A Randomized Trial Using 3Mixtatin Compared to MTA in Primary Molars with Inflammatory Root Resorption: A Novel Endodontic Biomaterial

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Objectives: *Novel methods for preserving primary teeth can help to maintain their developmental, esthetic, and functional capabilities. The aim of this study was to assess the success of the repair of bony defects, caused by pre-treatment perforations, with a mixture of three antibiotics combined with simvastatin (3Mixtatin) compared to MTA in hopeless primary molars.* ***Study design****: In this randomized clinical trial, 80 teeth from 65 healthy children aged 3–6 years with interradicular or periapical root resorption and/or perforation in primary molars were treated either with 3Mixtatin or MTA before conventional pulpectomy and restoration. The subjects were followed up clinically and radiographically for 4, 6, 12 and 24 months after pulp treatment to evaluate and compare the healing process. The data were compared using chi-square test at a significance level of 0.05.* ***Results****: By the end of 24 months in 3Mixtatin group, 31 (96.8%) teeth revealed no clinical signs or symptoms with arrested resorption progress in radiographs. In MTA group, clinical signs and symptoms including pain, mobility and sinus tract were observed in 18 (48.6%) teeth with cessation of root/ interradicular radiolucency in 7 (18.9%) teeth without bone repair.* ***Conclusions:*** *Radiographic and clinical healing occurred more successfully following 3Mixtatin treatment compared to treatment with MTA, it may lead to a paradigm shift in the pulpal treatment of primary teeth in the future.*

Key words: 3Mix, Primary teeth, Regenerative treatment, Root resorption

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INTRODUCTION

Primary teeth are significantly different from permanent teeth with regards to the cellular content of undifferentiated mesenchymal stem cells. They contain a rich supply of stem cells in their dental pulp compared to the permanent teeth [1]. Mesenchymal cells may give rise to odontoclastic cells in response to either the caries process or the pulp-capping material, resulting in the exaggerated inflammatory response and consequently internal resorption in primary teeth [2]. Pathologic root resorption is the most common cause of premature tooth loss in primary dentition, with long-term harmful effects such as space problems in the dental arch, problems in the eruption of the successor tooth and alterations in tongue posture [3].

Modern pediatric dentistry seeks novel methods for regeneration of remaining dental tissues in order to preserve primary teeth and maintain their developmental, esthetic, and functional capabilities. For this purpose, the biocompatible materials such as bone morphogenetic proteins (BMPs) [4], osteogenic protein–1 (OP-1) [5], demineralized dentin [6], and mineral trioxide aggregate (MTA) [7,8] have been studied previously. Statin components are emerging materials in regenerative processes. Local application of simvastatin gel can stimulate the regeneration of alveolar bone defects [9]. Statins might also improve the function of odontoblasts, thus dentin formation [10]. In addition, statins have an anti-inflammatory effect by decreasing the production of interleukin-6 and interleukin-8 [11].

Bacterial microleakage as well as the remaining bacteria in the root canal system may cause recurrent symptoms after previous endodontic treatment [12]. Therefore, the use of antibiotics with the primary objective of eliminating causative bacteria is quite reasonable. A combination of metronidazole, minocycline and ciprofloxacin (3Mix) has been used as an endodontic material under the concept of lesion sterilization and tissue repair (LSTR) therapy. LSTR using 3Mix has been shown to provide an excellent outcome in treatment of infected canals in primary teeth with periradicular lesions and those with physiological root resorption [13,14]. An *in vivo* study has shown that 3Mix penetrates previous root canal obturation and disinfects lesions [13]. It has been also demonstrated that periradicular radiolucent lesions disappeared or reduced, thus the lesions repaired [14].

This previously unstudied combination was applied with the aims of suppressing bacteria, preventing pulp inflammation, and inducing hard tissue formation, all leading to preservation of the primary teeth that are otherwise indicated for extraction according to current guidelines [15]. Therefore, the purpose of this ZOE pulpectomy study was to assess success of pre-treating perforations in hopeless primary molars with a mixture of three antibiotics combined with simvastatin compared to MTA on the repair of bony defects resulting from pulpal infections. We propose the term "3Mixtatin", an acronym of 3Mix and simvastatin, for the new combination. We expected to detect significantly superior main outcomes in 3Mixtatin treated teeth compared to that of MTA at 24-month follow-up.

