ORIGINAL RESEARCH

# Clinical and Radiographic Evaluation of Mineral Trioxide Aggregate and Human Amniotic Membrane Pulpotomy in Primary Molars: A Randomized Clinical Trial

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## Abstract

**Objectives:** To clinically and radiographically compare the outcomes of pulpotomy with mineral trioxide aggregate (MTA) and human amniotic membrane (HAM) in primary molar teeth at 1, 3, 6, and 12 months.

**Materials and methods:** The study was a randomized clinical trial with two arms. One arm consisted of participants whose pulpotomy was conducted using MTA, called group I, and the other arm, using HAM, was designated group II.

**Results:** Both MTA and HAM exhibited 100% clinical success. Also, there were no signs of external resorption, periapical bone destruction, or internal resorption in both the MTA and the HAM groups at all four time intervals in this study. However, periodontal ligament widening was seen in 30% of the participants in the MTA group at 1-month and at the 12-month follow-up, whereas in the HAM group, periodontal ligament widening was found to reduce significantly from 22.2% at 1-month to 11.1% at the 12-month follow-up.

**Conclusion:** The HAM exhibited favorable clinical and radiographic outcomes in the present study.

**Clinical significance:** Mineral trioxide aggregate is the most preferred choice as a pulpotomy agent for deciduous teeth. However, various drawbacks associated with MTA have been fueling the need for newer, effective agents. HAM is not only easily available, cost-effective, and easy to handle but also favors tissue regeneration. The positive outcome of the present study strongly advocates the use of HAM as an alternative to MTA for pulpotomy in primary teeth.

**Keywords:** Amniotic membrane, Clinical evaluation, Deciduous teeth, Mineral trioxide aggregate, Pulpotomy, Radiographic evaluation.

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## Introduction

Pulpotomy is the removal of the inflamed coronal pulp followed by the placement of a suitable material, thereby maintaining healthy radicular pulp and restoring tooth function.[1] Historically, many agents have been used in pulpotomy of primary teeth, such as sodium hypochlorite, calcium hydroxide, formocresol, ferric sulfate, and mineral trioxide aggregate (MTA). Studies[2–5] showed that when MTA was utilized as a pulpotomy agent in primary teeth, it outperformed other materials and had a greater success rate. However, disadvantages such as long setting time, discoloration of the tooth, difficult manipulation, and the cost associated with MTA have been fueling the need for newer, effective pulpotomy agents.[4,5]

The thick foundation membrane and the avascular stromal matrix make up the human amniotic membrane (HAM), also known as the amnion, which is the placenta's deepest layer. Additionally, it is said to lessen discomfort and inflammation, stop infections, and encourage epithelialization.[6] The use of HAM isn't new in the health care setting, with HAM being used for the treatment of burned and ulcerated skin surfaces[7,8] as a dural substitute after skull base surgery[9] and in ophthalmology.[10]

Although the use of HAM is relatively novel in pediatric dentistry, it has been used in flap surgery and temporomandibular joint surgery because of its pain-reducing and antimicrobial properties. HAM has also been used for ridge preservation following tooth extraction, as a graft material for reconstruction of the buccal mucosa, and as an intraoral dressing material.[8]

Human amniotic membrane has also been used as a scaffolding material in a wide range of tissue engineering applications[11] and has also been demonstrated to promote the regeneration of periodontal tissue and maintain its structural and anatomical configuration.[12] It has also been demonstrated that the amniotic membrane enhances the healing process following periodontal surgery.[13]

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**Conflict of interest:** None

Endogenous stem cells from induced periapical bleeding and scaffolds comprised of platelet-rich fibrin, platelet-rich plasma, or blood clots have also been employed in regenerative endodontics.[14] A thorough search of the literature turned up just one study[15] that used HAM as a pulpotomy agent in primary molars. The amniotic membrane functioned clinically and radiographically on par with formocresol. Nonetheless, res earch[3–5,16] has demonstrated that pulpotomy utilizing MTA had a notably higher radiographic success rate in comparison to pulpotomy utilizing formocresol.

