	2487.39 ± 648.19	0.083[a] <0.001[b] 0.009[c]
IL-6**	62.52 (42.78–71.03)	73.76 (34.49–214.44)	189.69 (22.81–221.20)	0.188[a] <0.001[b] 0.007[c]
IL-8**	55.47 (30.73–63.86)	63.0 (27.85–179.84)	140.88 (41.36–197.74)	0.212[a] <0.001[b] 0.019[c]
TNF-α*	97.08 ± 27.28	156.3 ± 95.73	223.95 ± 43.33	0.043[a] <0.001[b] 0.003[c]

*Values are presented as mean ± standard deviation or median (range: minimum–maximum). a: comparison of pain versus absence of pain and pain with stimulus; b: comparison of pain versus absence of pain and spontaneous pain; c: comparison of pain with stimulus versus spontaneous pain. *: One-way ANOVA; **: Mann-Whitney U. IL: Interleukin; TNF: Tumor Necrosis Factor.*

TABLE 2. Comparison of IL-1β, Presepsin, IL-6, IL-8, and TNF-α concentrations in teeth categorized by bleeding duration, severity, and coloration of the radicular pulp.

	IL-1β*	Presepsin*	IL-6**	IL-8**	TNF-α*
Radicular pulp bleeding time					
<3 min (n = 36)	1261.14 ± 542.32	1488.49 ± 786.78	63.93 (22.81–208.88)	55.47 (27.85–179.84)	139.34 ± 80.02
>3 min (n = 24)	1756.22 ± 440.43	2608.74 ± 736.64	191.04 (69.11–221.20)	143.38 (61.33–197.74)	219.77 ± 60.04
p	<0.001	<0.001	<0.001	<0.001	<0.001
Radicular pulp bleeding severity					
Normal (n = 45)	1232.69 ± 399.62	1541.02 ± 685.29	70.20 (22.81–197.79)	61.96 (27.85–146.32)	141.73 ± 68.79
Severe (n = 15)	2138.62 ± 380.37	3123.29 ± 492.03	203.22 (79.06–221.20)	151.57 (69.19–197.74)	260.87 ± 47.96
p	<0.001	<0.001	<0.001	<0.001	<0.001
Radicular pulp bleeding color					
Light (n = 28)	1312.67 ± 517.13	1630.32 ± 786.55	74.92 (22.80–208.90)	65.31 (27.85–148.28)	150.95 ± 73.94
Dark (n = 32)	1587.36 ± 566.18	2204.58 ± 993.36	121.14 (34.50–221.20)	71.92 (29.20–197.74)	189.51 ± 86.19
p	0.056	0.017	0.175	0.118	0.070

*Values are given as mean ± standard deviation or median (minimum–maximum). *: T test in independent samples; **: Mann-Whitney U. IL: Interleukin; TNF: Tumor Necrosis Factor; min: minute.*

TABLE 3. Comparative analysis of biomarker concentrations in coronal and radicular pulp.

	IL-1β*	Presepsin*	IL-6**	IL-8**	TNF-α*
RP					
Coronal pulp (n = 30)	1365.99 ± 424.42	1905.21 ± 695.78	70.46 ± 16.16	65.49 ± 14.20	142.17 ± 52.38
Radicular pulp (n = 30)	1003.51 ± 266.09	1211.46 ± 564.05	58.04 ± 15.29	50.53 ± 13.36	102.75 ± 46.65
p	<0.001	<0.001	0.003	<0.001	0.003
IRP					
Coronal pulp (n = 30)	1386.64 ± 419.49	2005.54 ± 719.64	181.31 (98.89–212.07)	128.13 (37.11–160.28)	178.61 ± 24.71
Radicular pulp (n = 30)	1914.83 ± 360.74	2661.71 ± 628.18	193.97 (111.90–221.20)	144.89 (48.10 –197.74)	240.28 ± 42.89
p	<0.001	<0.001	<0.001	<0.001	<0.001

*Values are given as mean ± standard deviation or median (minimum–maximum). *: T test in independent samples; **: Mann-Whitney U. RP: reversible pulpitis; IRP: irreversible pulpitis; IL: Interleukin; TNF: Tumor Necrosis Factor.*

TABLE 4. Comparative analysis of biomarker concentrations in coronal and radicular pulp specimens from RP and IRP teeth.

