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Assessment of MMP levels in reversible and irreversible pulpitis and a randomized controlled trial comparing clinical success of two different calcium-silicate cements in pulpotomy treatment of primary molars with an 18-month follow-up

Hazal Ezgi Gerihan[1,4], Dilşah Çoğulu[1*], Özant Önçağ[1], Asude Durmaz[2] and Elif Hasibe Kuru[3]

Abstract

Background Matrix metalloproteinases (MMPs) are critical enzymes involved in the remodeling and defense mechanisms of dental pulp tissue. While their role in permanent teeth has been extensively studied, research focusing on MMPs in primary teeth remains limited. This gap highlights the need for further investigations to understand the specific contributions of MMPs to pulpal defense in primary teeth. Moreover, the clinical efficacy of Biodentine as a pulpotomy material in primary teeth warrants further exploration through well-designed studies to establish its success and long-term outcomes in pediatric dentistry.

Aim This study aims to compare the expression levels of MMP-2, MMP-8, and MMP-9 in cases of reversible and irreversible pulpitis. Additionally, it seeks to evaluate the clinical success of Mineral Trioxide Aggregate (MTA) and Biodentine when used as pulpotomy agents in primary molars. By analyzing the differential expression of these MMPs, the study will contribute to a better understanding of their role in pulpal inflammation and the potential therapeutic outcomes of MTA and Biodentine in primary molars.

Design In this parallel randomized controlled trial, 63 mandibular primary second molars were assigned to two main groups: Group 1, consisting of 42 teeth diagnosed with reversible pulpitis, and Group 2, consisting of 21 teeth diagnosed with irreversible pulpitis. Group 1 was further divided into two randomized subgroups, each containing 21 teeth. The expression levels of MMP-2, MMP-8, and MMP-9 were evaluated in all samples. Pulpotomy treatments were performed using MTA and Biodentine in Group 1. Clinical and radiographic evaluations were conducted over an 18-month follow-up period. Statistical analyses were carried out using The Kolmogorov-Smirnov test, t-test and Fisher's exact test ($p < 0.05$).

*Correspondence:
Dilşah Çoğulu
dilsah.cogulu@ege.edu.tr

Full list of author information is available at the end of the article

Results The study revealed that MMP-2 and MMP-9 expression levels were significantly elevated in specimens with irreversible pulpitis ($p=0.01$), indicating a potential correlation between these matrix metalloproteinases and the severity of pulpal inflammation. However, no significant difference was observed in the clinical success rates of pulpotomies performed with MTA and Biodentine, suggesting that both materials are equally effective in the treatment of primary molars with reversible pulpitis.

Conclusions The expression of MMP-2 and MMP-9 in pulpal blood presents a promising biomarker for assessing the degree of pulpal inflammation in primary teeth, offering a potentially valuable diagnostic tool. Additionally, the clinical success of Biodentine in pulpotomy procedures supports its viability as an effective alternative to MTA, providing a reliable option.

Clinical Trial Registration ID The study protocol has been registered with an ID: NCT05145686. Registration Date: 9th November 2021.

Keywords Biodentine, Irreversible pulpitis, Matrix metalloproteinase, MTA, Reversible pulpitis

Background

Accurate assessment of pulp condition is critical to the success of pulp therapy in primary teeth [1]. Primary teeth, characterized by thinner hard tissue, wider dentinal tubules, and larger pulp chambers compared to permanent teeth, are more susceptible to pulp involvement [2]. Diagnosing pulpal diseases in children generally relies on the evaluation of clinical symptoms and radiographic findings. However, primary teeth do not possess a fully developed Raschkow plexus within the pulp-dentin complex, rendering thermal and electric pulp tests unreliable. Additionally, conducting proper radiological and clinical examinations can be particularly challenging in anxious pediatric patients or children with special needs, as their responses may lead to false positives [3, 4].

Treatment for these patients is often performed under general anesthesia, with pulpal diagnosis mainly relying on operative findings where pulp bleeding control is managed [1, 2]. The clinician is now at a point where they have to choose between two options, root canal treatment and pulpotomy. Particularly in cases of partial pulpal necrosis, it is challenging to determine the extent of necrosis by clinical observation. Moreover, during the late stages of primary teeth, bleeding control may not be enough for a safe diagnosis in some cases [3, 4]. In this instance, there is a need for a diagnostic tool that uses biomarkers from pulpal bleeding.

Various biomarkers have been studied for assessing pulpal inflammation, such as interleukins, matrix metalloproteinases (MMPs), tissue growth factors, neurokinins, vasoactive intestinal peptides, the receptor for advanced glycation end products [5–7]. Recently, there has been a growing interest in investigating matrix metalloproteinases (MMPs), a subgroup of calcium and zinc-dependent endopeptidases, for their potential role in diagnosing dental inflammatory processes [8].