The paucity of studies in the area led to the conceptualization of this study with the aim of clinically and radiographically comparing the outcomes of MTA pulpotomy and pulpotomy with HAM in primary molar teeth at 1, 3, 6, and 12 months.

## Materials and Methods

The study was a randomized clinical trial with two arms. One arm consisted of participants whose pulpotomy was conducted using MTA, called group I, and the other arm, using HAM, was designated group II.

All children between the ages of 4 and 9 years visiting the Department of Pediatric Dentistry who fulfilled the inclusion criteria were included in the study.

### Inclusion Criteria

- Children aged 4–9 years.
- Parents willing to give written informed consent and children who gave informed assent.

A deciduous tooth was indicated for pulpotomy based on the following criteria:

Participants with carious lesions that showed either of the following clinical or radiographic findings:

- Clinical findings: Cavity involving more than half the occlusal surface (International Caries Detection and Assessment System score 4 or 5)[17] and showing clinical signs of reversible pulpitis.
- Radiographic findings: Teeth with deep dentinal caries radiographically, with the possibility of pulp exposure during caries excavation.

Participants with physiologic tooth mobility, existing abscess or fistula, extraoral swelling, or teeth that showed signs of continuous bleeding from the radicular pulp after coronal pulp amputation were excluded from the study. Informed consent was obtained from parents, and informed assent was obtained from children prior to the study. Ethical clearance was obtained from the Institutional Ethics Committee (Protocol Ref No. 16101). The study was registered with The Clinical Trials Registry of India (CTRI/2017/07/009108).

### Sample Size Calculation

The sample size was calculated using the resource equation:

E = Total number of specimens – total number of groups
(Where E should be between 10 and 20)
$= (20 \times 2) - 2 = 40 - 2$
$= 38/2 = 19$

Adding a 5% rate of attrition, the final sample size was calculated to be 20, with 10 participants in each arm.

Out of the 50 participants who fulfilled the inclusion criteria, the lottery method was used to select the sample and randomly allocate them into two arms, group I and group II.

### Procedure

The pulpotomy procedure was carried out by the principal investigator. Topical local anesthetic gel (Precaine, Pascal Int. Bellevue, United States of America) was applied to the dried oral mucous membrane with a cotton swab and left for 2 minutes before administering a local anesthetic solution (lignocaine hydrochloride 2% with epinephrine 1:200,000) (Dentocare Pvt. Ltd.). Maxillary teeth were anesthetized with local infiltration, and mandibular teeth with an inferior alveolar nerve block. After achieving local anesthesia, isolation was achieved with a rubber dam. All carious tissue was removed using a round diamond bur at high speed under water coolant, following which access to the pulp chamber was gained with a safe end bur. The coronal pulp was extirpated using a sharp sterile spoon excavator. The pulp chamber was irrigated with normal saline. Hemostasis was achieved by applying pressure for 4 minutes on the radicular root stumps using a sterile cotton pellet moistened with saline.

#### *Group I (Mineral Trioxide Aggregate)*

Mineral trioxide aggregate powder (MTA-Angelus) was mixed with distilled water in a 3:1 ratio on a mixing pad using an agate spatula and placed on the floor of the pulp chamber over the amputated pulp tissue. The surface of the MTA was then covered with a slightly moist cotton pellet. The setting of the material was confirmed after 15 minutes with an explorer. The cavity was then restored with type II glass ionomer cement (GC Corporation, Tokyo, Japan).

#### *Group II (Human Amniotic Membrane)*

The HAM (1 × 1 mm) was a processed and stored amniotic membrane obtained from Tata Memorial Hospital, Mumbai, Maharashtra, India (Fig. 1). A small piece was cut using scissors, slightly moistened with saline, and placed over the amputated pulp. A thick mix of zinc oxide eugenol cement was placed on the membrane surface, following which the tooth was restored using type II glass ionomer cement (GC Corporation, Tokyo, Japan), and a stainless steel crown (3M™ ESPE™) was cemented using type I GIC (GC Corporation, Tokyo, Japan).