	IL-1β*	Presepsin*	IL-6**	IL-8**	TNF-α*
Coronal pulp					
RP	1365.99 ± 424.42	1905.21 ± 695.78	70.13 (22.76–104.68)	63.19 (46.4–119.59)	142.17 ± 52.38
IRP	1488.39 ± 651.36	1946.98 ± 1020.68	104.84 (22.81–221.2)	66.62 (27.85–168.38)	174.92 ± 83.60
p	0.393	0.854	0.095	0.379	0.075
Radicular pulp					
RP	1003.51 ± 266.09	1211.46 ± 564.05	59.48 (22.81–79.33)	49.46 (27.85–70.07)	102.75 ± 46.65
IRP	1914.83 ± 360.74	2661.71 ± 628.18	193.97 (111.93–221.2)	144.89 (48.08–197.74)	240.28 ± 42.89
p	<0.001	<0.001	<0.001	<0.001	<0.001

*Values are given as mean ± standard deviation or median (minimum–maximum). *: T test in independent samples; **: Mann-Whitney U. RP: reversible pulpitis; IRP: irreversible pulpitis; IL: Interleukin; TNF: Tumor Necrosis Factor.*

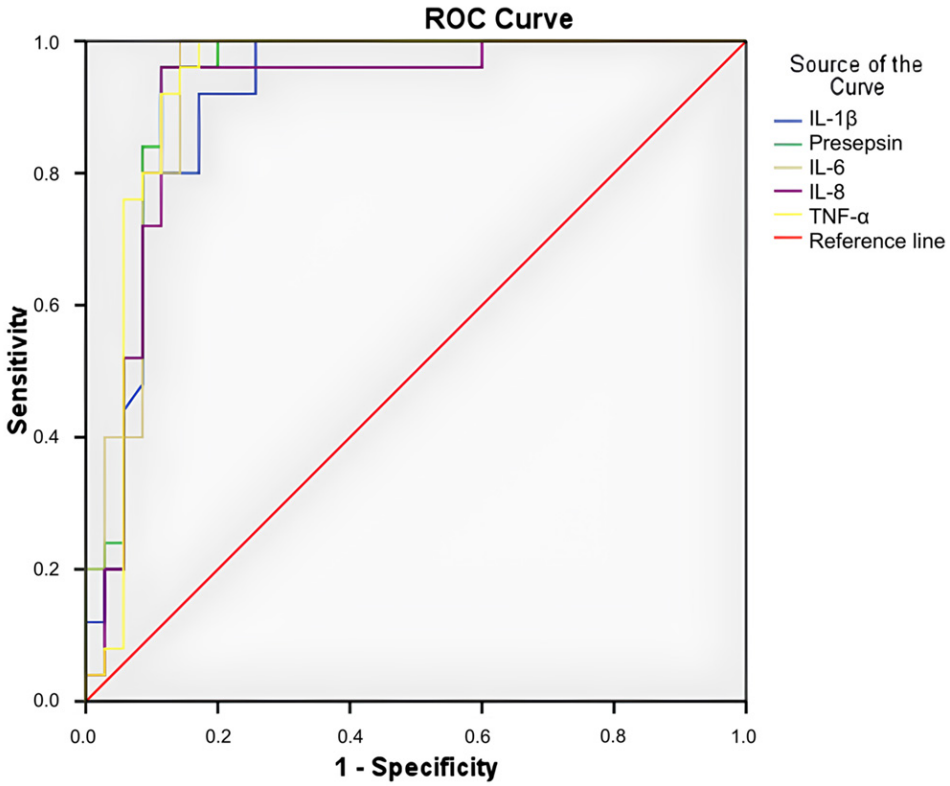

FIGURE 1. Assessment of the sensitivity and specificity of IL-1β (AUC = 0.913 and $p < 0.001$), Presepsin (AUC = 0.934 and $p < 0.001$), IL-6 (AUC = 0.931 and $p < 0.001$), IL-8 (AUC = 0.909, $p < 0.001$), and TNF-α (AUC = 0.930 and $p < 0.001$) concentrations in differentiating IRP from RP teeth using ROC curve analysis. ROC: Receiver Operating Characteristic; AUC: Area Under the Curve; RP: Reversible Pulpitis; IRP: Irreversible Pulpitis; IL: Interleukin; TNF: Tumor Necrosis Faktor.

