CLINICAL RESEARCH

Assessment of Inflammatory Cytokines in Molar Incisor Hypomineralization-affected First Permanent Molars: A Clinical Study

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ABSTRACT

Introduction: Molar incisor hypomineralization is a developmental enamel defect that weakens tooth structure, increasing susceptibility to caries, hypersensitivity, and treatment challenges. This study aimed to evaluate pro- and anti-inflammatory cytokine levels in the pulp of molar incisor hypomineralization-affected and nonmolar incisor hypomineralization (MIH) first permanent molars. **Methods:** This study analyzed pulpal inflammatory mediator levels in blood samples collected from 85 first permanent molars (38 (44.7%) with MIH and 47 (55.3%) without) requiring pulp treatment in systemically healthy children aged 8–13 years. During routine treatment, sterile cotton pellets were placed on the pulp exposure site for approximately 1 minute to obtain samples. Based on clinical findings and bleeding time, teeth were classified as reversible pulpitis, symptomatic or asymptomatic irreversible pulpitis. Clinical assessment included the Schiff cold air sensitivity score, periapical index and cold test. The collected samples were stored in lithium heparin–coated tubes at −70 °C, and the levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-4, IL-6, IL-8, IL-10, IL-13, matrix metalloproteinase (MMP)-3, MMP-8, and MMP-9 were analyzed using the enzyme-linked immunosorbent assay method. **Results:** In the molar incisor hypomineralization group, 18 (47.4%) teeth had reversible pulpitis, 8 (21%) symptomatic, and 12 (31.6%) asymptomatic irreversible pulpitis; corresponding numbers in the non–MIH group were 20 (42.6%), 11 (23.4%), and 16 (34%), respectively. Molar incisor hypomineralization-affected molars showed higher TNF-α, IL-4, IL-13, MMP-3, and MMP-8 levels than non-MIHs ($P < .05$). Subgroup analyses demonstrated that inflammatory marker levels were higher, particularly in MIH-related pulpitis subgroups ($P < .05$). These differences were mainly observed in biomarkers such as IL-4, IL-13, and MMP-8. Tooth maturity showed no overall differences, though IL-4 was higher in immature MIH teeth ($P < .05$). **Conclusion:** Elevated cytokine levels in molar incisor hypomineralization-affected teeth suggest severe pulp inflammation (TNF-α), a strong anti-inflammatory response (IL-4, IL-13), and inflammation-related tissue destruction (MMP-3, MMP-8), potentially leading to subclinical inflammation and increased pulpal damage. *(J Endod 2026;■:1–11.)*

KEY WORDS

Developmental defects of enamel; inflammatory cytokine; molar incisor hypomineralization; pulpitis; pulp tissue

SIGNIFICANCE

This study demonstrates that MIH-affected molars show elevated pulpal cytokine levels, indicating an enhanced inflammatory response. Understanding this biological mechanism may guide clinicians in managing hypersensitivity and pulpal complications in affected teeth.

Molar incisor hypomineralization (MIH) is a qualitative defect of tooth enamel, characterized as a developmental enamel disorder whose etiology is not yet fully understood[1]. First described as a clinical condition in the 1980s[2], the term MIH was introduced into the literature by Weerheijm et al.[1]. Clinically, MIH is primarily characterized by hypomineralization of permanent first molars, but similar defects can also be seen in incisors[3]. The global prevalence of MIH has been reported to range from 2.9% to 44%, and it is considered a significant oral health problem as it negatively affects the quality of life in children and creates challenges for dentists during treatment[4]. Furthermore, studies in Türkiye have shown that the prevalence

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of MIH ranges from 7.7% to 24%, with regional variations[5–8]. Regarding the etiology of MIH, it is believed to be multifactorial, involving a complex interaction of genetic, environmental, and systemic factors[9]. Etiologic studies have focused on prenatal factors such as maternal illnesses, stress exposure, drug use, alcohol consumption, and tobacco use; perinatal factors such as low birth weight, birth asphyxia, prematurity and congenital anomalies; and postnatal factors such as childhood illnesses, environmental toxin exposure and long-term or frequent drug use[3,10].

Since MIH is a developmental defect, the tooth is affected during enamel formation. Due to the resulting weakness in the enamel structure, posteruptive enamel breakdown may occur after eruption, and then secondary consequences such as caries development and subsequent pulpal involvement may follow. Enamel in MIH-affected teeth has been shown to exhibit decreased mineral content, with hydroxyapatite crystals transformed into more fragile carbonate forms and microhardness values significantly lower compared to healthy enamel[11]. This structural weakness is strongly associated with posteruptive enamel breakdown, caries development, and restorative failures. In addition to these structural enamel defects, MIH-affected teeth frequently present clinical consequences such as heightened hypersensitivity and rapid caries progression, which may increase the risk of early pulpal involvement[12]. Accordingly, it has been suggested that impaired enamel integrity may exert secondary effects on the dentin–pulp complex. Supporting this concept, Rodd et al.[13] suggested that MIH-affected teeth may be associated with a subclinical inflammatory response within the pulp, described as a persistent, low-level but biologically active inflammatory condition occurring without clear clinical manifestations of pulpitis[14]. According to this concept, the increased permeability of hypomineralized enamel may allow continuous diffusion of bacterial by-products and external stimuli through the dentin, leading to inflammatory changes within the pulp despite a clinically asymptomatic presentation. Consequently, structural and biological differences in enamel, dentin, and pulp tissues in MIH-affected teeth reduce their resistance to caries, while hypersensitivity, rapid pulpal involvement of caries, and inflammation pose additional difficulties for clinicians in the management of pulpal diseases[12,14].

Although MIH presents several clinical challenges, the current literature includes only a limited number of studies evaluating inflammatory markers such as cytokines and matrix metalloproteinases (MMPs) in MIH-affected teeth. One previous study examined the relationship between MIH and periodontal health by analyzing gingival crevicular fluid volume and biomarkers such as MMP-8, MMP-20, and TGF-β1; however, these studies did not assess pulpal inflammation at the biochemical level[15]. Consequently, a significant gap remains regarding the inflammatory profile of the pulp in molars affected by MIH. To address this gap, this study aimed to evaluate the levels of pro-inflammatory and anti-inflammatory cytokines, as well as matrix metalloproteinases, in pulp blood samples obtained from first permanent molars diagnosed with MIH and to compare these findings with those from first permanent molars without MIH.