MMPs are proteolytic enzymes involved in various pathophysiological conditions including inflammatory processes [9]. It is believed that MMP-2, MMP-8, and MMP-9 are specifically involved in dental pathologies. Additionally, they are suspected to be involved in extracellular matrix (ECM) degradation and growth factor release in pulpal inflammation [10–12]. These MMPs are essential for tissue remodeling as they destroy protein components of the ECM [13].

Current literature indicates that pathological pulp tissue in permanent teeth exhibits significantly higher levels of MMP-1 and MMP-2 compared to healthy pulp tissue [12, 18, 19]. However, there is a significant lack of studies investigating the potential changes in intrapulpal MMP expression in primary teeth. Prospective assessment of pulpal inflammation based on these inflammatory biomarkers could enhance clinicians' ability to formulate more accurate treatment plans, thereby improving prognosis, reducing the likelihood of treatment failures, and preventing the need for recurrent interventions.

Preserving the vitality of primary teeth affected by inflammation of the coronal pulp is commonly achieved through a vital pulpotomy. This procedure is only used when the radicular pulp is vital and not affected [4, 14]. Different methods and medications are used to treat the surface of the remaining vital pulp tissue. These include formocresol, ferric sulfate, calcium hydroxide, glutaraldehyde, sodium hypochlorite, bone morphogenic proteins, dentin bonding agents, enamel matrix derivatives, growth factors, MTA (Mineral Trioxide Aggregate), Biodentine, electrosurgery, and lasers [15–19].

Among the materials described before, MTA first appeared in dentistry literature in 1993 as a calcium silicate cement [20]. This material has several beneficial properties, including biocompatibility, bioactivity, hydrophilicity, radiopacity, sealing properties, and low solubility [21, 22]. Yet, due to its suboptimal manipulation qualities and cost-intensive nature, novel calcium silicate-based materials with enhanced attributes were subsequently introduced [16, 23].

Biodentine, a novel calcium-silicate cement was first introduced in 2009, specifically designed as a dentin

replacement material [24]. Leveraging the MTA-based cement technology, Biodentine refines certain characteristics, including its physical attributes and ease of manipulation. Moreover, Biodentine exhibits resistance to microleakage and exerts an antimicrobial influence. In comparison to MTA, it stands as a relatively more user-friendly material for clinicians [25, 26]. In a systematic review in 2021 [27] and a meta-analysis in 2019 [28], Biodentine was found to be as effective as MTA in mature permanent teeth. While MTA is considered as the gold standard material for pulpotomy, more studies with long-term results are needed for Biodentine in pulpotomies in primary teeth.

Therefore, this two-step in vivo study aimed to compare the expression levels of MMP-2, -8, and -9 in primary molars with reversible and irreversible pulpitis and to compare the two different pulpotomy agents (MTA and Biodentine) clinically and radiographically for 18 months. According to our null hypothesis, neither medicament type has a significant difference in terms of clinical success; and similar MMP-2, -8, and -9 expressions are present in primary molars with both reversible and irreversible pulpitis.

Methods

This single-center, single-blinded, parallel randomized controlled trial was conducted according to Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines [29]. The flowchart of the study is presented in Fig. 1. The registration number of the study protocol is NCT05145686. The study received approval from the University's Ethical Committee of the Medical School (Reference No: 16-7/14). Comprehensive information about the treatment protocol was provided to both the children and their parents, and written informed consent was obtained.

The primary second molar teeth of patients aged between 5 and 10 years were evaluated. Preceding any interventions, periapical radiographs were obtained to establish a baseline. Teeth exhibiting clinical or radiographical evidence of a profound carious lesion, extending close to or infiltrating the pulp chamber, were identified as potential candidates for inclusion in the study.

Inclusion criteria

- Cooperative, healthy pediatric patients within the age range of 5 to 10 years.
- A profound carious lesion without the existence of pathological root resorption and/or bone defect in the mandibular primary second molars.
- The participants were not administered any anti-inflammatory medication within three months before the commencement of the study.
- Restorable coronal structure.
- Positive parental informed consent.

The sample size determination indicated that a total of 63 teeth would be required to achieve 80% statistical power to detect differences at a 5% significance level, assuming an effect size of 0.4 [35]. This sample size includes 21 teeth in the irreversible pulpitis group and 21 teeth in each of the reversible pulpitis subgroups treated with MTA and Biodentine.

A total of 63 mandibular primary second molar teeth from 57 healthy and cooperative children, aged between 5 and 10 years, who visited the pediatric dentistry department were included in this study. The teeth were assigned to different groups according to the clinical diagnosis. When the dental pulp was exposed during caries removal, the following clinical and radiographic diagnostic criteria were applied:

Group I (teeth with reversible pulpitis): mild clinical symptoms of minor intensity, slightly exaggerated reaction to cold or sweet stimuli, no history of spontaneous pain, no sensitivity to chewing or percussion, bleeding time from the exposed pulp tissue less than 5 min, and no widening of the periodontal ligament space. In the pulpotomy group teeth with pulpal exposure smaller than a pinpoint, teeth with purulent/viscous, dark-colored exudate detected at the exposure site, and necrotic teeth were excluded.