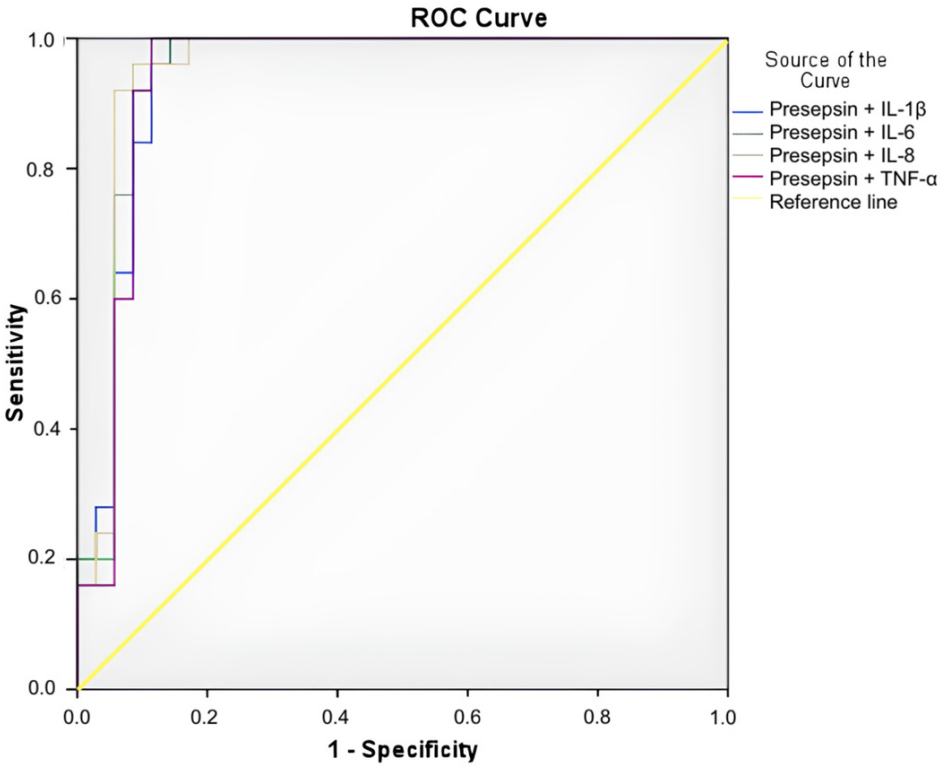

FIGURE 2. ROC analysis of the different combinations of biomarkers studied. ROC: Receiver Operating Characteristic; IL: Interleukin; TNF: Tumor Necrosis Factor.

criteria.

Pulpotomy is a crucial critical pulp therapy involving the whole or partial excision of infected pulp tissue [5]. Coronal pulpotomy is suggested for young permanent teeth with advanced pulp inflammatory signs [16]. However, there is no conclusive evidence for the appropriate treatment in cases where the pulp roof is exposed to caries, albeit exhibiting minor signs of pulp inflammation, contingent upon the extent of exposure. Currently, it is indicated that partial pulpotomy procedures may be performed prior to coronal pulpotomy in instances of moderate pulp inflammation, within the framework of minimally invasive endodontic practices [5, 16]. Partial pulpotomy treatment presents difficulties, including the necessity for technical precision in excising the infected coronal pulp, the requirement for adequate magnification, the risk of fragmenting the residual pulp, and an extended procedural duration [5]. In light of these circumstances, this study involved coronal pulpotomy therapy on teeth exhibiting significant pulp exposure due to caries, along with severe and moderate pulp inflammation, with bleeding samples collected from both the coronal and radicular pulp for biomarker assessment. In pulpotomy treatment, the precision of treatment and prognosis assessment relies on observations in the radicular pulp; thus, clinical parameters were assessed in relation to biomarker levels in the radicular pulp.