Accordingly, the null hypothesis of the study was formulated as follows: "There is no statistically significant difference in the levels of proinflammatory and anti-inflammatory cytokines in the pulp tissue between permanent first molars affected by MIH and those non-MIH."

MATERIALS AND METHODS

Ethical Approval

The protocol for this study was reviewed and approved by the Clinical Research Ethics Committee of the Faculty of Dentistry, Marmara University, on October 12, 2022, with the protocol number 2022/96. Furthermore, the study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki, as revised in 2013. The study has been registered on ClinicalTrials.gov with the number NCT07008599. This study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines[16].

Sample Size and Classification of Groups

Since no previous study has evaluated inflammatory cytokine levels in MIH-affected teeth, the sample size calculation was based on the most comparable work available in the literature. The primary outcome for the sample size calculation was pulpal MMP-9 concentration, selected from the available biomarkers in the reference study as it was the only biomarker also assessed in the present study. Based on the data from Mente et al.[17], the MMP-9 levels (ng/mL) in the 3 pulpal diagnosis groups used for sample size calculation were as follows: asymptomatic teeth, 267–807 (median = 386); teeth with reversible pulpitis, 300–1396 (median = 938); and teeth with irreversible pulpitis not influenced by NSAID intake, 1011–3055 (median = 1701). Pairwise comparisons between these groups were used to calculate the required sample sizes with a 95% confidence level ($\alpha = 0.05$) and 90% test power (1-β), using a two-tailed t-test. The calculated sample sizes were 4 teeth for asymptomatic vs. reversible pulpitis, 5 teeth for reversible vs. irreversible pulpitis, and 8 teeth for asymptomatic vs. irreversible pulpitis. Taking the largest of these estimates (8 teeth per group) ensured adequate statistical power for all comparisons. Accordingly, a minimum total of 48 teeth was included, divided into MIH and non-MIH groups, and further categorized according to pulpal status (reversible pulpitis, symptomatic irreversible pulpitis, and asymptomatic irreversible pulpitis) in accordance with the AAE diagnostic framework[18].

MIH evaluation was performed as part of the routine oral examination using a dental mirror and explorer, supplemented by air drying and adequate lighting conditions. Diagnosis was established according to the criteria defined by the European Academy of Paediatric Dentistry[19]. All individuals included in the MIH group presented hypomineralization involving both first permanent molars and permanent incisors. The diagnostic process included exclusion of enamel defects unrelated to MIH, identification of clearly demarcated white–cream or yellow–brown opacities, assessment of posteruptive enamel breakdown, evaluation of atypical restorations and carious involvement, identification of tooth loss associated with MIH, and recording of lesions that could not be conclusively categorized. Furthermore, the teeth included in the study were evaluated using the Schiff cold air test[20], the periapical index (PAI)[21], and the cold test.

The Schiff cold air test[20] was performed by applying the air-water spray from the dental unit to the dentin surface from a distance of 2–3 mm, at a 90° angle, with continuous pressure of 45–60 psi for 2–3 seconds, after isolating the adjacent teeth. Patients' sensitivity levels were scored by a pediatric dentist using a predetermined scale. In addition, PAI[21] was used to evaluate the periapical regions of the teeth included in the study and for their grouping. To assess and classify the pulp vitality of the teeth, the cold vitality test (Cerkamed Pulp Spray, Cold Vitality Test Spray) was applied.

First permanent molars with deep dentin caries were included in the study and classified into three groups according to their pulpal status. Molars with a history of spontaneous moderate or severe pain, prolonged or lingering pain lasting 30 seconds or longer in response to cold testing, sensitivity to percussion or palpation, carious exposure of the pulp with pulpal bleeding lasting longer than 5 minutes, and widening of the lamina dura (PAI $\leq$ 2) were classified as the symptomatic irreversible pulpitis group.