MATERIALS AND METHOD

This randomized clinical trial was performed at the Department of Pediatric Dentistry, Tabriz University of Medical Sciences, between April 2012 and April 2013. During a one-month period, 475 children were screened during the routine examination of children to match the inclusion criteria:

- 3-6 year old children with complete physical health without any confounding medical history including no history of allergic reactions to local or systemic drugs.
- Presence of at least one restorable primary molar with adequate bone support and no pathology of the succedaneous permanent tooth follicle, indicated for extraction due to interradicular or periapical root resorption and/or perforation in the coronal third of roots as a result of infective or inflammatory conditions, based on radiographic examination.
- Child's parents willing to participate in the study.

As such teeth are indicated for extraction, the study procedure and its alternatives as well as probable risks and benefits of the pulpectomy treatment were explained to the parents and written informed consents were taken. The study design which was in accordance with the Helsinki Declaration of Human Rights was submitted to and approved by the Committee for Ethics in Research on Humans at Tabriz University of Medical Sciences (Trial Number: IRCT2013071714031N1).

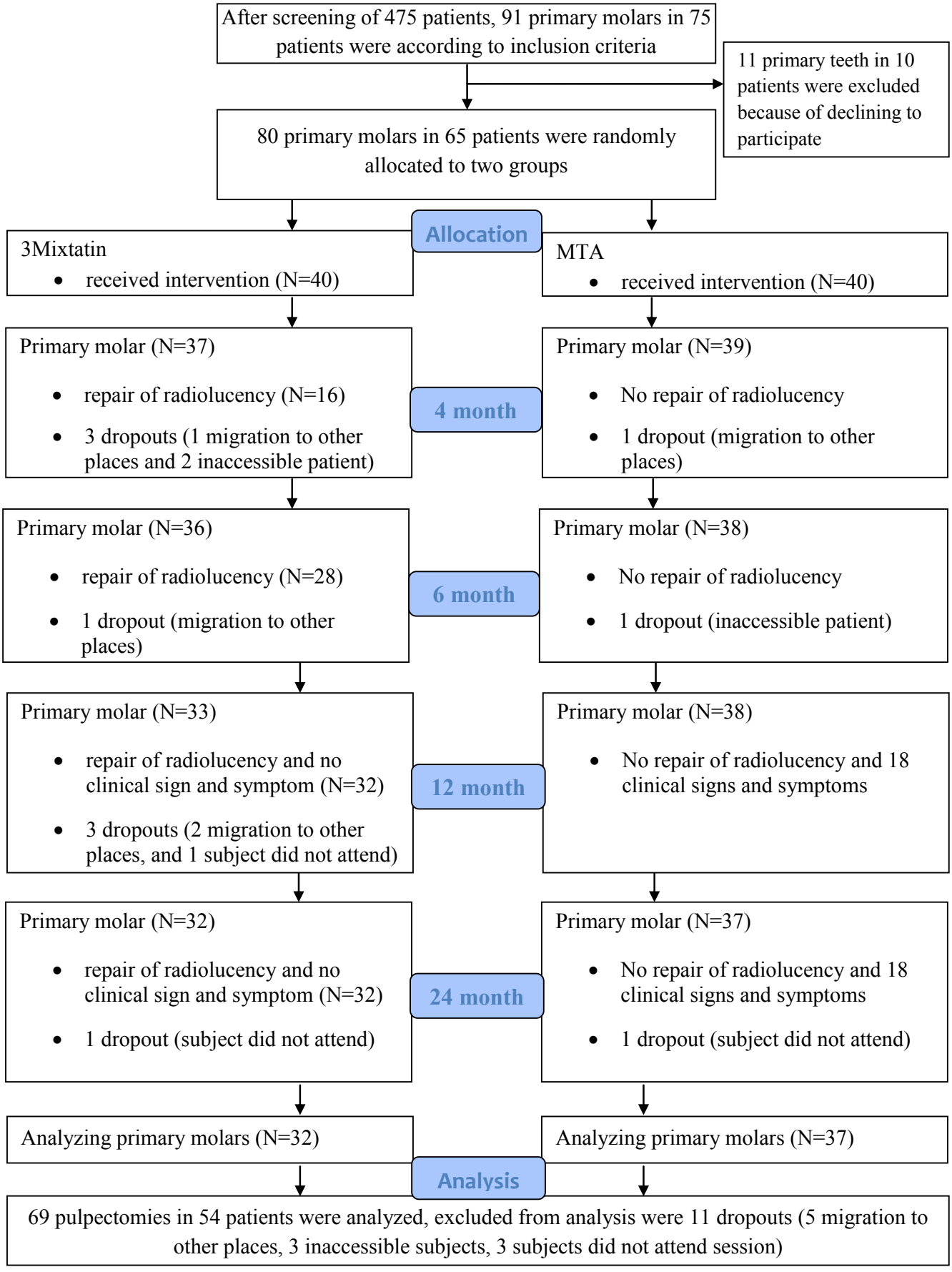
According to the pilot study which was conducted by a post-graduate student on 14 teeth equally distributed in two groups and followed up for three month, the success rates for sinus tract healing as the primary outcome were 58% with 3Mixtatin and 28% with MTA. The primary outcome of the pilot study was only used to calculate the sample size and pilot cases were not included in the study, in which all procedures and follow-ups were performed by an experienced pediatric dentist. Considering $\alpha = 0.05$ and power = 80%, the 15% outcome difference lead to a required sample size of 32 for each group, which was increased to 40 to improve the validity of the study and compensate for probable lost to follow-up. Other variables including pain, mobility, and radiographic repair were considered as secondary outcomes.