Group II (teeth with irreversible pulpitis): Spontaneous pain, sensitivity to chewing or percussion, bleeding time from the exposed pulp tissue of more than 5 min, and widening of the periodontal ligament space, periapical lesion.

For ethical reasons, there was no control group with blood samples from healthy, caries-free teeth. All primary molars were distributed between the two treatment groups as pulpotomy (Group I, $n=42$) and pulpectomy (Group II, $n=21$).

The pulpotomy procedures and all sampling were performed by the same operator (H.E.G.) following the procedure as follows: local anesthesia (2% lidocaine (1:000,000)- Jetokain Simplex, Adeka, Turkey) was administered, and teeth were isolated by a rubberdam (Roeko Dental Dam, Coltane, Whaledent, Germany). The carious dentin was removed from the cavity, working from the periphery toward the center using a low-speed round bur (no:4). The roof of the pulp chamber was removed, and coronal pulp tissue was taken using a sharp excavator. All pulp samples were placed in 1 ml Tri Pure Reagent transport medium in pre-weighed Eppendorf tubes. The samples were weighed again and their weights

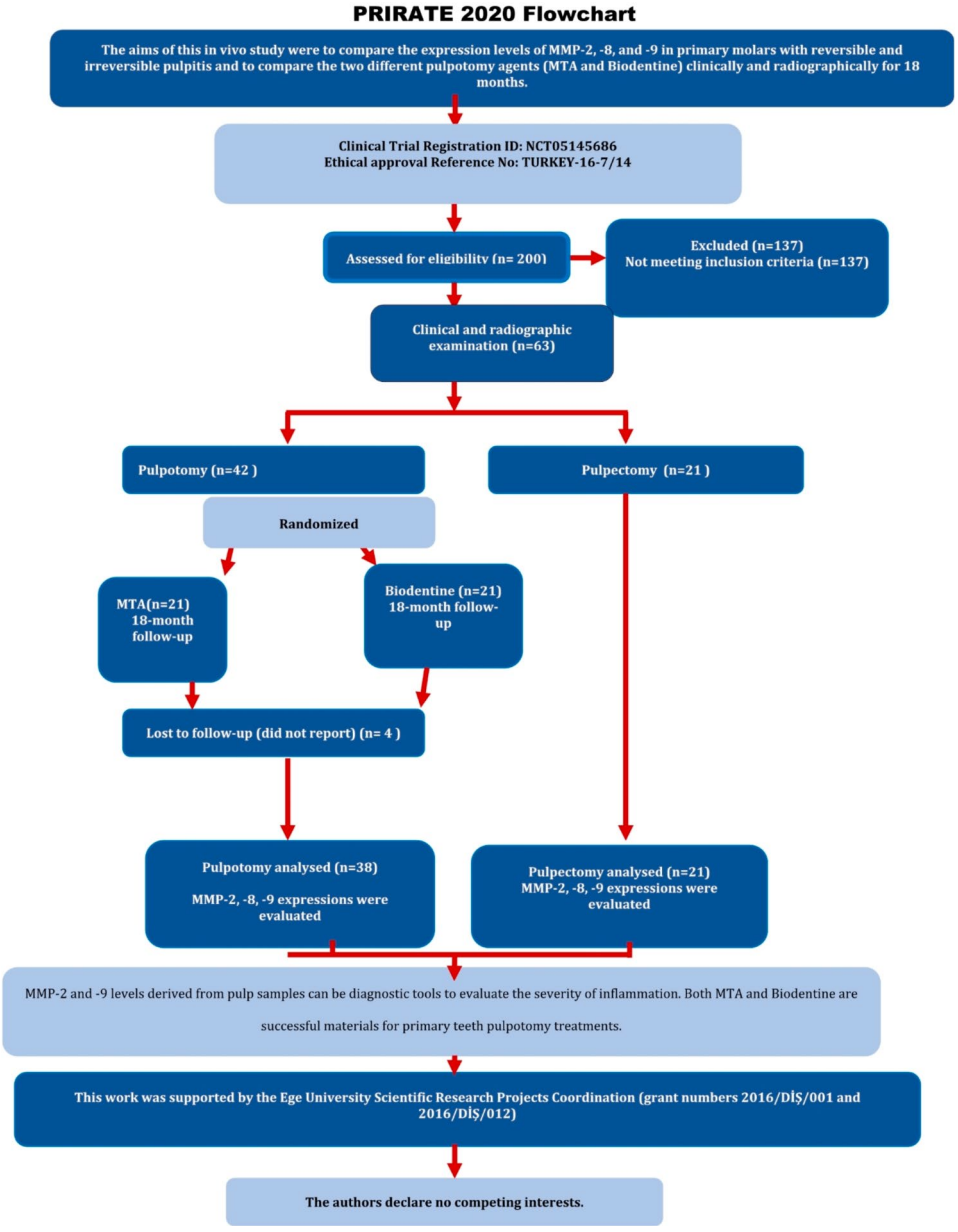

Fig. 1 The PRIRATE flowchart of the study [34]

were noted before they were stored at -80 °C until the laboratory procedures. In the pulpectomy group (Group II), all sampling procedures were performed as same in the pulpotomy group.