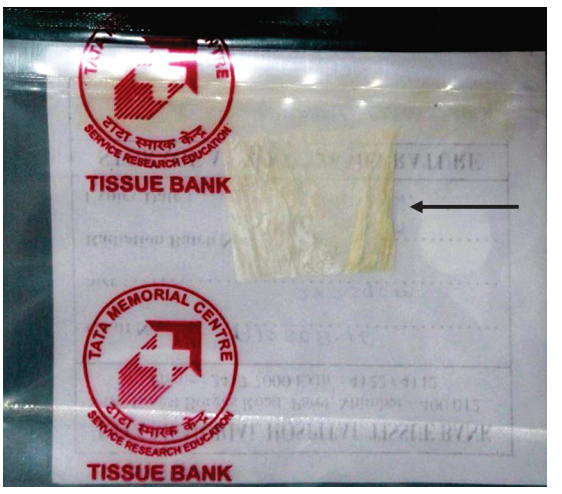
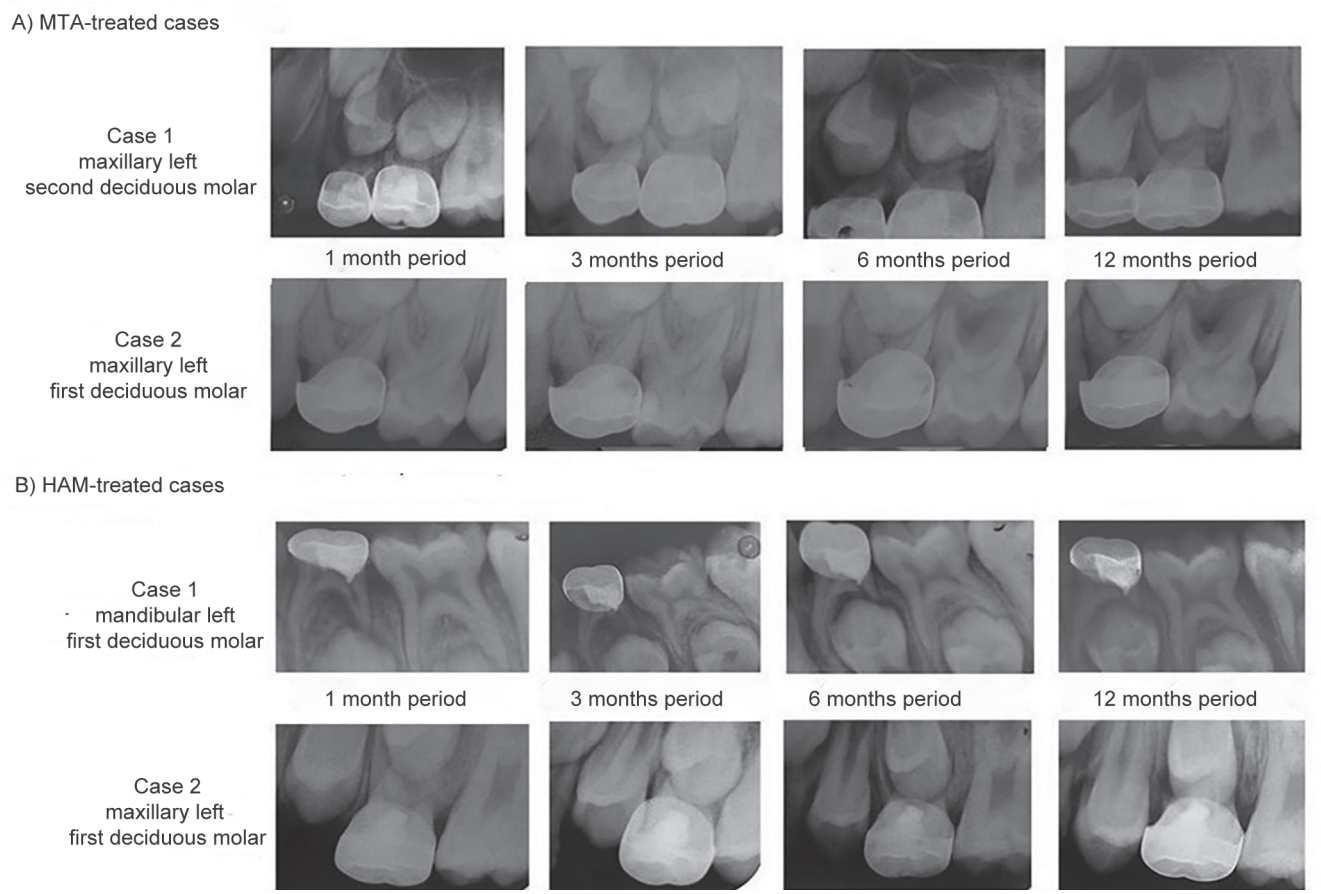