4.1 Clinical assessment parameters findings

In contemporary clinics, examining the duration and intensity of pain, as well as the precipitating factors, is crucial for estimating the extent of pulp inflammation and informing treatment decisions and prognosis [3]. Analysis revealed markedly elevated biomarker levels in teeth exhibiting spontaneous pain in contrast to those experiencing pain following stimulation or exhibiting no pain. This outcome aligns with prior studies examining pain and biomarker concentrations in primary teeth [13]. Ricucci *et al.* [17] did a histology analysis revealing that pain is a significant factor in differentiating between normal and reversible pulpitis teeth. In addition, a study assessing the relationship between biomarkers and pain found significant differences between the presence of pain and IL-1α, IL-6, and IL-8 [18]. The European Society of Endodontology asserts that the existence and characteristics of pain must serve as a clinical criterion for differentiating between reversible and irreversible pulpitis [5]. Currently, there is no evidence indicating that the presence and characteristics of pain serve as reliable measures for assessing the degree of pulp inflammation [19]. This is due to the potential emergence of painless pulpitis following distinct specific pathophysiological processes [20]. Studies indicates that 14% to 60% of permanent pulpitis cases may be asymptomatic [20, 21].

During the clinical assessment of the level of pulpal inflammation, both pain and signs of pulpal hemorrhage should be assessed. A comprehensive analysis by Donnermeyer *et al.* [7] indicated that bleeding discoveries in the pulp are more reliable indicators than pain for assessing pulp inflammation, with cases of chronic or severe pulp bleeding suggesting irreversible pulpitis. The study results indicated a substantial correlation between the elevation of biomarker levels and the duration and intensity of pulp bleeding, as well as its coloration. This is anticipated, as heightened vascularization and rapid blood flow during pulp inflammation, along with disturbance of the coagulation process, results in prolonged and intensified bleeding time [7, 22]. Currently, there is no clear data regarding the duration and severity of pulp haemorrhage in determining the level of pulp inflammation. The latest guidelines of the American Academy of Paediatric Dentistry on pulpotomy emphasise that bleeding in teeth with reversible pulpitis should be controlled within a few minutes [15]. In a clinical study conducted by Caredde and Duncan, it was stated that pulp bleeding lasting longer than 2 minutes reflects severe pulpitis [14]. In our previous study on deciduous teeth, it was demonstrated that biomarker levels were significantly elevated in deciduous teeth with pulp bleeding lasting longer than 3 minutes [13]. Based on these data, in our present study, pulp bleeding lasting longer than 3 minutes was considered an indication of severe pulp inflammation. Although our study found a significant difference between increased bleeding time and increased biomarker levels, there are also studies in the literature showing no correlation [18, 23]. When evaluating current clinical studies, although there is a significant correlation between increased bleeding time and pulpal inflammation levels, it is believed that this does not affect treatment success [24].

No significant difference was observed between the elevation of biomarker levels and the darkening of pulp hemorrhage. Aminabadi *et al.*'s [25] study, which meticulously examined the color of pulp hemorrhage in connection to inflammation levels, demonstrated a substantial association between the two variables. This study assessed the color of pulp bleeding samples using a specialized instrument, whereas the present study utilized visual inspection, potentially accounting for the observed discrepancy. The current study identified a substantial link between elevated biomarker levels and the majority of clinical measures, aligning with recent studies assessing the association between clinical parameters and pulp inflammation [13, 17]. Currently, there is no conclusive data supporting the efficacy of any clinical findings in identifying pulp inflammation [7, 19].

Based on these findings, when our initial hypothesis was evaluated, it led to the partially rejection of the hypothesis that "clinical findings are insufficient to indicate the level of pulp inflammation". This is because spontaneous pain, prolonged pulp bleeding exceeding 3 minutes, and severe bleeding were found to be associated with high biomarker levels.

4.2 Biomarker findings

Pulp exposure to caries leads to primary bacterial infections, resulting in an elevation of different biomarkers [3, 26]. A comprehensive review has assessed biomarkers applicable in assessing pulp inflammation, with various studies previously undertaken on this subject [27]. In the present study, the proinflammatory cytokines IL-1β and TNF-α, which are crucial in modulating immunity during pulp inflammation, as well as IL-8, a significant chemokine in the acute pulp inflammatory response, were assessed [3]. Furthermore, Presepsin, a cytokine known for its efficacy in the swift and accurate assessment of

bacterial sepsis in clinical settings, was incorporated into the study under the premise that it would similarly yield prompt and dependable results in pulp inflammation, typically induced by bacterial activity.

