Study design and ethics

It was a randomized, triple-blinded, split-mouth, active-controlled clinical trial. This trial was conducted in accordance with the Declaration of Helsinki as revised in 2013 and the Consolidated Standards of Reporting Trials (CONSORT) statement. Ethical approval was provided by the Biomedical Research Ethics Committee (1297/2024). It was performed at the Department of Pediatric Dentistry and the Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Damascus University, between August 2023 and January 2024. The treatment plan was explained in detail. Participation was voluntary and confidential. Patients' legal guardians signed written informed consent before enrollment, and they can withdraw consent at any time. No child was excluded based on their race, gender, and socioeconomic status. Each child received complete other required dental treatments.

Sample size calculation

The sample size was calculated using G*Power version 3.1.9.4 (G*Power 3.1.9, Heinrich Hein Universität Düsseldorf, Düsseldorf, Germany). A sample size of n = 48 achieved a large effect size f (0.55), 80% Power (1 - β err prob), and a significance level of 0.05. A pilot study on 4 samples was performed to determine the effect size.

Eligibility criteria and sampling

The inclusion criteria were as follows:

- Children aged 8-10 years.
- Children with at least 2 bilateral carious first primary molars indicated for pulpotomy.
- Children require serial extraction of first primary molars for orthodontic reasons.

The exclusion criteria were as follows:

- Children with systematic diseases and/or allergies to the anesthetic agents.
- Children with clinical and radiographical signs of pulp necrosis in the targeted teeth and/or unrestorable teeth.
- Children with nocturnal and/or spontaneous pain.

The CONSORT flow diagram is illustrated in Figure 1. Two experienced pediatric dentists assessed 29 patients who were referred to the Department of Pediatric Dentistry for eligibility. Periapical radiographic image was performed using intraoral periapical sensor (i-sensor, Guilin Woodpecker Medical Instrument Co., LTD., Guilin, China). According to the inclusion criteria, five patients were excluded. A total of 24 patients with 48 first primary molars indicated for pulpotomy were randomly assigned into two groups (n=24) according to the pulp dressing material used:

- Group 1 (WMTA + DW): WMTA mixed with distilled water (Rootdent, TehnoDent Co., Belgorod, Russia), this was considered the control group.
- Group 2 (WMTA + NaOCl gel): WMTA mixed with 2.25% NaOCl gel (LET'S CLEAN Concentrated Chlorine, DTIC®, Damascus, Syria), this was considered the interventional group.

Each group was sub-divided into three sub-groups (n=8) according to the follow-up period:

- Sub-group I: The serial extraction was scheduled after 7 days.
- Sub-group II: The serial extraction was scheduled after 30 days.
- Sub-group III: The serial extraction was scheduled after 90 days.

Blinding and randomization

It was a triple-blinded trial where dentists, participants, and outcome assessors were masked to group allocations. A simple randomization technique was applied by flipping a coin for each patient by a blinded investigator, and then the first primary molars were randomly allocated to either the control or interventional group.

Procedure

Topical anesthetic (Iolite, Dharma Research Inc., Florida, United States) was applied at the site of needle insertion then local anesthetic solution (2% Lidocaine HCL Injection, Huons Co., Ltd, Seongnam, Korea) was deposited using a dental carpule syringe (Dental carpule syringe, Dental Laboratorio, china) and a 27-gauge x 21 mm needle (Disposable Dental Needle, Shanghai Dochem Industries Co., Ltd., Shanghai, China). Rubber dam (Sanctuary®, Perak, Malaysia) and saliva ejector (Disposable Transparent Surgical Dental Saliva Ejectors China, Andent Dental Co., ltd., Hebei) were used for isolation. Caries lesions were removed, and the pulp chamber was deroofed using round tungsten carbide cavity bur (Round E 0123, Dentsply Maillefer, Ballaigues, Switzerland) in an air turbine handpiece (NSK PANA-AIR, NSK Nakanishi Inc., Tochigi-ken, Japan) with copious irrigation. Coronal pulpotomy was performed using a slow–speed endodontic opening cutter carbide bur (Excavabur E123A, Dentsply Maillefer, Ballaigues, Switzerland) in a contra-angle handpiece (NAC-EC, NSK Nakanishi Inc., Tochigi-ken, Japan). The pulp chamber was thoroughly irrigated, and hemostasis was achieved using a moist cotton pellet with normal saline (SODIUM CHLORIDE 0.9% MIAMED, Miamed Pharmaceutical Industry, Damascus, Syria) for 5 m. In the control group, WMTA powder was mixed with distilled water in a 3:1 powder-to-liquid ratio, and then the pulp was stamped with a 3mm thick layer of MTA. In the interventional group, WMTA was mixed with 2.25% NaOCl gel in a 3:1 powder-to-gel ratio. In both study groups, the cavity was sealed with glass ionomer cement (RX Glass Ionomer Cement, Stardent Equipment Co., Ltd., Guangdong, China), and then the tooth was restored with a stainless-steel crown (Kids Crown, Shinhung, Seoul, Korea) at the same appointment. Extraction was scheduled after 7, 30, and 90 days for histological evaluation.