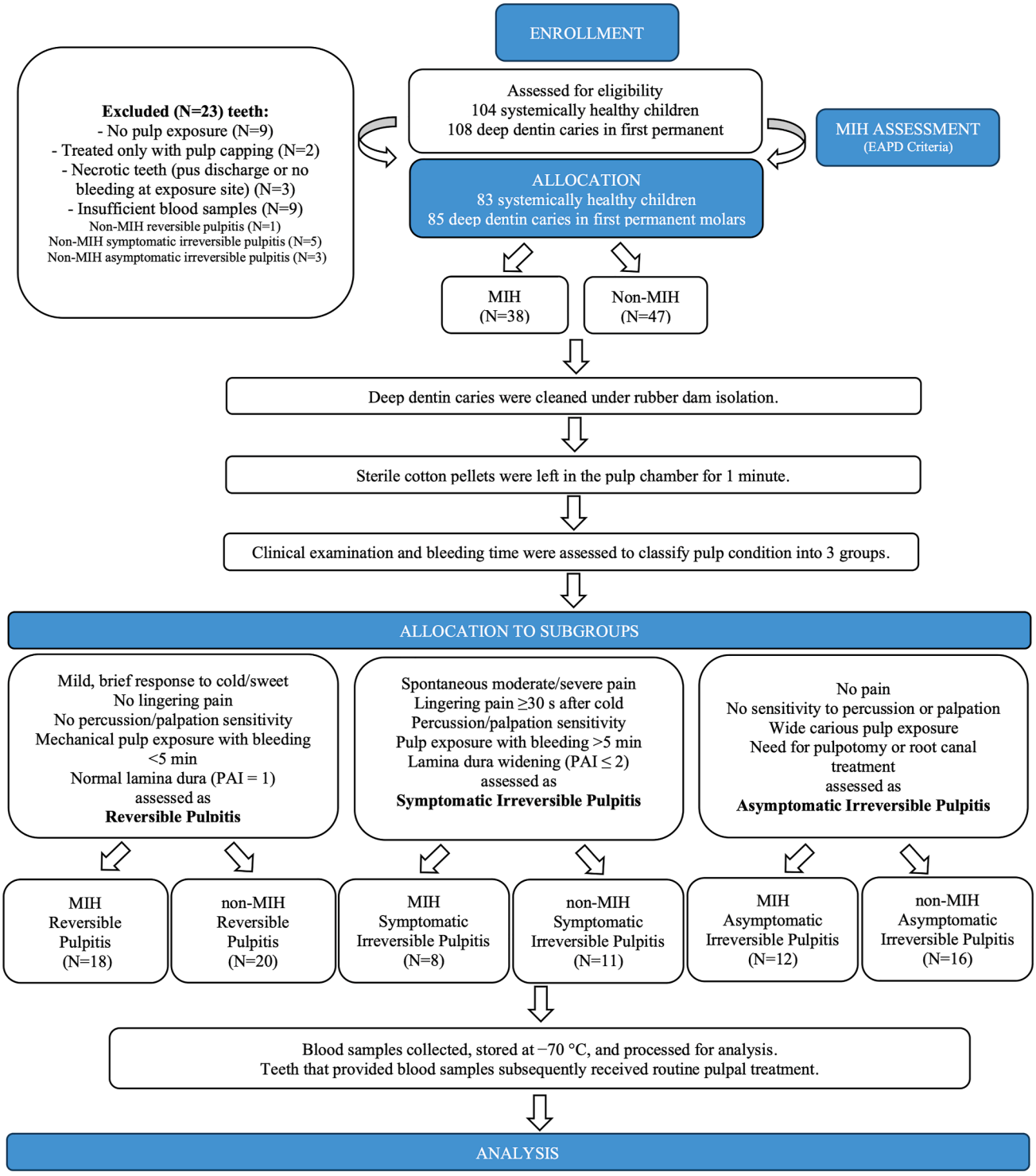


FIGURE 1 – The flow chart of the progress of the study. MIH, molar incisor hypomineralization; PAI, periapical index.

Additional diagnostic characteristics included sharp pain to thermal stimuli, referred pain, pain exacerbated by postural changes, and limited response to over-the-counter analgesics. Molars presenting these findings were included in the symptomatic irreversible pulpitis group. Molars showing mild reactions to cold or sweet stimuli, demonstrating normal and nonlingering responses to cold testing that resolved within a few seconds after stimulus removal, exhibiting no sensitivity to percussion or palpation, presenting mechanical pulp exposure during caries removal with pulpal bleeding lasting less than 5 minutes, and showing no widening of the lamina dura (PAI = 1) were classified as the reversible pulpitis group. Molars presenting no pain or sensitivity to percussion or palpation but exhibiting a wide pulp exposure due to caries and requiring pulpotomy or root canal treatment were categorized as the asymptomatic irreversible pulpitis group[17,18]. The study flowchart is shown in Figure 1. Representative examples of clinical intraoral

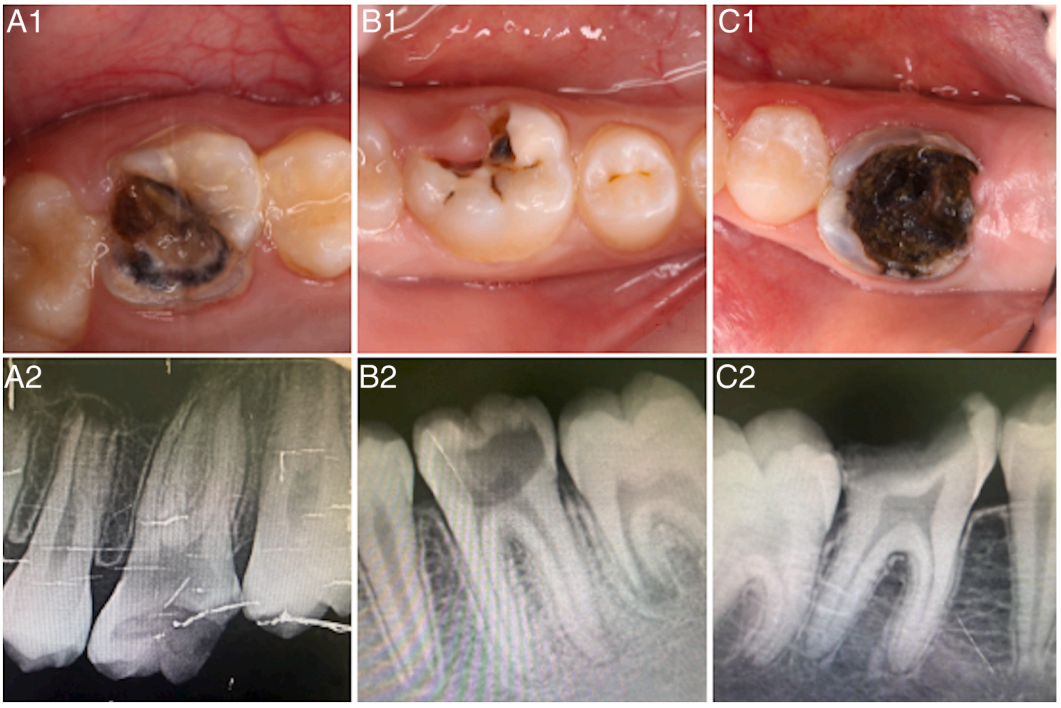


FIGURE 2 – Examples of clinical intraoral images and periapical radiographs of first permanent molars.

images and corresponding periapical radiographs from some of the groups were presented in Figure 2.

Tooth maturity was determined based on apical development. Teeth that were fully developed with closed apices were classified as "mature," while teeth with incomplete apical development and open apices were classified as "immature." These assessments were independently performed by 2 experienced pediatric dentists with 11 and 3 years of clinical experience, respectively, using periapical radiographs.