In Group I, after removal of the coronal pulp, the cavity was irrigated with sterile saline, and initial bleeding was controlled by placing sterile cotton pellets of moisturizer with saline over the radicular pulp stump exerting slight pressure, and waiting for 5 min. The teeth for which hemostasis at the canal orifices could be achieved in 5 min were included in this group. The randomization was performed in two groups in a 1:1 ratio by using free online software (http://www.random.org). Following the randomization procedure, patients were assigned sequential numbers in the order of enrollment and the operator was informed about the material, either with MTA ($n=21$) or with Biodentine ($n=21$).

MTA group

According to the manufacturer's instructions, MTA (Pro Root White MTA, Dentsply, Tulsa Dental Specialties, USA) powder was mixed with sterile water in a 3:1 powder/water ratio to obtain a thick creamy paste, then placed on the floor of the pulp chamber. The MTA mixture was covered with a moistened cotton pellet and then temporarily sealed using glass ionomer cement

(GIC-Ketac Molar Easymix, 3 M Espe, Seefeld, Germany) until the second visit.

Biodentine group

Biodentine (Septodont, Saint-Maur-des-Fossés, France) capsule was gently tapped on a hard surface to diffuse powder, five drops of liquid from the single dose dispenser were poured into the capsule and then mixed with 4200 rpm 30 s and placed over the pulp stumps using an appropriate spatula advised by the manufacturer. After allowing Biodentine to set for 12 min, the teeth were temporarily sealed with GIC as same in the MTA group. After 24 h, the teeth in both groups were restored with preformed stainless-steel crowns (3 M Espe, Stainless Steel Crowns, Seefeld, Germany).

Group II was treated with pulpectomy. A single dentist (H.E.G) treated each involved molar over two visits. During the first appointment, the molar was isolated with a rubber dam after administering local anesthesia. The pulp chamber was accessed by removing all carious tooth structures, and pulpal debris was cleared using barbed broaches. The working length was determined by Electronic Apex Locator (Root ZX mini) and a Mani K files (MANI Inc., Tochigi, Japan). Cleaning and shaping of the root canals were performed using Mani K files (MANI Inc., Tochigi, Japan) in a pullback direction, with sizes ranging from 35 to 40. Continuous irrigation with 2.5% sodium hypochlorite was conducted throughout the procedure, and sterile paper points were used to dry the root canals. Calcium hydroxide (Multi-Cal, Pulpdent Corporation, Watertown, MA, USA) was then injected into the root canal, and a sterile cotton ball was placed in the pulp chamber, which was sealed with Cavit (3 M ESPE, St. Paul, MN, USA) as a temporary sealing material. At the second visit, typically occurring two weeks later, the root canal was irrigated again with 2.5% sodium hypochlorite and dried with sterile paper points. The prepared molars were then randomly allocated to Vitapex (Neo Dental Chemical Products Co. Ltd., Tokyo, Japan), SSC restoration will be performed, and finally, a post-treatment radiograph will be taken.

The participants were recalled at 6, 12, and 18 months after the treatment. At each follow-up visit, clinical and radiographic evaluations were performed by two calibrated blinded examiners. All teeth were evaluated clinically and radiographically according to the criteria: (1) absence of spontaneous pain and/or sensitivity to pressure, (2) absence of sinus, fistula, edema, and/or abnormal mobility, (3) absence of radiolucency at the inter radicular and/or periapical regions, (4) absence of internal or external root resorption. The absence of spontaneous pain, pathologic mobility, tenderness to percussion, swelling, fistula, and gingival inflammation was considered a clinical success, whereas the absence of internal/external root resorption and periapical/furcal radiolucency was considered a radiographic success. Calcific metamorphosis of the pulp was not considered a failure.

Pulp tissue samples obtained from the patients were stored at -80 ºC until RNA extraction. 50–100 mg of each sample was used for RNA extraction using RNAiso Plus (TaKaRa Bio, Shiga, Japan). RNA concentration and quality were evaluated by A260/A280 and A230/A280 absorbance ratios using the Nanodrop (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized from 400 ng of total RNA using the PrimeScript RT reagent kit (TaKaRa Bio, Otsu, Japan) according to the manufacturer's instructions. One-tenth of the cDNA obtained was added for a final volume of 20 µL for each reaction containing SYBR Green I (TaKaRa Bio, Shiga, Japan). The primers for MMP-2, MMP-8, and MMP-9 were as follows (sense/antisense):

MMP-2: 5′-ATC CTG GCT TTC CCA AGC TC-3′/3′-CAC CCT TGA AGA AGT AGC TGT G-5′. MMP-8: 5′-CTGTTGAAGGCCTAGAGCTGCTGCTCC3′/5′CA TCTTCTCTTCAAACTCTACCC-3′.