Clinical failure parameters were presence of sinus tract, provoked or spontaneous pain, and pathologic tooth mobility. Radiographic failure parameter was expanding periapical or furcation radiolucency. Clinical and radiographic re-examination was independently performed at 12 and 24-month follow-up by two experienced dentists (not the operator) blinded to the technique. In case of disagreement, the examination of a third examiner was recorded as the treatment outcome. Tooth mobility and root/furcation radiolucency with or without the succedaneous permanent tooth follicle involvement were considered as corresponding clinical and radiographic criteria to assess the inter-examiner reliability using Kappa agreement coefficient. Teeth were screened for 4 and 6 month intermediary follow-ups. The flow of participants and pulpectomies were followed from allocation to the final data analysis after 24 months (Fig. 1).

Finally, 80 teeth in 65 children were randomly divided into two groups. Random allocation list was generated using randomization software (RandList version 1.2; DatIng GmbH, Tübingen, Deutschland; seed number: 1,901,365,632). In 15 children with two teeth enrolled in the study, teeth were randomly assigned to one of the treatment groups using allocation blocks defined in the software. The operator was not blinded to the treatment because of different manipulation techniques implemented for the studied groups. All other contributors to the study were blinded to generation and implementation of the treatment assignment.

MTA (ProRoot, Dentsply/Tulsa Dental, Tulsa, OK, USA) and 3Mixtatin were used in two intervention arms. MTA is widely used in permanent teeth to seal perforations because of its biocompatibility and sealability[16,17]. Further, microscopic examinations of periodontal tissues after perforations in the furcal area and subsequent sealing with MTA has demonstrated repair of the periodontium, and new cementum formation(cementogenesis) over the material[17,18]. 3Mixtatin was prepared by mixing three commercially available antibiotics with simvastatin powder. After removal of the capsules or coating materials that enclosed the drug products, they were pulverized to fine powders using porcelain mortars and pestles [13,19]. 100 mg ciprofloxacin (Ruzdarou, Tehran, Iran), 100 mg metronidazole (Tehranshimi, Tehran, Iran), and 100 mg cefixime (Farabi, Tehran, Iran) were mixed in a ratio of 1:1:1 [13,19]. Minocycline was replaced by cefixime because of its contraindication in children. Pure simvastatin powder was provided by the Faculty of Pharmacy. The measurements were done by an analytical balance with 0.1 mg accuracy. The 3Mixtatin preparation process was trained and supervised by a consultant pharmacist. 2 mg of simvastatin were added to the powdered drug mix, which was then stored in a tightly capped porcelain container with a small amount of silica gel in a bag to maintain low humidity. The powder and normal saline were mixed to form a paste of 3Mixtatin upon its clinical application.

Figure 1. Flow of participants and pulpectomized teeth.



One pediatric specialist with twelve years of experience (the operator) performed all pulpectomies. In both groups, following administration of local anesthesia and standard isolation using rubber dam, caries were removed using a no. 330 bur mounted in a water-cooled high-speed handpiece. A round bur in a slow speed handpiece was used to excavate caries. The roof of the pulp chamber was removed by joining the pulp horns with the high speed bur cuts. Remaining radicular pulp was removed using Hedstrom files #15-30 (Dentsply/Maillefer, Ballaigues, Switzerland). A light flow of sterile 0.9% normal saline solution was delivered by a syringe and needle to wash away remaining tissues. The resoption area was cleaned with 2% chlorhexidine followed by another sterile saline rinse. Thereafter, 1% NaOCl was applied using cotton pellet and after achieving homeostasis the pulp chamber and canals were rinsed with normal saline and dried using paper cones. If bleeding could not be controlled, a dry cotton pellet was placed in the pulp chamber and tooth was temporized with a temporary filling material (Cavit, 3MESPE DentalAG, Seefeld/Oberbay, Germany). The treatment was continued in a second appointment.