Collection of Pulpal Blood Samples

As part of the blood sample collection procedure, routine caries removal protocols were applied. After the teeth were classified according to the presence or absence of MIH, routine treatments of the teeth requiring therapy were initiated. First, anesthesia was achieved using topical anesthesia (Vemcaine, 50 mL spray containing 10% lidocaine HCl, Vem İlaç, Türkiye) and local anesthesia without a vasoconstrictor (infiltrative or mandibular) (SAFECaine, 30 mg/mL solution containing 3% mepivacaine HCl, Adeka, Türkiye). The teeth were then isolated using a rubber dam. Caries removal was started with high-speed rotary instruments using round and/or fissure burs. The cavity margins were first defined, followed by the removal of caries on the cavity walls, and finally the infected dentin in the center of the cavity was cleaned.

Teeth without pulp exposure, those treated only with pulp capping, and necrotic teeth with pus discharge or absence of bleeding at the exposure site were excluded from the study. For blood sample collection, a sterile cotton pellet was placed at the pulp exposure site for 1 minute. The pellets that yielded an insufficient amount of blood for biochemical analysis were excluded from the study[22]. After blood samples were obtained, pressure was applied using a moist sterile cotton pellet for 5 minutes to control bleeding. Pulpal bleeding time at the exposure site was used as an adjunctive criterion, with bleeding lasting less than or more than 5 minutes indicating reversible or irreversible pulpitis, respectively.

Following this classification, routine pulpal treatments required for the teeth were continued. During the procedures, cotton pellets containing blood samples, collected by a pediatric dentist, were transferred into lithium-heparin-coated tubes containing 0.8 mL phosphate-buffered saline. To prevent clotting of the collected blood samples, the tubes were gently shaken to activate the

TABLE 1 - Demographic and Clinical Characteristics of the Study Population

	MIH N (%)	Non-MIH N (%)	Total N (%)	*P* value
Gender				
Girls	26 (52.0)	24 (48.0)	48 (57.8)*	.106
Boys	12 (34.3)	23 (65.7)	35 (42.2)*	
Apical maturity				
Mature	12 (35.3)	22 (64.7)	34 (40)	.154
Immature	26 (51.0)	25 (49.0)	51 (60)	
Pulpal status				
Reversible pulpitis	18 (47.4)	20 (52.6)	38 (44.7)	.905
Symptomatic irreversible pulpitis	8 (42.1)	11 (57.9)	19 (22.4)	
Asymptomatic irreversible pulpitis	12 (42.9)	16 (57.1)	28 (32.9)	
Total	38 (44.7)	47 (55.3)		

MIH, molar incisor hypomineralization; N, number of teeth.
values marked with * refer to the number of participants.

TABLE 2 - Evaluation of Biochemical Mediators in the MIH Group Versus the Non-MIH Group

	MIH group (n = 38)		Non-MIH group (n = 47)		
	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	P value
TNF-α	241.78 ± 112.72	252.46 (34.22–553.39)	159.78 ± 114.27	129.84 (42.17–569.17)	**.001**[*]
IL-4	165.10 ± 74.96	149.36 (32.14–356.24)	90.90 ± 36.00	87.66 (32.48–206.45)	<**.001**[*]
IL-6	160.08 ± 78.18	146.76 (44.18–384.33)	153.06 ± 77.90	154.12 (25.82–312.09)	>.05[†]
IL-8	235.14 ± 122.41	269.52 (40.03–468.60)	181.63 ± 101.25	149.46 (5.74–380.40)	>.05[*]
IL-10	391.87 ± 139.95	352.61 (203.44–714.00)	405.78 ± 131.20	384.00 (184.56–748.44)	>.05[†]
IL-13	44.53 ± 10.19	44.66 (25.44–61.89)	39.74 ± 9.75	39.01 (2.89–61.68)	<**.05**[†]
MMP-3	4.67 ± 1.18	4.56 (2.49–6.74)	4.05 ± 1.01	3.95 (2.35–6.32)	<**.05**[†]
MMP-8	1.59 ± 0.87	1.28 (0.57–3.95)	0.78 ± 0.26	0.75 (0.34–1.31)	<**.001**[*]
MMP-9	2286.74 ± 1121.22	2248.5 (738.00–4628.00)	1928.32 ± 975.83	1888.00 (538.00–4628.00)	>.05[†]

IL, interleukin; MIH, molar incisor hypomineralization; Min–Max, minimum–maximum; MMP, matrix metalloproteinase; N, number of teeth; SD, standard deviation; TNF, tumor necrosis factor.
Bold values indicate a significant difference.
[*]Mann-Whitney U Test.
[†]Independent Samples t-test.

heparin. Each tube was labeled with the group information determined by clinical examination and intraoperative bleeding time. Blood samples were stored at −70 °C until laboratory analysis. After thawing the samples on ice, the blood samples were centrifuged for separation.

ELISA Test Analysis

The levels of tumor necrosis factor-α (TNF-α), interleukin-4 (IL-4), IL-6, IL-8, IL-10, IL-13, MMP-3, MMP-8, and MMP-9 cytokines were analyzed using the enzyme-linked immunosorbent assay (ELISA) test at the Biochemistry Laboratory of the Faculty of Dentistry, Marmara University. The measurements were performed using specific ELISA kits provided by BT Lab (Bioassay Technology Laboratory, Shanghai, China) and Elabscience (Houston, TX, USA).

Statistical Analysis

All statistical analyses were performed using the IBM SPSS version 26.0 software (IBM SPSS, Chicago, Illinois, USA). Categorical data were presented as frequency (n) and percentage (%), while continuous data were expressed as mean ± standard deviation (SD) or median (minimum–maximum) according to their distribution characteristics. The normality of distribution for continuous variables was assessed using the Kolmogorov–Smirnov test and visual inspection.

Categorical baseline variables, including gender, apical maturity, and pulpal status, were compared between MIH and non-MIH groups using Pearson's chi-square test. For comparisons between two groups (MIH–non-MIH, mature-immature teeth), the Independent Samples t-test was used for normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data. In comparisons among three or more groups, One-Way ANOVA was used for data with normal distribution, while the Kruskal–Wallis test was applied for data that did not follow a normal distribution. Post-hoc pairwise comparisons following the ANOVA test were performed using the Tukey test, whereas pairwise comparisons following the Kruskal–Wallis test were carried out using the Dunn test. A P-value of <0.05 was considered statistically significant for all tests.