Histological evaluation

Each sample was stored in 10% buffered formalin solution (10% Neutral Buffered Formalin, Thomas Scientific LLC, New Jersey, United States) for 48 h at room temperature for fixation, and then it was demineralized in Morse's solution (Morse Solution, FUJIFILM Wako Pure Chemical Co., Hong Kong, China), which is an aqueous solution of 22.5 % formic acid and 10 % sodium citrate. Each specimen was embedded in a paraffin wax block (Clear Paraffin Block, EverBio Technology INC., New Taipei City, Taiwan), and then the paraffin-embedded wax blocks were sectioned at 5 µm using a semi-motorized rotary microtome (Leica RM2145 Microtome, GMI, New Jersey,

United States). The sectioned samples were stained with hematoxylin and eosin (H&E Staining Kit, Abcam, England, United Kingdom), and the histological samples were evaluated using a light microscope (Leica Microscope DM2500, Leica, Hesse, Germany) at 400× magnification by two blinded operators. Cohen's Kappa coefficient values of intra-examiner and inter-examiner reliability were > 0.8. The following primary outcome measures were considered:

Odontoblastic integrity

- Grade 0 = Normal tissue morphology.
- Grade 1 = Mild odontoblastic disorganization. Normal morphology of central pulp tissue.
- Grade 2 = Moderate odontoblastic disorganization.
- Grade 3 = Severe odontoblastic disorganization. Complete morphological disorganization of pulp tissue.
- Grade 4 = Pulp necrosis.

Pulp tissue hemorrhage

- Grade 0 = No hemorrhage.
- Grade 1 = Mild hemorrhage. A few scattered red blood cells.
- Grade 2 = Moderate hemorrhage. Some clusters or red blood cells.
- Grade 3 = Severe hemorrhage. Extensive infiltration of red blood cells.

Pulp fibrosis

- Grade 0 = No pulp fibrosis.
- Grade 1 = Mild pulp fibrosis. Thin collagen fibers.
- Grade 2 = Moderate pulp fibrosis.
- Grade 3 = Severe pulp fibrosis. Thick collagen fibers.

Dentin bridge formation

- Grade 0 = No dentin bridge formation.
- Grade 1 = Initial dentin bridge formation. Dentin bridge extended to < ½ of the exposure site
- Grade 2 = Partial dentin bridge formation. Dentin bridge extended to > ½ of the exposure site.
- Grade 3 = Complete dentin bridge formation. Continuity of dentin bridge.

Pulp calcification

- Grade 0 = No pulp calcification.

- Grade 1 = Single small calcification.
- Grade 2 = Multiple small calcifications.
- Grade 3 = Single large calcification.
- Grade 4 = Multiple large calcifications.

Statistical analysis

Data were analyzed by IBM SPSS software version 24 (IBM SPSS Statistics® version 24, IBM Corp., New York, USA). Descriptive statistics were presented as frequency and percentage. The chi-square test was performed to compare categorical data, and post hoc (Z test) test was performed when the overall test showed a significant difference. Adjusted residuals were extracted and each one was multiplied by itself to calculate chi-square values, and then chi-square values were transformed to obtain p-values. Statistical significance was adjusted at $p < 0.05$.