MMP-9: 5′-GGG CTT AGA TCA TTC CTC AGT G-3′/3′-GCC ATT CAC GTC GTC CTT AT-5′.

Reactions were run on an ABI Step One Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with the following program: 45 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20s. The expression levels of each mRNA were normalized by the expression level of internal control gene G6PDH using primers 5′-ATGG GGAAGGTGAAGGTCG-3′ (forward) and 5′-GGGGTC ATTGATGGCAACAATA-3′ (reverse). For relative comparisons of each gene, we analyzed the threshold cycle (Ct) value of the real-time PCR data using the 2–ΔΔCt method (Fig. 2) [12, 30, 31].

Data analysis and statistical methods

The statistical analysis was performed by an independent statistician using SPSS for windows version 25.0 (SPSS, Chicago, IL, USA). A p-value of <0.05 was considered statistically significant. The Kolmogorov-Smirnov test was used to assess the normality of the data for MMP-2, MMP-8, and MMP-9 expression levels in each group. Since MMP-2, MMP-8, and MMP-9 expression levels were found to be normally distributed within each group, an independent samples t-test was used to compare the results. The clinical, and radiographic results were analyzed by the Fisher's exact test ($p<0.05$).

Results

The study was conducted on a total of 63 mandibular primary second molars in 57 children (29 males and 28 females) with a mean age of 6.53 ± 1.32 years. The reversible pulpitis group included 42 teeth and the irreversible

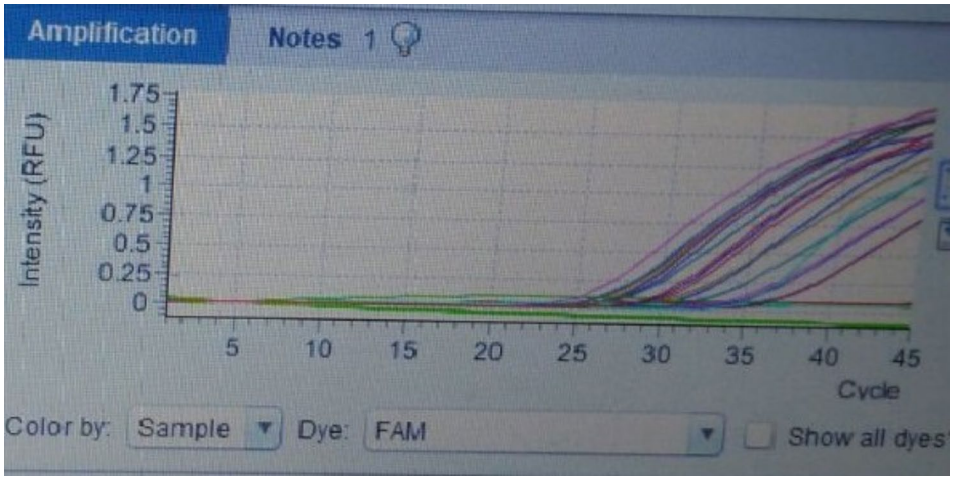

Fig. 2 Representative graphical presentation of RT-qPCR results

Table 1 The mean MMP-2, -8 and -9 expression levels according to the reversible and irreversible pulpitis groups. (μg/L) ($p < 0.05$)

MMP	Group	Mean	Std. Deviation	*P*
MMP-2	Reversible	25.88	3.82	0.000
	Irreversible	33.03	1.91	
	Total	28.26	4.73	
MMP-8	Reversible	27.76	1.22	0.767
	Irreversible	27.86	1.42	
	Total	27.79	1.28	
MMP-9	Reversible	32.17	2.35	0.000
	Irreversible	38.55	0.68	
	Total	34.30	3.60	

group included 21 teeth. In the reversible pulpitis group, a total of 21 mandibular primary second molar teeth were subjected to vital pulpotomy using mineral trioxide aggregate (MTA), whereas an additional 21 teeth underwent the same procedure applying Biodentine. A total of 18 left and 24 right mandibular primary second molars were treated in the reversible pulpitis group.

The mean MMP-2, -8, and -9 expression levels according to the reversible and irreversible pulpitis groups are shown in Table 1. Irreversible pulpitis specimens exhibited significantly higher MMP-2 and MMP-9 mRNA gene expression than the reversible pulpitis group ($p=0.01$). No significant difference was found between the MMP-8 expression level and the treatment groups ($p>0.05$).