RESULTS

The study was conducted with a total of 85 pulpal blood samples collected from 83 children aged 8–13 years (mean ± standard deviation= 9.58 ± 1.48). Of the participants, 57.8% (n= 48) were girls and 42.2% (n= 35) were boys. In the sample, 55.3% of the teeth (n= 47) were in the non-MIH group, while 44.7% (n= 38) were in the MIH group, and all pulp exposures in both groups were caries-related (Table 1). The mean age was 9.26 ± 1.50 years in the MIH group (median: 9, range: 8–13) and 9.77 ± 1.54 years in the non-MIH group (median: 10, range: 8–13), with no statistically significant difference between the groups (P= .077) (Table 1).

Levels of TNF-α, IL-4, IL-13, MMP-3, and MMP-8 were significantly higher in the MIH group compared with the non-MIH group, while no significant differences were observed for the remaining biochemical mediators (Table 2).

When the MIH and non-MIH groups were compared according to pulpal status (reversible pulpitis, symptomatic irreversible pulpitis, and asymptomatic irreversible pulpitis), significant differences were found in TNF-α, IL-4, IL-13, MMP-3, and MMP-8 levels (P< .05) (Table 3). The results shown in Table 3 are graphically presented as box plots in Figure 3. Post hoc analyses revealed that IL-4 levels in the MIH reversible pulpitis subgroup were significantly higher than those in the non-MIH reversible pulpitis subgroup (P< .05). Regarding IL-13, levels in the MIH asymptomatic irreversible pulpitis subgroup were significantly higher than those in the non-MIH asymptomatic irreversible pulpitis subgroup (P< .05). For MMP-8, concentrations in the MIH reversible pulpitis subgroup were significantly higher than those recorded in all non-MIH subgroups (P< .05), including reversible pulpitis, symptomatic irreversible pulpitis, and asymptomatic irreversible pulpitis (Table 3).

When the MIH and non-MIH groups were evaluated separately based on root development, no significant differences were observed between mature and immature teeth for most biochemical mediators. However, within the MIH group, IL-4 levels were significantly higher in immature teeth compared with mature teeth (Table 4).

DISCUSSION

This study aimed to contribute to the understanding of potential subclinical inflammation observed in MIH-affected teeth by evaluating the levels of inflammatory mediators. Significant differences were detected in the levels of TNF-α, IL-4, IL-13, MMP-3, and MMP-8 between the MIH and non-MIH groups as well as among the 6 subgroups determined according to pulpal status. Therefore, the null hypothesis stating that there is no statistically significant difference in the levels of proinflammatory and anti-inflammatory cytokines measured in the pulp tissue of teeth with and without MIH was rejected.