Presepsin is a soluble subtype of Cluster of Differentiation (CD)14 that is released when the CD14 receptor, responsible for presenting bacterial products to the cell, is detached from the cell membrane or synthesized by the cell [28]. Presepsin levels are believed to rise swiftly in the initial stages due to the upregulation of CD14 receptors, which facilitate the presentation of bacterial products to cellular receptors during pulp inflammation. Bacterial products binding to Toll-Like Receptor (TLR)-4 on the cell surface through the CD14 receptor trigger a cascade of physiological responses, activating the innate immune system and promoting the secretion of cytokines including TNF-α, IFN-γ, IL-1β, IL-8, and IL-6 [28, 29]. Consequently, the elevation of IL-1β, IL-8, IL-6, and TNF-α, which are additional biomarkers alongside Presepsin within the present study of pulp inflammation, can be elucidated by this sequence of occurrences.

4.3 Findings regarding biomarker levels in the coronal and radicular pulps of teeth classified as having RP and IRP

In assessing the severity of pulp inflammation according to its localization, the biomarker concentration in the coronal pulp was observed to exceed that in the radicular pulp in RP teeth, whereas in IRP teeth, the inflammation level in the radicular pulp surpassed that in the coronal pulp. To illustrate this scenario, a comprehensive understanding of the events associated with the pulp's inflammation process is essential. Acute pulpitis induces substantial vasodilation, especially in the subodontoblastic capillary plexus, relative to healthy pulp tissue, facilitating the infiltration of different immune cells, predominantly neutrophils, into the affected area. The inflammatory response is modulated by several cytokines and chemokines through vasodilation [3]. In the case of RP, this largely ongoing process may persist with moderate chronic inflammation confined to the coronal pulp [17]. Consequently, the present study anticipated that inflammation indicators in the coronal pulp of teeth exhibiting RP symptoms would surpass those in the radicular pulp.

The ongoing inflammatory process leads to heightened tissue perfusion, causing edema in the pulp chamber encased by dentin, which, due to the lack of expansion capacity, exerts pressure on the venous and lymphatic arteries. This disorder may result in pulp damage and potentially necrosis [3]. A histology study by Ricucci *et al*. [17] revealed that partial or total necrosis was observed in the coronal pulp in the context of RP. In this scenario, inadequate blood circulation in the coronal pulp would result in a diminished quantity of immune cells, thereby leading to a reduction in biomarker levels. The present study indicates that the markedly reduced concentration of biomarkers in the coronal pulp of IRP teeth, in comparison to the radicular pulp, may be attributable to this factor. Aminabadi *et al*. [25] reported no disparity in inflammation levels between the coronal and radicular pulp in teeth with irreversible pulpitis, contrary to the current study's findings, in their histological assessment of deciduous teeth post-pulpectomy. This condition may arise from the assessment of pulp inflammation levels through various methodologies.

Zanini *et al*. [30] conducted a systematic assessment indicating that biomarker levels may facilitate the clinical differentiation between RP and IRP teeth. Consequently, the present study quantified biomarker levels to clarify the distinctions between RP and IRP teeth. When the pulp is afflicted with caries, bacteria and their byproducts that have not yet infiltrated the pulp can elicit a natural and adaptive immune-inflammatory response within the pulp [31]. When bacteria invade the pulp, necrotic regions entirely lacking immune defense mechanisms will form within the pulp [17]. Pulp tissue possesses innate and adaptive immune defense systems that inhibit the dissemination of germs and inflammation from the necrotic region. The inflammatory process will have disseminated from the coronal pulp to the radicular pulp. In reversible pulpitis, the inflammatory response initiates in the coronal pulp, but in irreversible pulpitis, it culminates in the coronal region and predominates in the radicular pulp [31]. Consequently, data from the present research indicates that biomarker levels in the coronal pulp of RP and IRP teeth are comparable; however, in the radicular pulp, they may be elevated in IRP cases. Prior studies have assessed biomarker concentrations in RP and IRP teeth without differentiating between coronal and radicular pulp, revealing elevated biomarker levels in IRP teeth [32–34]. Nonetheless, in light of the occurrences during the pulp inflating process, we contend that it is more precise to assess the pulp independently regarding its radicular and coronal dimensions.

Based on these findings, the second hypothesis "There is no difference in the level of inflammation between the coronal and radicular pulps of teeth with reversible and irreversible pulpitis", was partially refuted, as biomarker levels in the radicular pulps of teeth with irreversible pulpitis were elevated, whereas the coronal pulps of both reversible and irreversible pulpitis exhibited comparable levels.