Evaluation of clinical and radiographic success

The clinical success outcomes for MTA and Biodentine at the 6, 12, and 18-month follow-up intervals are outlined in Table 2. At the 6-month follow-up, 40 out of 42 teeth were assessed for the reversible pulpitis group (2 dropouts), while the count was 38 for both groups at the 12 and 18-month follow-ups (4 dropouts). The clinical success rate stood at 100% for both MTA and Biodentine at the 6 and 12-month assessments, declining to 95% for MTA and 94.4% for Biodentine at the 18-month assessment.

Regarding radiographic success (Table 3), MTA showed 100% success rate at the 6-month follow-up, while Biodentine exhibited a rate of 94.7%. At the 12-month and 18-month follow-ups, MTA maintained a radiographic success rate of 100% and 95%, respectively. However, Biodentine's radiographic success rate was 83.3% for both the 12 and 18-month assessments. An example of radiographs taken at baseline and 18 months after pulpotomies are shown in (Fig. 3).

Analysis of total success rate and MMP expression

Comparing the overall success rate from the start of treatment to the 18-month mark, no significant difference found between the two material groups ($p>0.05$). Radiographic failure was notably more prevalent than clinical failure in both material groups. Although the

Table 2 The 6, 12, and 18 months clinical success of MTA and Biodentine

	6 months clinical evaluation			12 months clinical evaluation			18 months clinical evaluation		
	Successful (n)	Failure	Total	Successful	Failure	Total	Successful	Failure	Total
MTA (n)	21	0	21	20	0	20	19	1	20
%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	95.0%	5.0%	100.0%
Biodentine (n)	19	0	19	18	0	18	17	1	18
%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	94.4%	5.6%	100.0%
Total (n)	40	0	40	38	0	38	36	2	38
%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	94.73%	5.27%	100.0%

Table 3 The 6, 12 and 18- month radiographic success of MTA and Biodentine

	6-month radiographic evaluation			12-month radiographic evaluation			18-month radiographic evaluation		
	Successful	**Failure**	**Total**	**Successful**	**Failure**	**Total**	**Successful**	**Failure**	**Total**
MTA (n)	20	0	21	20	0	20	19	1	20
%	100.0%	0.0%	100.0%	100.0%	0.0%	100.0%	95.0%	5%	100.0%
Biodentine (n)	18	1	19	15	3	18	15	3	18
%	94.74%	5.26%	100.0%	83.3%	16.7%	100.0%	83.3%	16.7%	100.0%
Total (n)	38	1	40	35	3	38	34	4	38
%	97.5%	2.5%	100.0%	92.1%	7.9%	100.0%	89.5%	10.5%	100.0%

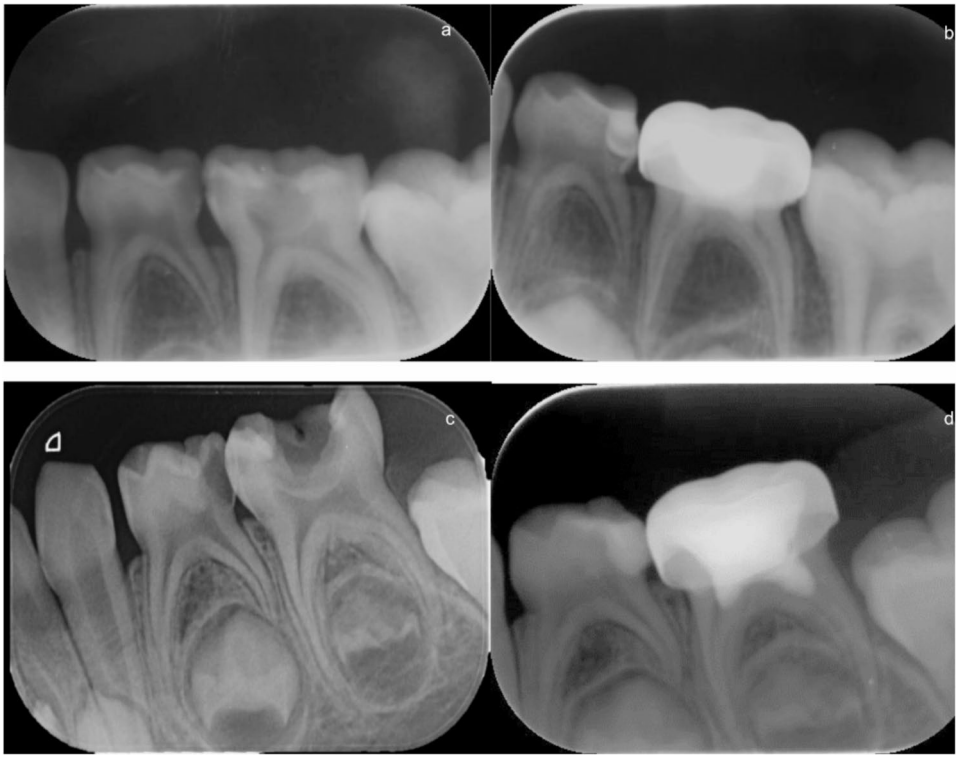

Fig. 3 Periapical radiographs of primary second molars before (**a**, **c**) and 18 months after (**b**, **d**) pulpotomy treatments. (**b**: pulpotomy with Biodentine, **d**: pulpotomy with MTA)