**Fig. 1:** Amniotic membrane




**Figs 2: A and B** Mineral trioxide aggregate and HAM treated pulpotomy cases with follow-up from 1 to 12 months

The procedure was evaluated using intraoral periapical radiographs.

### Follow-up and Evaluation

The participants were then followed up at 1, 3, 6, and 12 months and evaluated for clinical and radiographic findings[18,19] (Fig. 2). The evaluations at the follow-ups were conducted by two blinded examiners. Clinical assessments were performed to determine whether the subjects experienced pain, swelling, abscess/fistula, and pathologic tooth movement. They were also evaluated radiographically for the presence of external resorption, periapical bone destruction, internal resorption, periodontal ligament widening, and interradicular bone destruction.

### Statistical Analysis

Version 16.0 of the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, Illinois) was used to compile and analyze the data. Frequencies and descriptive statistics were calculated. The Kaplan–Meier test was utilized to evaluate the statistical difference in the results between the two groups. A statistical significance threshold of $p < 0.05$ was applied.

## Results

The Consolidated Standards of Reporting Trials (CONSORT) guidelines were followed throughout the study (Fig. 3). The study was initiated by stating the null hypothesis that there is no difference in the clinical and radiographic outcomes between MTA pulpotomy and HAM pulpotomy in primary molar teeth.

The 20 participants selected for the study were randomly allocated into the two arms with 10 participants each using the lottery method. Among the 20 participants, 7 were male and 13 were female. In group I included in the study, 6 were maxillary molars and 4 were mandibular molars, whereas in group II, there was only one maxillary molar and the remaining 9 were mandibular molars.

Interexaminer reliability for both clinical and radiological examinations was tested, yielding a Cronbach's α score of 0.75 and 0.86, indicating good agreement. There was one loss to follow-up in the first month in group II, the HAM group.

When the participants were examined clinically at all the follow-ups, none of the participants showed any signs of pain, swelling, abscess/fistula, or pathologic tooth mobility in both the mineral trioxide and amniotic membrane groups, indicating 100% success rates at 1, 3, 6, and 12 months.

When radiological findings were analyzed, although both groups did not show any signs of external resorption, periapical bone destruction, or internal resorption, periodontal ligament widening and interradicular bone destruction were seen in both groups at 1, 3, 6, and 12 months.