4.4 Findings regarding the sensitivity and specificity of Presepsin

According to a comprehensive systematic analysis conducted by Hirsch *et al*. [9], studies have found significant increases in IL-1β, IL-2, IL-6, IL-8, and TNF-α levels in teeth with IRP compared to normal pulp samples, with IL-6 and TNF-α being noted as more promising. The present study also showed that IL-1β, IL-6, IL-8, TNF-α, and Presepsin levels increased in IRP. Furthermore, when assessing the ROC analysis results, the highest sensitivity and specificity in distinguishing IRP teeth from RP teeth was demonstrated in the Presepsin biomarker. This outcome aligns with the findings of a prior study on deciduous teeth, and the evidentiary support for Presepsin in assessing pulp inflammation is on the rise [13]. The study by Sorsa *et al*. [35] demonstrates that matrix metalloproteinase-8 (MMP-8) is effectively utilized for diagnosis, monitoring treatment efficacy, and assessing treatment prognosis through its measurement in gingival crevicular fluid, implant sulcular fluid, or saliva during periodontal assessment. This study further substantiates the notion that

pulp inflammation can be assessed at the patient's chairside. Currently, the identification of extremely sensitive and specific biomarkers like Presepsin, along with the creation of patient-specific measurement kits, allows for the quantification of pulp inflammation during treatments, facilitating precise diagnosis and suitable intervention. As a result of these findings the final hypothesis, "there is no difference between Presepsin and other markers in indicating pulp inflammation", was refuted based on the ROC analysis results, as Presepsin exhibited the highest sensitivity and specificity values, proving to be more effective than other biomarkers.

Currently, histological analysis is considered the gold standard for determining the inflammatory status of the pulp [19]. Due to our limited resources, we were unable to compare histological analyses with biomarker levels in our study, which may be considered a limitation. Furthermore, evaluating the prognosis of the treatments performed is also clinically significant data. The fact that the effect of increased biomarker levels on prognosis was not evaluated in our study can be considered another limitation of the study. There is a clear need for new studies that evaluate the histological findings of pulp inflammation and biomarker levels together and assess teeth prognostically based on biomarker levels.

5. Conclusions

Clinically assessing the degree of pulp inflammation is a multifaceted process, and there is presently no evidence-based conclusion. The current study indicates that the presence of spontaneous pain in young permanent teeth undergoing pulpotomy, pulp bleeding exceeding three minutes, and elevated biomarker levels correlated with bleeding severity are effective predictors of pulp inflammation levels. Moreover, these data indicate that when teeth were categorized as RP and IRP, the biomarker levels in the coronal pulp of RP teeth were elevated, although those in the radicular pulp of IRP teeth were also elevated. This outcome suggests that pulpotomy procedures conducted on IRP teeth exhibit a higher propensity for failure and necessitate more regular monitoring. Another important finding of this study is that Presepsin is more successful than the IL-1β, IL-6, IL-8, and TNF-α biomarkers in distinguishing between RP and IRP teeth. As it is anticipated that pulp inflammation will be measurable at the patient's chairside using molecular methods in the future, the identification of biomarkers with high sensitivity and specificity, such as Presepsin, contributes significantly to the literature.

ABBREVIATIONS

TNF, Tumor Necrosis Factor; IL, Interleukin; RP, Reversible Pulpitis; IRP, Irreversible Pulpitis; NaOCl, Sodium Hypochlorite; ELISA, Enzyme-Linked Immunosorbent Assay; CV, Coefficient of Variation; ANOVA, Analysis of Variance; ROC, Receiver Operating Characteristic; AUC, Area Under the Curve; CD, Cluster of Differentiation; TLR, Toll-Like Receptor; MMP, matrix metalloproteinase.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ABO, MA, SD—Conceptualization; Project Administration. ABO, PC, FS—Methodology. ABO, FS, EL—Software. ABO, MA, FS—Validation. FS, EL—Formal Analysis. ABO, MA, PC—Investigation. ABO, PC, SD—Resources. MA, PC—Data Curation. ABO, EL—Writing–Original Draft Preparation. ABO—Writing–Review & Editing; Funding Acquisition. MA—Visualization. FS, SD—Supervision.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has been approved by the Ataturk University Faculty of Medicine Ethics Committee and conducted in accordance with the Declaration of Helsinki (Decision no.: 70/Decision date: 26 October 2023). Informed consent was obtained from all patients' parents involved in the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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