Table 4 The mean MMP-2, -8 and -9 expression levels according to the clinical success of the treatments (µg/L)

		6-month clinical evaluation			12 month clinical evaluation			18 month clinical evaluation		
		n	**Mean**	**Std Deviation**	**n**	**Mean**	**Std Deviation**	**n**	**Mean**	**Std Deviation**
MMP-2	Success	40	26.05	3.83	38	26.18	3.86	36	25.81	3.62
	Failure							2	32.86	0.26
MMP-8	Success	40	27.77	2.39	38	27.80	1.24	36	27.78	1.25
	Failure							2	28.22	1.28
MMP-9	Success	40	32.21	2.39	38	32.21	2.45	36	31.86	1.97
	Failure							2	38.61	0.49

radiographic failure rate was higher in the Biodentine group compared to MTA at 12 and 18 months, this difference did not attain statistical significance ($p>0.05$).

In terms of MMP-2 and -9 expression levels, clinically and radiographically unsuccessful pulpotomy treatments exhibited notably higher levels compared to the successful treatment group, regardless of the material applied ($p=0.01$). Conversely, no significant difference was observed in MMP-8 expression levels concerning the unsuccessful pulpotomy treatments ($p>0.05$) (Tables 4 and 5).

Table 5 The mean MMP-2, -8 and -9 expression levels according to the radiographical success of the treatments (µg/L)

		6 month radiographic evaluation			12 month radiographic evaluation			18 month radiographic evaluation		
		n	Mean	Std Deviation	n	Mean	Std Deviation	n	Mean	Std Deviation
MMP-2	Success	39	25.76	3.41	35	25.33	2.51	34	25.10	2.15
	Failure	1	37.42	–	3	36.10	3.00	4	35.34	2.89
MMP-8	Success	39	27.75	1.26	35	27.84	1.25	34	27.80	1.25
	Failure	1	28.61	–	3	27.35	1.24	4	27.79	1.35
MMP-9	Success	39	32.02	2.10	35	31.66	1.58	34	31.45	0.95
	Failure	1	39.56	–	3	38.69	0.75	4	38.76	0.63

Discussion

In the present study, in the reversible pulpitis group, there was no significant difference between MTA and Biodentine pulpotomy treatments in clinical and radiologic evaluation with 18-month follow-up. Thus, the first hypothesis was accepted. Also, MMP-2 and -9 expressions were significantly higher in the irreversible pulpitis group than the reversible pulpitis group; therefore, the second hypothesis was rejected. Based on our findings, MMP-2 and -9 levels were successful in determining the pulp inflammation status in primary teeth, and Biodentine showed similar success as MTA in pulpotomy treatments in primary teeth.

Matrix metalloproteinases (MMPs) are a family of 24 zinc-dependent endopeptidases involved in the degradation of extracellular matrix (ECM) components, essential for both normal tissue remodeling and pathological processes [7, 32]. In the context of dental pulp tissue, Pulp tissue destruction is partially regulated by MMPs and tissue inhibitors of MMPs (TIMPs) [32]. MMP family proteins have dual roles in inflammation pathogenesis, stimulation of protective innate and adaptive immune functions and tissue destruction [33]. These enzymes are secreted by various cell types, including neutrophils, odontoblasts, and other cells within the dental pulp. During pulpal inflammation, MMPs, such as MMP-2, MMP-8, and particularly MMP-9, are activated and participate in the breakdown of ECM components like collagen, leading to pulp tissue degradation [7].

Our findings indicate that MMP-2 and MMP-9 expressions were significantly higher in the irreversible pulpitis group and in cases of pulpotomy failure, consistent with previous research showing increased MMP-9 levels in inflamed dental pulp tissues [34–36]. However, there was no significant difference in MMP-8 levels between the two groups. In contrast, Wahlgren et al. identified a role for MMP-8 in pulpal and periapical inflammation, contributing to ECM degradation and tissue remodeling [11]. This discrepancy in findings may be due to differences in methodologies, with Wahlgren's study analyzing periapical exudates from permanent teeth undergoing root canal treatment (RCT), while our study focused on pulp samples from primary molars.