3Mixtatin paste was delivered to the perforation or resorption site using small endodontic amalgam carrier and plugger instrument. MTA paste (MTA powder mixed with normal saline) was delivered to the perforation site or resorption region using an MTA carrier (Sybro Endo, Orange, CA, USA), and packed with a cotton pellet moistened with sterile distilled water. A layer of glass-ionomer cement (Fuji IX, GC, Tokyo, Japan) was applied to seal 3Mixtatin or MTA taking care not to compromise the isolation, in order to prevent 3Mixtatin or MTA contamination. Conforming to the standard technique for pulpectomy, a thick mix of zinc oxided-eugenol (ZOE) was condensed into other areas including the canals using a root canal plugger and then the pulp chamber using a condenser to reach an at least 2 mm thickness [20]. Teeth were subsequently restored with a stainless steel crown (Unitek SS Crown-3M Co, Monrovia, USA), restorative glass ionomer (Dentsply, Weybridge, UK), glass-ionomer reinforced amalgam (Permite; SDI Limited, Bayswater, Australia) or composite resin (Filtek Z250; 3M-ESPE GmbH, Neuss, Germany) restoration according to standard indication. A periapical radiograph was taken immediately after treatment.

Data were described as numbers (%). The main statistical assessment addressing the research question was chi-square test or Fisher's Exact test to compare qualitative data. Data were analyzed using SPSS software (version 16). $P < 0.05$ was considered statistically significant.

RESULTS

Prior to treatment, pathologic-clinical and radiographical findings in 3Mixtatin group were recorded as pain, mobility and sinus tract in 31 (93.9 %), 19 (57.5%) and 19 (57.5%) teeth respectively. Root resorption and perforation were detected radiographically in 19 teeth (57.5%) and 21 teeth (63.6%) respectively. In MTA group, findings were recorded again as pain, mobility and sinus tract in 29 (76.3%), 27 (71.0%) and 27 (71.0%) teeth respectively. Radiographically, 17 teeth (44.7%) had root resorption and 23 teeth (60.5%) showed perforations. The differences in baseline characteristics were not statistically significant ($P > 0.05$).

71 teeth (Table 1) in 56 subjects (23 males, 33 females; mean age = 5.36) were re-evaluated at 12-month follow-up. The agreement between the examiners at baseline and 12- month follow-up were excellent (Baseline Kappa=0.91, $P < 0.001$ and 12-month follow-up Kappa = 0.94, $P < 0.001$). There was a statistically significant difference in the clinical ($P = 0.034$) and radiographical ($P < 0.01$) characteristics between the two groups in the 12-month follow-up.

At the end of 12 months, in 32 (96.9%) teeth within 3Mixtatin group, the clinical symptoms including gingival swelling and sinus tract disappeared and the patients did not report any pain. Radiographically, resorption progress arrested and bone healing was detected in the same 32 (96.9%) teeth (Fig 2). After 12 months, one of the teeth in 3Mixtatin group remained symptomatic (Table 2). Absolute risk reduction values were 0.76, 0.34, 0.34 and 0.96 for pain, mobility, sinus tract and repair of radiolucency respectively.

After 12 months in MTA group, clinical symptoms, seen in 18 (47.3%) teeth, included pain, mobility and sinus tract in 9 (23.6%), 13 (34.2%) and 13 (34.2%) teeth respectively. All teeth with pain and four teeth with mobility also showed sinus tract. In MTA group, bone and root/interradicular radiolucency were ceased in 8 (21.0%) teeth but none of them were repaired (Table 2) (Fig 2).

69 teeth in 54 subjects (23 males, 31 females; mean age = 5.72) were re-evaluated at 24 month follow-up (Table 1). The agreement between the examiners at baseline and 24- month follow-up was good (Baseline Kappa=0.91, $P < 0.001$ and final follow-up Kappa = 0.86, $P < 0.001$). There was also a statistically significant difference in the clinical ($P = 0.03$) and radiographical ($P = 0.01$) characteristics between the two groups in the 24-month follow-up (Table 2).