The most striking finding of the present study was the presence of an enhanced

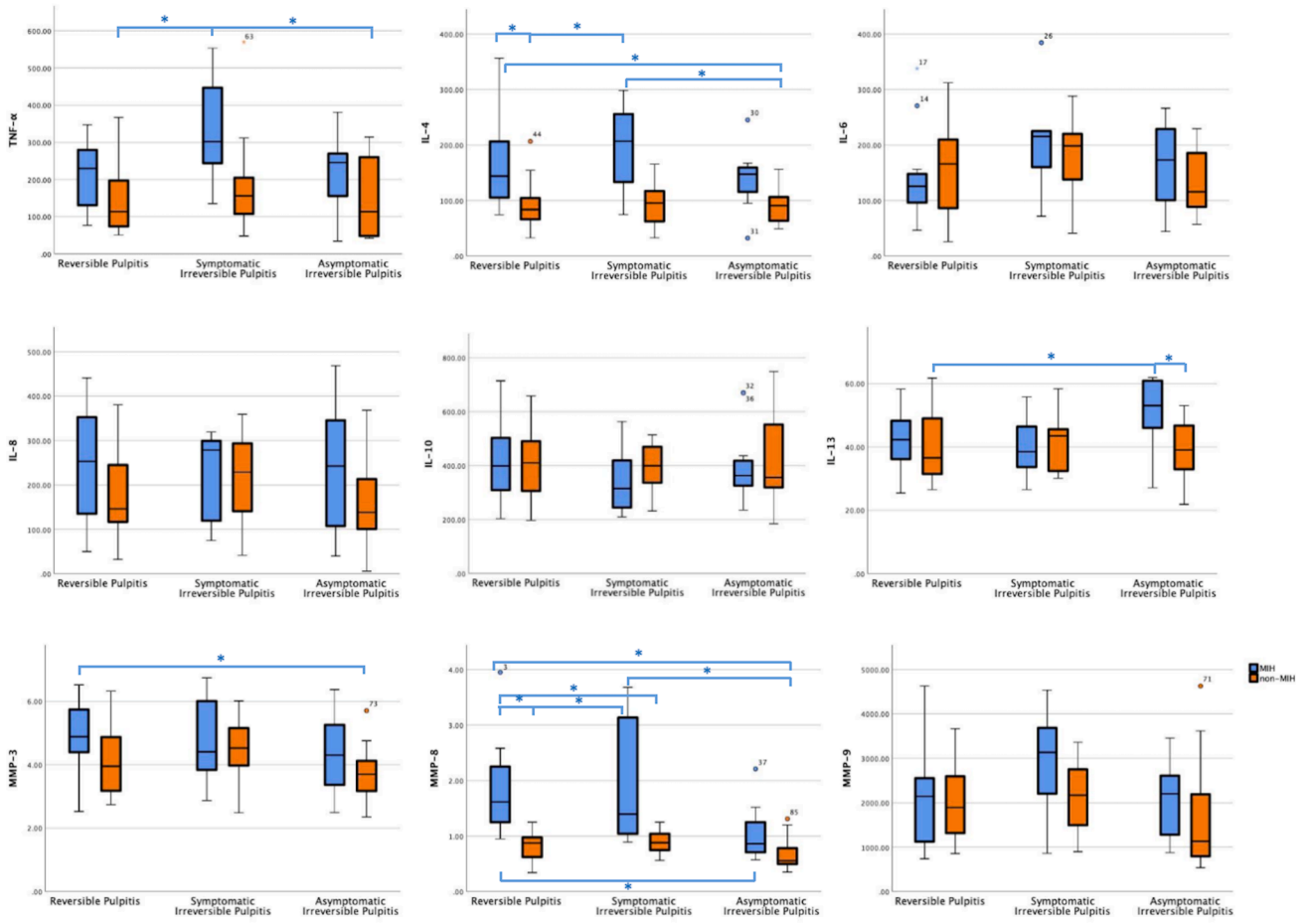


FIGURE 3 – Graphical representation of biochemical mediators in reversible and symptomatic/asymptomatic irreversible pulpitis in the MIH and non-MIH groups. MIH, molar incisor hypomineralization; TNF-α, tumor necrosis factor-α; IL, interleukin.

inflammatory mediator profile in MIH-affected teeth compared to non-MIH teeth, even when similar clinical pulpal diagnoses were present. Previous studies have shown that inflammatory cytokines and matrix metalloproteinases increase with the severity of pulpal inflammation; however, their levels do not always correspond precisely with clinical symptoms or conventional pulpal status classifications. In this context, the significantly elevated levels of selected pro- and anti-inflammatory cytokines as well as tissue-degrading enzymes observed in MIH-affected teeth suggest that hypomineralized dental tissues may predispose the pulp to sustained low-grade or subclinical inflammatory stimulation, indicating a biologically distinct pulpal microenvironment in MIH-affected teeth.

TNF-α is a central regulator of inflammatory responses and promotes extracellular matrix degradation through the induction of matrix metalloproteinases such as MMP-3, MMP-8, and MMP-9[23,24]. Consistent with previous reports suggesting its utility as a biomarker for pulpal status[17,23], the present study demonstrated significantly elevated TNF-α levels in MIH-affected teeth compared with non-MIH teeth, with the highest levels observed in the symptomatic irreversible pulpitis subgroup. Overall, the data indicate that pulp tissue in MIH-affected teeth is characterized by a more pronounced inflammatory profile.

IL-4 is a cytokine regulated by Th2 cells, known for its anti-inflammatory and tissue-healing effects; although IL-4 levels are low in healthy pulp tissue, a significant increase can be observed during inflammatory processes, and it has been previously reported that IL-4 levels are higher in irreversible pulpitis compared to reversible pulpitis and healthy pulp[24,25]. Similar to these findings, this study also showed a significant increase in IL-4 levels in symptomatic irreversible pulpitis. In addition, a marked increase was observed in the MIH group compared to the non-MIH group. Particularly, IL-4 levels in the MIH reversible pulpitis subgroup were found to be significantly higher compared to the non-MIH reversible pulpitis subgroup. This may indicate that the hypomineralized enamel and dentin structures in MIH teeth, with their increased dentin permeability, are continuously exposed to antigenic stimulation and may trigger anti-inflammatory cytokines such as IL-4 to regulate the early inflammatory response. Elevated levels of the Th2-associated cytokines IL-4 and IL-13 may also indicate activation of type 2 immune pathways. In this context, the structurally compromised and more permeable enamel characteristic of MIH-affected teeth may allow continuous low-grade antigen penetration, thereby facilitating the activation of type 2–related immune responses within the pulpal microenvironment. Li[26] reported that IL-13 levels increased in irreversible pulpitis compared to control groups. In parallel, this study also observed increased IL-13 levels in reversible pulpitis teeth. Furthermore, significantly higher IL-13 levels were observed in the MIH asymptomatic irreversible pulpitis subgroup compared to the non-MIH reversible pulpitis and asymptomatic

TABLE 3 - Comparison of Biochemical Mediators in Reversible and Symptomatic/Asymptomatic Irreversible Pulpitis in the MIH and Non-MIH Groups

