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ORIGINAL ARTICLE

A Bacteriological study on mineral trioxide aggregate and calcium hydroxide as materials against *Streptococcus mutans*

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Mineral trioxide aggregate (MTA) and Calcium hydroxide ($CaOH)_2$, indirect pulp-capping, Streptococcus mutans

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ABSTRACT

Background: *Streptococcus mutans play an important role in occurrence of dental caries* ***objective:*** *to compare the clinical and microbiological antibacterial outcomes of Mineral trioxide aggregate (MTA) and Calcium hydroxide cement $Ca(OH)_2$.* ***Methodology****: 20 primary molars in 10 children with year's 5-9 y .For each child one tooth was treated with $Ca(OH)_2$ and the other with MTA. Finally, all the cavities were restored using compomer restorative material, and then microbiological parameters were recorded.* ***Results:*** *MTA treated teeth did not show any clinical sign or symptoms of failure. While three teeth treated with $Ca(OH)_2$ were excluded because of necrosis. Changes in color and consistency of dentin were nearly the same for both groups. Microbiological evaluation showed a decrease in the count of Str. mutans with calcium hydroxide and complete killing of bacteria with MTA.* ***Conclusion****: The treatment showed satisfactory results of MTA as it was more potent inhibitor of bacterial-growth than $Ca(OH)_2$.*

INTRODUCTION

Maintaining the deciduous teeth in the dental arch till it exfoliates is pivotal in maintaining the integrity of the arch and establishing occlusion and function of the permanent dentition. The main reasons for premature loss of primary teeth are dental trauma or dental caries. [1]

We must maintain the vitality of the pulp as it gives good treatment outcomes[2]; indirect pulp treatment (IPT) is very important method [3] and removal of the outer layer of dental caries is the base of IPT. [4]

Calcium hydroxide was considered as the best material for indirect pulp capping for over 200 years. It served as the gold standard for the newly introduced pulp capping materials [5]. It can be used as a pulp capping agent because it allows the formation of repairative dentin through cellular differentiation, extracellular matrix formation, and subsequent mineralization.[6,7]

Furthermore, it protects the pulp against thermo-electrical stimuli as well as antimicrobial action [8] However, long-term failure rates of this material increases with time as it does not provide close adaptation to dentin. Gradual degradation, does not promote consistent odontoblast differentiation and has been shown to be cytotoxic in cell culture; tunnel defect in newly form dentin bridges may provide a pathway for the penetration of microorganisms to activate circulating immune cells, induce pulpal irritation and produce subsequent dystrophic calcification. [9,10,11]

So introduction of newer materials with bioactive properties such as MTA helped surpass the demerits of Ca $(OH)_2$ such as non-adherence to dentin, dissolution over time, and tunnel defects and ensured increased success rate for IPT procedure.[3,5]

As an alternative to Ca $(OH)_2$, MTA is composed of a mixture of tricalcium silicate, dicalcium silicate, tricalcium aluminate, and calcium oxide with an addition of bismuth oxide in a 4:1 ratio [12, 13] . This agent has many advantages as its sealing ability, alkaline pH which acts as a strong inhibitor of bacterial growth, and slow release of calcium ions [14]. It also induces pulp cell proliferation, cytokine release, and subsequent hard tissue formation with the synthesis of a mineralized dentin similar to that of biological hydroxyapatite.[15]

Dental caries is defined as an infectious, transmissible diseases which is caused by interactions between acidogenic bacteria, substrate and environmental factors along with the host's individual characteristics. [16]

Focusing on the bacterial factor of dental caries, *Streptococcus mutans* (*Streptococcus mutans* and *Streptococcus sorbrinus*) is considered an important cause of human dental caries. [17]