At 24-month follow-up, one tooth in the MTA group had extensive mobility because of early root resorption and was extracted. In 3Mixtatin group, although one tooth showed slight furcal rarefaction, it was not considered treatment failure (Table2) (Fig 2). Absolute risk reduction values in 3Mixtatin group for pain, mobility, sinus tract and repair of radiolucency were 0.76, 0.34, 0.34 and 0.91 respectively. In two cases, composite resin restorations (one case in each group) were replaced with stainless steel crowns due to the failure of restorations (Table 1).

In overall, considering the clinical and radiographical signs and symptoms in the MTA group during all follow-up visits, 18 (48.6%) teeth were deemed to be endodontic treatment failures. Of these, nine (24.3%) teeth were extracted due to pain and sinus tract and one tooth because of premature root resorption. In the remaining 9 teeth with only sinus tract, the parents did not consent to an extraction due to no pain. In 3Mixtatin group during the 24-month follow-up, 31 (96.8%) teeth revealed no clinical signs or symptoms with arrested resorption progress in radiographs. However, one tooth in 3Mixtatin group remained symptomatic between the two follow-ups and was deemed as failure.

DISCUSSION

Structural and molecular differences are reflected by a higher susceptibility to root resorption seen in primary teeth. Understanding of the mechanisms that protect, control and regulate root resorption may help in maintaining a primary tooth as long as it is necessary. Thus, this study aimed to preserve the primary teeth with pathologic interradicular or periapical root resorption and/or perforation by targeting the undifferentiated mesenchymal cells leading to odontoblast and osteoblast differentiation and activation. Simultaneously, action needs to be taken to reduce or

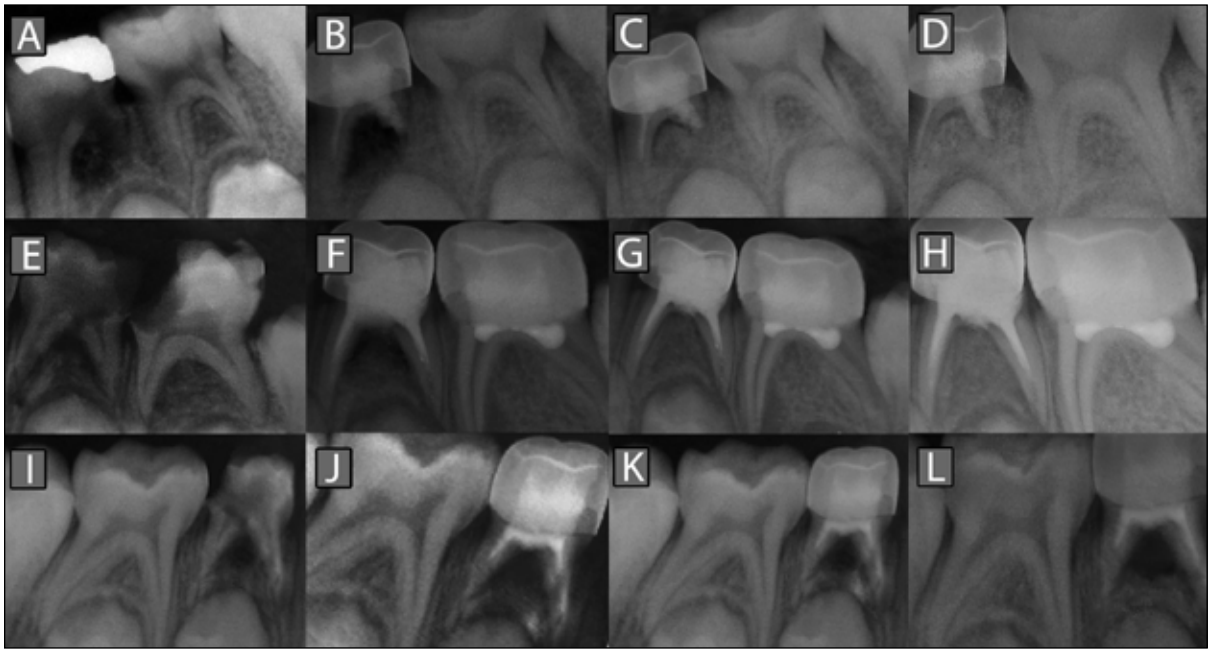
Table 1. Clinical characteristics of the study samples at 12 and 24-month follow-up