	MIH group						Non-MIH group						
	Reversible pulpitis ($n = 18$)		Symptomatic irreversible pulpitis ($n = 8$)		Asymptomatic irreversible pulpitis ($n = 12$)		Reversible pulpitis ($n = 20$)		Symptomatic irreversible pulpitis ($n = 11$)		Asymptomatic irreversible pulpitis ($n = 16$)		*P* value
	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	Mean ± SD	Median (Min–Max)	
TNF-α	216.51 ± 88.93	229.85 (76.55–346.76)^{ab}	334.15 ± 137.69	301.72 (135.16–553.39)^{a}	218.10 ± 102.88	245.48 (34.22–380.91)^{ab}	153.72 ± 103.20	113.95 (50.32–366.89)^{b}	188.58 ± 146.39	156.35 (47.96–569.17)^{ab}	147.56 ± 107.10	113.69 (42.17–314.13)^{b}	**<.05**†
IL-4	168.99 ± 85.07	143.88 (74.33–356.24)^{a}	195.63 ± 76.45	206.83 (74.68–298.32)^{a}	138.91 ± 50.43	147.24 (32.14–245.20)^{ab}	89.99 ± 38.81	83.65 (32.48–206.45)^{b}	95.45 ± 42.59	95.40 (32.55–165.40)^{ab}	88.92 ± 28.96	90.77 (48.67–156.40)^{b}	**<.001**†
IL-6	134.90 ± 70.09	125.67 (46.42–338.06)	207.05 ± 91.47	215.41 (71.55–384.33)	166.54 ± 70.41	172.97 (44.18–266.12)	157.21 ± 90.49	165.82 (25.82–312.09)	175.23 ± 74.72	198.50 (41.04–287.91)	132.63 ± 60.71	115.56 (56.78–229.33)	>.05†
IL-8	237.56 ± 120.82	253.15 (49.74–440.89)	223.59 ± 102.71	278.62 (74.60–319.17)	239.21 ± 144.93	242.60 (40.03–468.60)	179.67 ± 100.96	146.17 (32.40–380.40)	213.58 ± 104.23	228.89 (41.74 - 359.17)	162.11 ± 100.62	138.03 (5.74–368.31)	>.05†
IL-10	410.05 ± 147.525	398.44 (203.44–714.00)	341.01 ± 128.87	314.83 (209.56–562.33)	398.52 ± 138.58	363.17 (235.11–670.11)	405.39 ± 130.01	409.56 (196.78–657.89)	396.55 ± 87.70	399.28 (231.78–513.44)	412.61 ± 161.68	355.67 (184.56–748.44)	>.05†
IL-13	42.06 ± 8.50^{ab}	42.24 (25.44–58.21)	38.91 ± 9.37^{ab}	38.45 (26.57–55.79)	51.32 ± 10.27^{a}	53.01 (27.14–61.89)	39.77 ± 10.89^{b}	36.54 (26.57–61.68)	40.62 ± 8.87^{ab}	43.45 (30.12–58.35)	39.09 ± 9.37^{b}	39.01 (21.89–53.03)	**<.05**†
MMP-3	4.86 ± 1.09^{a}	4.88 (2.53–6.52)	4.76 ± 1.35^{ab}	4.41 (2.87–6.74)	4.32 ± 1.23^{ab}	4.30 (2.49–6.37)	4.08 ± 1.03^{ab}	3.95 (2.74–6.32)	4.52 ± 1.00^{ab}	4.52 (2.48–6.01)	3.68 ± 0.89^{b}	3.70 (2.35–5.70)	**<.05**†
MMP-8	1.79 ± 0.78	1.61 (0.95–3.95)^{a}	1.96 ± 1.17	1.39 (0.89–3.68)^{ab}	1.02 ± 0.47	0.86 (0.57–2.21)^{bc}	0.80 ± 0.25	0.87 (0.34–1.25)^{c}	0.89 ± 0.22	0.88 (0.56–1.25)^{bc}	0.67 ± 0.28	0.55 (0.35–1.31)^{c}	**<.001**†
MMP-9	2129.83 ± 1206.78	2144.50 (738.00–4628.00)	2930.25 ± 1177.24	3136.00 (858.00–4538.00)	2093.08 ± 844.70	2200.50 (877.00–3457.00)	1987.00 ± 818.76	1892.50 (855.00–3668.00)	2133.45 ± 875.70	2171.00 (898.00–3362.00)	1713.94 ± 1214.65	1128.50 (538.00–4628.00)	>.05†

IL, interleukin; MIH, molar incisor hypomineralization; Min–Max, minimum–maximum; MMP, matrix metalloproteinase; N, number of teeth; SD, standard deviation; TNF, tumor necrosis factor.
*Kruskal–Wallis Test with Dunn test.
†One-Way ANOVA Test with Tukey Test. Bold values indicate a significant difference. Groups sharing the same letter within the same row are not significantly different ($P < .05$).

irreversible pulpitis subgroups. This difference between the MIH and non-MIH groups suggests that MIH teeth may exhibit subclinical inflammatory activity.

In contrast, comparisons between MIH and non-MIH groups, as well as among different pulpal diagnostic categories, did not reveal statistically significant differences in IL-6, IL-8, or IL-10 levels. Although IL-8 has been suggested as an indicator of symptomatic pulpal inflammation and IL-10 has been associated with advanced stages of chronic pulpitis[26–29], the current results align with previous findings indicating that these cytokines, when evaluated individually, do not possess adequate discriminatory power to reliably reflect the severity of pulpal pathology[30,31].

MMPs are key regulators of pulpal inflammatory responses due to their roles in extracellular matrix degradation, tissue remodeling, and modulation of inflammatory progression. Produced by various cell types within the dental pulp, MMPs are involved both in pathological matrix breakdown observed in chronic inflammatory conditions such as pulpitis and periodontitis and in normal tissue turnover and reparative processes. Among these enzymes, MMP-3 occupies a central position due to its capacity to activate latent forms of other MMPs[32,33]. In agreement with previous studies reporting elevated MMP-3 expression in acute pulpitis relative to healthy pulp tissue[34], this study found significantly higher MMP-3 levels in the MIH reversible pulpitis subgroup compared with the non-MIH asymptomatic irreversible pulpitis subgroup, supporting the presence of an early yet biologically active inflammatory state in MIH-affected teeth.

MMP-8, a neutrophil-derived collagenase, is primarily associated with acute inflammatory responses and reflects active collagen degradation within pulpal and periapical tissues. Elevated MMP-8 levels have been reported in symptomatic irreversible pulpitis and periapical inflammation[35,36]. In line with these observations, significantly higher MMP-8 levels were detected in the MIH group in the present study, supporting the presence of a more pronounced inflammatory response in MIH-affected teeth.

Histologic analyses have demonstrated the presence of a cell-rich region between the pulp and the apical papilla[37]. Structural differences between the apical papilla and root pulp have also been examined in immature teeth that have not yet completed root development. In the present study, no significant differences in inflammatory mediator levels were found between mature

TABLE 4 - Comparison of Biochemical Mediators in the MIH and Non-MIH Groups Based on Root Maturity (Immature/Mature)