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There is a strong correlation between the amount of the oral cavity *Streptococcus mutans* and the incidence and progress of the dental caries. [18] Several selective culture media such as *Mitis salivarius* agar have been developed for the isolation and enumeration of the two species of *Streptococcus mutans* in saliva and plaque Bacitracin and high concentrations of sucrose are common ingredients in these growth media, which make it easy to identify *streptococci mutans*. Bacitracin has a selective important role against the oral *streptococci* except *mutans streptococci*. [19]

As the key to pulp survival after capping is a well-sealed restoration, our study aim is Clinical and bacterial evaluation of MTA and $(CaOH)_2$ as material against *streptococci mutans*. [20]

METHODOLOGY

Our study was carried out clinically at Pediatric Dentistry Department, Faculty of Dentistry, Tanta University. 10 children (2 boys and 8 girls) with mean age 5-9 years, each child has two primary molars with a deep caries lesion. The total 20 primary molars were divided into two groups, each composed of 10 primary molars. Group I: in which MTA was used for IPT and group II: in which Ca $(OH)_2$ was used for IPT. Approval for this research was obtained from Ethics Committee of Faculty of Dentistry, Tanta University.

The selection of the children was based on the following inclusions criteria: Absence of spontaneous pain and/or sensitivity to pressure; absence of fistula, edema, and/or abnormal mobility. Absence of radiolucencies at the interradicular and/or peri- apical regions, absence of internal or external root resorption that was not compatible with the expected resorption due to the exfoliation process. Any tooth presented with clinical or radiographic signs or symptoms of irreversible pulp pathology or necrosis was either pulpectomized or extracted and recorded as treatment failure.

The 20 teeth were divided into two groups. Indirect pulp capping was done by MTA and Ca $(OH)_2$, respectively. Each child received regional anesthesia and the arch of the treated tooth was isolated with rubber dam. Access to the dentin carious lesion was done with a high speed diamond bur with sufficient cooling arrangement. Removal of carious tissue in the lateral walls was done with low speed round burs and spoon excavators. Necrotic tissue with soft and fragmented appearance located at the pulpal wall was removed with spoon excavators, with no rotary instruments. At this moment, the cavity was washed. With sharp excavator the dentin piece was transported to Microbiology laboratory.

In group I: MTA (Proroot, Dentsply, Tulsa Dental, USA) powder was mixed with sterile water in a 3:1 ratio, according to the manufacturer recommendation and placed on the operative site with amalgam carrier up to 2-3 millimeter thickness and applied by light pressure with moist cotton pellets.

In group II: Ca $(OH)_2$ (Hydro C Dentsplay) was mixed equal quantities of both the catalyst and the base paste to a homogenous paste and applied to the sites with ball ended condense into the base of the prepared cavity up to 0.5 to 1 mm thickness. All the cavities in both groups were restored using compomer restorative material (Composan Cream Promedica Dermany).

Clinical follow up: was carried out at baseline, 1 and 3 months. For clinical evaluation, remaining carious dentin was divided into [21]:

1. Color: Dark brown; Light brown; and Yellow.
2. Hardness: Hard, Leathery, and Soft

Bacteriological evaluation

***Samples processing*:** were conducted at Microbiology Department, Faculty of Medicine, Tanta University. In which sterile tubes each contains 2 ml of reduced transport fluid medium (0.4% agar, 0.15% thioglycollate/phosphate buffered saline) were used to transfer dental plaques samples from all children included in the study for further processing where the tubes were centrifuged at 2500 rpm for 10 minutes for homogenization of samples.

***Streptococcus mutans isolation from children premolars*:** After homogenization, a volume of 0.1 mL was spread onto *Mitis-salivarius* (MS) agar (supplemented with 200 µg bacitracin and 15% sucrose per liter). The plates were then incubated at 37 °C under anaerobic condition for 48 hours. After incubation, plates were checked for bacterial growth and colony count was done. Colonies of *Streptococcus mutans* were identified by routine bacteriological methods. The growth ability of the isolates in 4% NaCl was tested by incubating the organism in trypticase soya broth anaerobically at 37˚ C for 48 hrs. After this, the isolates were sub-cultured on MS agar to obtain pure colonies for further identification by API 20 *Strept*. This was followed by in-vitro testing of the antibacterial effect of different concentrations of MTA and calcium hydroxide against *Streptococcus mutans*. (Fig. 1).