Periodontal ligament widening was observed in 30% of the participants in the MTA group compared to 22.2% in the HAM group during the 1st month. In the next follow-up at 3 months, the percentage of affected participants remained at 30% in

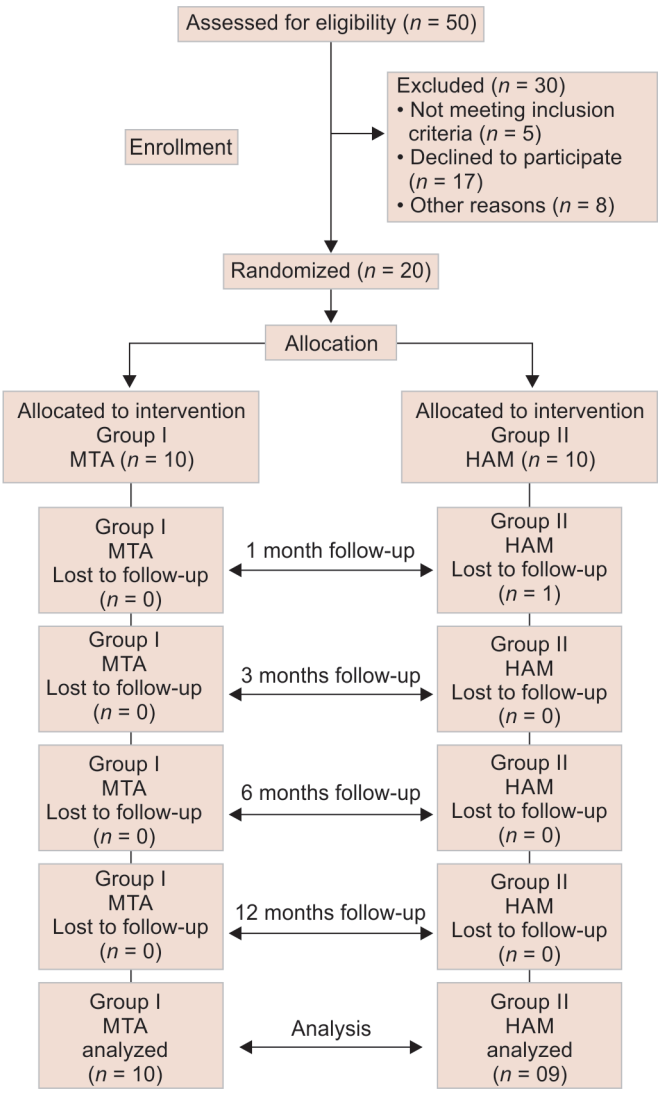



**Fig. 3:** Consolidated Standards of Reporting Trials flow diagram

the MTA group but increased to 33.3% in the HAM group. By 6 months, periodontal ligament widening was seen in 50% of the MTA group participants but had reduced to 22.2% in the HAM group. At the 12-month follow-up, although periodontal ligament widening had decreased to 30% in the MTA group, it stood at 11.1% in the HAM group. In the MTA group, there was no reduction in the presence of periodontal ligament widening from the 1st month to the 12-month follow-up. In contrast, in the HAM group, it reduced from 22.2% at 1-month to 11.1% at the 12-month follow-up, which was found to be statistically significant ($p < 0.175$) (Table 1).

Similarly, when we compared the presence of interradicular bone loss between the two groups, we found that in the MTA group, the number of participants with interradicular bone loss remained the same at 1-month and at the 12-month follow-up. However, in the HAM group, we found that the number of participants with interradicular bone loss had reduced from 22.2% at the 1-month follow-up to 11.1% at the 12-month follow-up. This reduction was found to be statistically significant ($p < 0.879$) (Table 2).

**Table 1:** Presence of periodontal ligament widening

| *Follow-up* | *Group I (MTA) (n = 10)* | *Group II (HAM) (n = 9)* |
|---|---|---|
| 1 month | 3 (30%) | 2 (22.2%) |
| 3 months | 3 (30%) | 3 (33.3%) |
| 6 months | 5 (50%) | 2 (22.2%) |
| 12 months | 3 (30%) | 1 (11.1%) |

Degree of freedom, 1; *p*-value = 0.175; $\chi^2$ value, 37.75

**Table 2:** Presence of interradicular bone destruction

| *Follow-up* | *Group I (MTA) (n = 10)* | *Group II (HAM) (n = 9)* |
|---|---|---|
| 1 month | 1 (10%) | 2 (22.2%) |
| 3 months | 2 (20%) | 2 (22.2%) |
| 6 months | 2 (20%) | 0 |
| 12 months | 1 (10%) | 1 (11.1%) |