Despite the methodological differences, our findings align with the broader understanding of MMPs in pulp diseases. Notably, there is a lack of studies on MMP activity in primary teeth compared to permanent teeth. However, existing evidence in permanent teeth suggests that MMP-9, a member of the gelatinase family, is predominantly secreted by neutrophils, which are more abundant in inflamed pulp tissues than healthy pulp tissues [34, 37]. Additionally, cytokines such as IL-8 and TNF-α, associated with neutrophil activity, are also present in primary tooth pulp inflammation, where they induce the rapid release of MMP-9 zymogen in human blood [38]. Scheffel et al. observed that while primary and permanent tooth tissues. initially exhibit similar MMP activity, collagen degradation occurs more rapidly and significantly in primary dentin over time [39]. These findings indicate that elevated levels of MMP-2 and MMP-9 could therefore serve as valuable diagnostic biomarkers for assessing pulpal status in primary teeth, though further studies are necessary to clarify these responses in pediatric populations.

In clinical part of the study, similar success rate of MTA and Biodentine can be attributed to the bioactive nature and odontoblast-promoting ability of calcium-silicate cements (CSC) [40]. Since its introduction in dentistry, MTA has mostly been used for endodontic applications, owing to its extended setting time and discoloration. On the other hand, Biodentine™ is a type of CSC with shorter setting time and color stability, which distinguishes it from the other CSC proposed for use as both endodontic and coronal restoration materials [41–43]. Similar results with different follow-up time were found in previous in-vivo studies [44–46]. While MTA is often considered the gold standard material for pulpotomies, Biodentine has similar success rates to other materials but offers a shorter setting time and better color stability, making it a viable alternative for primary teeth for both pulpotomy and dentin restorations.

The other factor in pulpotomy success is final coronal restoration. In the present study, in both groups, the final restorative material was stainless steel crowns (SSC). SSCs are more enduring and reliable restorations for endodontically treated primary molars [31] and

recommended by American Association of Pediatric Dentistry [47].

Even though there was no statistically significant difference between the groups, low success rate over time in Biodentine group suggested that the follow-up period's extension might have had an impact on the success rate differences. This is especially true given that numerous clinical studies have indicated that variations in follow-up duration affect success rates [38, 48–50]. In our study, we determined our follow-up period as 18 months to observe the pulpal healing.

Our study has several strengths, as it is one of the few recent randomized clinical trials with MMP expression in the primary tooth with both irreversible and reversible pulpitis, as well as clinical and radiographic evaluation of Biodentine and MTA as pulpotomy materials with 18-month follow-up. Combining immunologic markers with clinical and radiologic evaluation led us to better understanding of material success and pulp healing mechanisms together. This clinical trial might be a groundwork for more research with more participants and longer follow–up times.

The present study has some limitations, such as restricted timeframe for follow up and strict criteria for participant selection. Operator blinding was unfeasible due to the materials requiring distinct manipulations, possibly introducing cognitive bias during procedures, although it did not affect follow-up assessments.

Conclusions

In conclusion, MMP-2 and -9 expression in pulpal blood shows potential for assessing pulpal inflammation and distinguishing between reversible and irreversible pulpitis in primary teeth. Furthermore, our study found similar success rates for pulpotomy treatments with MTA and Biodentine in primary molars over an 18-month follow-up. Biodentine's clinical success and improved properties make it a viable alternative for pulpotomy and dentin restorations. Accurate biomarker-based diagnostics may reduce unnecessary RCTs by providing clinicians with better prognostic certainty for pulpotomy treatments in primary teeth.

Clinical relevance

- MMP-2 and MMP-9 expression levels are significantly higher in primary teeth with irreversible pulpitis than reversible pulpitis.
- MMP-2 and MMP-9 expressions are important to assess pulpal inflammation severity and can be used as prospective pulpal diagnosis tools in primary molars.
- Biodentine is a successful pulpotomy material for carious primary teeth.

Abbreviations

MMP	Matrix-metalloproteinases
MTA	Mineral Trioxide Aggregate
GIC	Glass ionomer cement
ECM	Extracellular matrix
IL	Interleukin

Supplementary Information

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Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
H.E.G. and D.C. conceived the ideas; D.C., H.E.G and E.K collected the data; A.D and Ö.Ö. analyzed the data, and E.K. and D.C. led the writing.

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

Declarations

Ethics approval and consent to participate
The Ethical Committee of the Medical Faculty of Ege University approved the study (Reference No: 16-7/14). All participants were comprehensively informed of the research objectives, and detailed information about the treatment regimen was provided to both the children and their parents. Written informed consent was obtained, ensuring all individuals' voluntary and consensual participation. Data will be used exclusively for research purposes, maintaining strict confidentiality and privacy.

Consent for publication
Informed written consent was obtained from the patients to publish the medical history and radiographs.

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This work is an original contribution, and no material has been reproduced from other sources.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Pediatric Dentistry, Ege University School of Dentistry, İzmir, Turkey
[2]Department of Medical Genetics, Ege University School of Medicine, İzmir, Turkey
[3]Department of Pediatric Dentistry, Usak University School of Dentistry, Usak, Turkey
[4]Present address: Avrupadent Dental Clinic, İzmir, Turkey

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