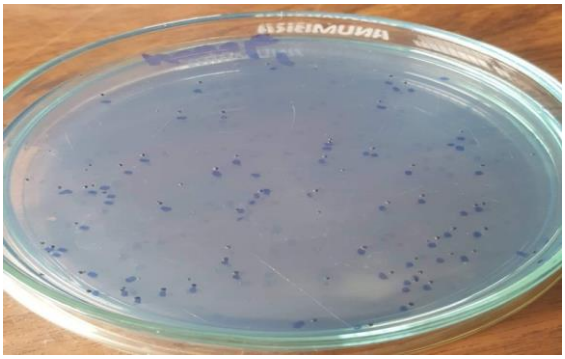

Fig. 1: Growth of *Streptococcus mutans* on *Mitis-salivarius* (MS) agar

Determination of antibacterial of effect of MTA and $Ca(OH)_2$ against Streptococcus mutans in-vitro:

Antibacterial activities of the selected materials were evaluated against *Streptococcus mutans* using both agar diffusion and broth dilution method.

Agar diffusion method[22] : the isolated *Streptococcus mutans* was inoculated in trypticase soy browth to produce a turbidity of 0.5 on the McFarland scale, which corresponds to 10^8 Colony Forming Unit(cfu). *Streptococcus mutans* was inoculated on blood agar plates. Four uniform wells (4 mm diameter, one for each tested material and two as a control) were punched at equidistant points in agar by means of a sterile copper coil. The cavities were filled with freshly prepared tested materials. While, the positive control was filled with doxycycline mixed with liquid agar to concentration of 1 mg/ml, and the negative control was filled with sterile water. The plates were kept at room temperature for 2 h for pre-diffusion of the tested materials and then incubated at 37˚C under anaerobic condition for 48 hours. A total number of 3 plates were employed to test the inhibitory activity of three concentrations of MTA, in which MTA powder were mixed with sterile saline in concentrations of 60%, 50%, and 40%. Also, the antibacterial effect of the Ca(OH)2 was tested in the same plates with three different concentration of 60%, 50% and 40%. The diameter of bacterial growth inhibition zones was measured with a millimeter ruler with accuracy of 0.5 mm in two perpendicular locations for each sample by an independent observer.

Broth dilution method: The MTA was dispersed in sterile water to prepare series concentration. Each concentration was mixed with equal volume of broth which was previously inoculated with streptococcus mutans. The final concentrations of MTA were 0.5, 0.25.0.125 % W/V.

The antibacterial activity of $Ca(OH)_2$ was detected by broth dilution method. The stock solution of calcium hydroxide was prepared in sterile water which was boiled to remove gases. The initial concentration was 0.5%w/v. This was used to prepare serial dilution with inoculated broth to produce concentration of 2.5mg/ml.

The MIC of both MTA and $Ca(OH)_2$ were determined using rezasurine dye which is a metabolic sensitive indicator that undergoes color change in response to bacterial activity reflecting the viability of the bacteria. The concentrations were incubated at 37°C for 24 hours at the end of which, 250 ml of rezasurine solution (0.1% W/V) was added and the mixture was incubated for 2 hours before determination of the MIC visually.

Bacteriological follow up: Bacteriological follow up of IPT with MTA and $Ca(OH)_2$ to detect their antibacterial effect was done by taking a sample from dentin by excavator from the cavity after one and three months of IPT. The samples of all the children were identified by a code number during the period of sample collection and processing. The same code number was used for the particular child during subsequent sample collection after three months. The sample was transported through sterile tubes each contain 2 ml of reduced transport fluid medium mentioned above, to the Microbiology laboratory immediately after collection and processed on the same day, where the tubes were centrifuged at 2500 rpm for 10 minutes for homogenization of samples. 0.1 mL was spread onto (MS) agar; the inoculated plates were left to dry. All inoculated plates were incubated for 48 h at 37 °C under anaerobic conditions. After incubation, colony characteristics were studied and the viable count was determined by calculating the number of colonies (cfu/ml).