Degree of freedom, 1; *p*-value < 0.872; $\chi^2$ value, 0.64

## Discussion

To preserve pulp vitality and encourage healing, several substitutes have been proposed.[5,20,21] One such substance that has demonstrated significant potential for healing is MTA. Initially suggested for use as a root-end filling material, MTA has since been employed for root canal fillings, pulp capping, pulpotomy, apexogenesis, apical barrier development in teeth with open apices, and root perforation repair. The hard tissue conductive, hard tissue inductive, and biocompatible properties of MTA have also been extensively studied.[22,23]

It was demonstrated by Adachi et al.[24] that periodontal ligament cells had differentiated, multiplied, and adhered to the amniotic membrane when grown on it. Due to its demonstrated capacity for multipotent differentiation, amniotic membranes can serve as a source of cells for cell transplantation therapy. This characteristic of amniotic membranes can also be advantageously applied to pulpal regeneration, making them extremely valuable in the field of dentistry.[17,25–27]

These characteristics led us to explore whether HAM could serve as a viable alternative to routinely used pulpotomy agents. Dried HAM was used in the study because it is not only very convenient to transport but can also be stored at room temperature while maintaining its regenerative properties.[28] It is available in the form of sheets and is prepared for use by soaking in normal saline for one minute.[15]

The present study showed that both MTA and HAM did not cause any pain, swelling, abscess/fistula, or pathologic tooth mobility in either group, indicating 100% clinical success rates. This finding was corroborated by the study conducted by Prasad et al.[15] When the radiological findings were analyzed, we found that there were no signs of external resorption, periapical bone destruction, or internal resorption in both the MTA and HAM groups at all four time intervals in this study. These results are consistent with the findings of Goyal et al.[29] The results of this investigation, however, differed with those of Prasad et al.,[15] who reported internal resorption in the amniotic membrane group at a 9-month follow-up. Furcal radiolucency, pathological nonperforated internal root resorption, perforated internal root resorption, and external root resorption were also noted by Biedma et al.[16] during a 48-month follow-up period.

When other radiological findings were analyzed in the present study, periodontal ligament widening was observed in 30% of the participants at 1-month and at the 12-month follow-up in the MTA group. In contrast, in the HAM group, periodontal ligament widening was found to significantly decrease from 22.2% at 1-month to 11.1% at the 12-month follow-up. In the study by Prasad et al.,[15] none of the teeth in the HAM group showed periodontal ligament widening.

Since we found a significant difference in the clinical and radiographic outcomes between the two materials, MTA and HAM, used for pulpotomy in primary molar teeth, we reject the null hypothesis and accept the alternative hypothesis.

The amniotic membrane is not only easily available, cost-effective, and easy to handle but also shows favorable outcomes. This strongly advocates for the use of HAM as an alternative to MTA for pulpotomy in primary molar teeth.

## Conclusion

The HAM is not only easily available, cost-effective, and easy to handle but also shows favorable outcomes in the present study, strongly advocating for the use of HAM as an alternative to MTA for pulpotomy in primary molar teeth.

### Why This Paper is Important to Pediatric Dentists?

- Clinical and radiographic evaluation of HAM has yielded a positive outcome compared to MTA.
- The human amniotic membrane is easy to store and work with and is also less expensive than MTA.
- Thus, this study supports using HAM instead of MTA for pulpotomy in primary molar teeth.

### Compliance with Ethical Standards

**Ethical approval:** Institutional Ethics Committee approval was obtained before the commencement of the study (Protocol Ref No. 16101).

**Informed consent:** Obtained from every participant.

## Author Contributions

Conceived the idea: EJ, AR, KYM.

Collection of data: EJ, KYM.

Data analysis: RS, SBS, ASR.

Manuscript writing: EJ, AR, ASR, SBS.

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