	Maturation	Mean ± SD	Median (Min-Max)	*P* value
MIH group				
TNF α	Immature	232.49 ± 104.71	254.98 (34.22–454.35)	>.05*
	Mature	261.91 ± 131.04	241.84 (76.55–553.39)	
IL-4	Immature	144.68 ± 60.41	133.83 (32.14–266.31)	**<.05***
	Mature	209.33 ± 86.52	181.73 (98.35–356.24)	
IL-6	Immature	157.80 ± 83.86	146.77 (44.18–384.33)	>.05†
	Mature	165.02 ± 67.37	152.03 (71.55–270.60)	
IL-8	Immature	234.00 ± 127.73	262.18 (40.03–468.60)	>.05*
	Mature	237.62 ± 115.37	269.52 (49.74–430.15)	
IL-10	Immature	410.46 ± 148.76	363.44 (203.44–714.00)	>.05†
	Mature	351.60 ± 114.00	343.72 (205.67–562.33)	
IL-13	Immature	45.23 ± 10.05	45.44 (25.44–61.89)	>.05†
	Mature	43.03 ± 10.76	43.31 (26.57–61.40)	
MMP-3	Immature	4.60 ± 1.11	4.67 (2.49–6.37)	>.05†
	Mature	4.82 ± 1.36	4.43 (2.74–6.74)	
MMP-8	Immature	1.47 ± 0.77	1.28 (0.57–3.68)	>.05*
	Mature	1.84 ± 1.05	1.29 (0.89–3.95)	
MMP-9	Immature	2091.85 ± 901.98	2145.00 (738.00–3844.00)	>.05†
	Mature	2709.00 ± 1447.48	2404.50 (858.00–4628.00)	
Non-MIH group				
TNF α	Immature	175.91 ± 131.76	129.84 (42.39–569.17)	>.05*
	Mature	141.45 ± 90.00	130.04 (42.17–366.89)	
IL-4	Immature	84.87 ± 32.16	78.65 (32.48–156.40)	>.05*
	Mature	97.76 ± 39.57	95.63 (45.33–206.45)	
IL-6	Immature	150.67 ± 80.56	164.63 (32.69–306.72)	>.05†
	Mature	155.77 ± 76.56	144.90 (25.82–312.09)	
IL-8	Immature	181.02 ± 111.30	149.46 (5.74–380.40)	>.05*
	Mature	182.32 ± 91.11	148.47 (41.74–345.46)	
IL-10	Immature	422.29 ± 138.25	409.00 (184.56–748.44)	>.05†
	Mature	387.02 ± 123.17	372.61 (186.22–632.89)	
IL-13	Immature	41.32 ± 9.29	42.67 (27.35–57.92)	>.05†
	Mature	37.93 ± 10.16	37.49 (21.89–61.68)	
MMP-3	Immature	3.97 ± 0.93	3.95 (2.35–5.64)	>.05†
	Mature	4.13 ± 1.11	4.18 (2.48–6.32)	
MMP-8	Immature	0.79 ± 0.27	0.86 (0.34–1.25)	>.05*
	Mature	0.78 ± 0.27	0.72 (0.35–1.31)	
MMP-9	Immature	1926.68 ± 895.87	2013.00 (538.00–3668.00)	>.05†
	Mature	1930.18 ± 1081.02	1613.00 (748.00–4628.00)	

IL, interleukin; MIH, molar incisor hypomineralization; Min–Max, minimum–maximum; MMP, matrix metalloproteinase; SD, standard deviation; TNF, tumor necrosis factor.
Bold values indicate a significant difference.
*Mann-Whitney U Test.
†Independent Sample t-test.

and immature teeth in either the MIH or non-MIH groups. These findings contribute to the literature emphasizing the regenerative potential of immature teeth on pulpal inflammation; however, further studies are needed to clarify this relationship more clearly.

From a clinical perspective, the findings of this study suggest that MIH-affected teeth may exhibit an enhanced inflammatory response even in the absence of overt clinical symptoms. This subclinical inflammatory activity may help explain the increased susceptibility of MIH-affected teeth to pulpal complications and their tendency to progress more rapidly toward advanced pulpal involvement. Accordingly, reliance on clinical symptoms alone may underestimate the underlying pulpal condition in MIH-affected teeth. For clinicians, these results underscore the importance of careful case selection and individualized treatment planning. There are certain limitations that should be taken into consideration when interpreting the findings of this study. As the research was conducted *in vivo* and blood samples were collected from patients undergoing routine treatment, it was not possible to include a negative control group consisting of caries-free teeth with healthy pulp tissue. Therefore, the levels of inflammatory response could not be directly compared with the normal physiology of healthy pulp. In addition, some samples were obtained from the same patients, which may introduce the potential for intrasubject correlation due to individual immune response characteristics. However, only 2 participants contributed more than one tooth. As the vast majority of participants provided a single sample, the resulting clustering was considered negligible. Therefore, statistical methods designed to account for within-subject correlation, such as generalized estimating equations, were not applied. Teeth with necrotic pulp tissue or those from which sufficient blood samples could not be collected for the ELISA assay were also excluded from the study. Finally, the fact that the study was conducted in a single center

limits the generalizability of the findings to the wider population.

In addition to these limitations, this study represents the first investigation to biochemically assess the inflammatory response in the pulp tissue of teeth affected by MIH. The diagnosis of pulpal diseases was established through a combination of medical history, clinical, and radiographic evaluations, which ensured a high level of diagnostic accuracy during the grouping process. One of the strengths of this study is that the blood samples were collected *in vivo* during treatment rather than *ex vivo* from extracted teeth, increasing the clinical relevance of the findings. Furthermore, the inclusion of asymptomatic teeth in addition to those with reversible and irreversible pulpitis allowed for the identification of associations between mediator levels and not only symptomatic inflammation but also subclinical or asymptomatic cases.

CONCLUSION

This study demonstrated that not only the enamel tissue but also the pulp tissue in MIH-affected teeth is impacted and that inflammatory processes occur within the pulp. The significantly higher levels of TNF-α, IL-4, IL-13, MMP-3, and MMP-8 observed in the MIH group compared to the non-MIH group indicate a more pronounced pulpal inflammatory response in these teeth. These findings suggest that clinical symptoms associated with MIH, such as hypersensitivity, pain, and difficulties in achieving anesthesia, may arise not only from structural defects but also from inflammation-related biological mechanisms. Overall, the results of this study provide important insights into the pathophysiology of MIH and may help guide future studies focusing on its effects on the pulp.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used ChatGPT-4 (OpenAI) solely for assistance with grammar and spelling. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Seray Sahin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Nihal Sehkar Oktay:** Methodology, Data curation, Writing – review & editing. **Sukriye Caliskan:** Methodology, Data curation, Writing – review & editing. **Betul Kargul:** Conceptualization, Data curation, Writing – review & editing. **Betul Sen Yavuz:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft.

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