Statistical analysis: was performed using the Statistical Package for Social Science (SPSS) version 8.0 software. A one-way ANOVA was used for the mean zones of growth inhibition among the materials tested. The Post Hoc test was run for multiple comparisons. Statistically significant differences among the groups were set at $p<0.05$.

RESULTS

Clinical evaluation:

Clinically, no teeth of group I (0%) treated with MTA showed any clinical sign or symptoms of failure. While in group II treated with calcium hydroxide three teeth (30%) were excluded because of necrosis. Most of the color of dentin in both groups was light brown at the beginning of the study which transfers to dark brown at the end of the study with no statistical difference between the two groups as shown in table 1. Similarly, table 2 shows the consistency of dentin which revealed the changing from soft to hard dentin at the end of the study with no statistical significance. The consistency of dentin revealed the changing from soft to hard dentin at the end of the study with no statistical difference between the two groups as shown in table 2.

Table 1: the dentine color at the beginning and the end of the study of both groups

MTA	**Baseline**		**Final**		**X^2**	**P-value**
	N	**%**	**N**	**%**		
Yellow	0	0	0	0	**7.203**	**0.007***
Light brown	8	80	2	20		
Dark	2	20	8	80		
Total	10	100	10	100		
Ca(OH)2	**Baseline**		**Final**		**13.602**	**0.001***
	N	%	N	%		
Yellow	2	20	0	0		
Light brown	8	80	2	20		
Dark	0	0	8	80		
Total	10	100	10	100		

Table 2: Dentin consistency in MTA and $Ca(OH)_2$ at baseline and final examination

MTA	**Baseline**		**Final**		**X^2**	**P-value**
	N	**%**	**N**	**%**		
Soft	9	90	0	0	**17.331**	**0.001***
Hard	0	0	8	80		
Leathery	1	10	2	20		
Total	10	100	10	100		
Ca(OH)2	**Baseline**		**Final**		16.002	0.001*
	N	%	N	%		
Soft	8	80	0	0		
Hard	0	0	8	80		
Leathery	2	20	2	20		
Total	10	100	10	100		

Bacteriological evaluation: The antibacterial activity of MTA and Ca $(OH)_2$ in three different concentrations (60%, 50%, and 40%) against *Streptococcus mutans* was shown in table 3, with increasing the concentration of the tested material, the antibacterial activity was increased as evidenced by change of diameter of the inhibition zones. The inhibition zone of the 60% concentration was 5.30 and 5.11 for MTA and calcium hydroxide respectively, while it was 4.30 and 3.28 in the 40% concentration for MTA and calcium hydroxide respectively.

Table 3: MTA and $Ca(OH)_2$ antibacterial effect in three different concentrations against *Streptococcus mutans* by agar diffusion method.

Tested dental material	**Inhibition zone of 40% concentration**	**Inhibition zone of 50% concentration**	**Inhibition zone of 60% concentration**
MTA	4.30 cm	4.90 cm	5.30 cm
Ca(OH)2	3.28 cm	4.37 cm	5.11 cm

Also, the MIC determination reflected the antibacterial activity of MTA with the recorded MIC being 0.25%. This indicates that MTA can provide antibacterial effect but at relatively high concentration.

The antibacterial activity of MTA in the in-vitro test was better than that of Ca $(OH)_2$. Moreover, after IPT using MTA and $Ca(OH)_2$, the antibacterial effect of MTA was superior to that of $Ca(OH)_2$ after one and three months of IPT as shown in table 4.

Table 4: Bacterial colony count (cfu/ml) at base line, after one month and three months of using MTA and $Ca(OH)_2$.

		Baseline	After 1 m.	After 3 m.	Baseline & 1 m.	Baseline & 3 m.	1 m. & 3 m.
MTA	Range	7–18	0–5	0–0	0.001*	0.001*	0.004*
	Mean±SD	12.7±3.4	1.7±1.6	0±0			
$Ca(OH)_2$	Range	7–18	3–9	0–5	0.001*	0.001*	0.001*
	Mean±SD	12.7±3.4	6.2±1.9	2.5±1.8			
T. test		-	31.586	17.737			
p. value		-	0.001*	0.001*			

DISCUSSION

$Ca(OH)_2$ can be used for killing bacteria present in carious dentin left after partial caries removal[23], IPT was a better technique than pulpotomy .[24, 25]

Careful diagnosis of the pulpal status is essential for the success of any conservative pulp treatment. [26]

In the present study there were changes in the color and consistency of dentin with no significant difference between calcium hydroxide and MTA group indicating the arrest of the carious process which agrees with the study of pinto et al. [23]

This is duo to the fact that both materials release calcium ions in sufficient quantities to promote reparative dentin formation.[27] This result is in agreement with other studies[27,28]. This may be attributed to antibacterial effect of $Ca(OH)_2$ that can minimize or eliminate bacterial penetration to the pulp. The high pH of $Ca(OH)_2$ producing irritation of tissue of the pulp, which stimulates repair duo to the release of bioactive molecules. It is known that a variety of proteins are incorporated into the dentin matrix during dentinogenesis. Bone Morphogenic Protein and Transforming Growth Factor-Beta One, have demonstrated the ability to stimulate pulp repair.

In group II of patients treated with $Ca(OH)_2$ three teeth (30%) of cases showed necrosis, this in agreement with [29] . According to the microbiology point of view in this study, the MTA used in IPT has a superior antibacterial activity over Ca $(OH)_2$ against *Streptococcus mutans* which is the main cause of dental caries. In Group I (MTA) reveals marked decrease in bacterial count after one month of IPT while no bacterial growth after 3 months of IPT. On the other hand group II $Ca(OH)_2$ reveals a decreased bacterial colony count after one month and marked decrease in the count after 3 months which means that MTA had a better antibacterial effect than $Ca(OH)_2$ especially on the long run, these results were supported by other studies which also consider MTA is one of the best used dental material[33],[34] and other studies mentioned that MTA has two major disadvantages: The setting time requires (2.75h) so it is considered a long time setting and also the moisture is needed during the setting. [32]The effect of MTA had been evaluated by many studies with different results[33] suggesting that the difference between studies might be due to different methods used for testing MTA [29]

Another study[30] reported that MTA has no antibacterial effect against any of the strict anaerobic bacteria. In contrast the present study showed that MTA has antibacterial effect in anaerobic condition against *Streptococcus mutans*, this can be due to the highly alkaline pH of MTA which leads to antibacterial activity even in anaerobic condition [31]

The antibacterial effect of two studied agents was also tested in three different concentrations in-vitro by using the agar diffusion method; MTA has a better antibacterial effect than calcium hydroxide against *Streptococcus mutans* in the three tested concentrations. This results was supported by the results of other studies[32] which tested the antibacterial effect of MTA and $Ca(OH)_2$ against *Streptococcus mutans* and found that MTA and bioceramics have the best antibacterial effect against *Streptococcus mutans*[33] Other studies showed fluctuating achievement rates, which is related to the cytotoxic impact and the persistence of an inflammatory process [34]

CONCLUSION

The treatment showed satisfactory results of MTA as it was more potent inhibitors of bacterial-growth than $Ca(OH)_2$, suggesting that this minimally invasive approach for carious lesion removal can replace the total removal, providing the tooth is asymptomatic and well-sealed, even if residual caries remains.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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