	12-month follow-up			24-month follow-up		
	Overall	**MTA**	**3Mixtatin**	**Overall**	**MTA**	**3Mixtatin**
Pulpectomy	71	38	33	69	37	32
Tooth type						
First molar	30	16	14	30	16	14
Second molar	41	22	19	39	21	18
Dental arch						
Maxilla	24	14	10	24	14	10
Mandible	47	24	23	45	23	22
Restoration method						
Stainless Steel Crown	34	18	16	36	19	17
Composite Resin Restoration	11	5	6	9	4	5
Amalgam Restoration	15	9	6	15	9	6
Glass Ionomer Restoration	11	6	5	11	6	5

Table 2. Frequency (%) of clinical and radiographical signs and symptoms in the study groups

Evaluation criteria	**Study groups**							
	Before treatment		**After 12-month**		**P value**	**After 24-month**		**P value**
	3Mixtatin (n=33)	**MTA (n=38)**	**3Mixtatin (n=33)**	**MTA (n=38)**		**3Mixtatin (n=32)**	**MTA (n=37)**	
Pain	31(93.9)	29(76.3)	0 (0)	9(23.6)	0.003	0 (0)	8(21.6)	0.006
Mobility	19(57.5)	27(71.0)	0 (0)	13(34.2)	0.005	0 (0)	13(35.1)	0.005
Sinus tract	19(57.5)	27(71.0)	0 (0)	13(34.2)	0.005	0 (0)	12(32.4)	0.005
Radiolucency	33(100)	34(89.4)						
Cessation of radiolucency progress			32(96.9)	8 (21)	<0.001	31(96.8)	7(18.9)	<0.001
Repair of radiolucency			32(96.9)	0 (0)	<0.001	31(96.8)	0(0)	<0.001

Figure 2. Pulp treatment of a primary molar (A) with 3Mixtatin, immediately after treatment (B) at 12-month (C) and 24-month follow-up (D). Another case (E) treated with 3Mixtatin, immediately after treatment (F) at 12-month (G) and 24-month follow-up (H). Pulp treatment of a primary molar (I) with MTA, immediately after treatment (J) at 12-month (K) and 24-month follow-up (L).



eliminate bacterial contamination and inflammation as the main cause of treatment failure in primary teeth. In 3Mistatin, simvastatin was used as an anti-inflammatory and bioinductive agent and 3Mix served as an antibacterial agent, in an effort to preserve hopeless primary teeth with root resorption or perforation.

The overall direction of the obtained results demonstrated an advantage of 3Mixtatin in preservation of hopeless primary teeth with pathologic root/interradicular resorption in the 24-month follow-up. Consistent with our hypothesis, a substantial number of teeth in 3Mixtatin group revealed marked healing in the root/interradicular resorption areas. This outstanding outcome could be attributed to the bioinductive effects of simvastatin in inhibition of bone resorption and osteocyte apoptosis and promotion of osteoblast proliferation and differentiation [21]. BMP is an inducer for osteoblastic differentiation from a population of undifferentiated cells [22]. Several studies have shown that statin drugs can specifically stimulate high levels of BMP-2 expression in osteoblasts [23-26] which in turn induces the transformation of mesenchymal stem cells into osteoblasts, and thereby, increases the formation of bone tissues[23,24]. It has been shown that statins promote mineralization in non-mineralizing osteoblasts through induction of BMP-2, suppression of osteoclast function and osteocalcin[27-29]. In addition, simvastatin is shown to increase cancellous bone volume, bone formation rate, and cancellous bone compressive strength [9]. Moreover, statins stimulate angiogenesis which contributes to the wound healing process [30]. Therefore, it is reasonable to assume that all of these pathways lead to the observed bone and root repair and the elimination of clinical symptoms including pain, sinus tract and mobility the primary teeth treated by 3Mixtatin.

Elimination of the pathogenic microorganisms is an integral part of pulp treatment in primary teeth. In our study, this goal was attained by using a mixture of three antibiotics. 3Mix has shown to be capable of eliminating bacteria from infected dental tissues in both permanent and primary dentitions[31]. The bactericidal efficacy of antibiotics has been shown previously in carious lesions of primary teeth, indicating the sterilizing effect of their topical application[13,14]. The results from in situ experiments suggest that mixed drugs penetrate into the lesions and sterilize them within one day [32]. Furthermore, triple antibiotic paste has been used successfully in regenerative endodontic treatment and in healing large periradicular lesions[33]. Current evidence indicates successful outcomes of 3Mix in the treatment of periradicular lesions of permanent[34] and primary teeth[21].

MTA is a more recent material used for pulpotomies and partial pulpectomy showing a superior rate of success. Currently available evidence suggests MTA compared with formocresol, ferric sulfate and calcium hydroxide as a pulpotomy medicament in primary teeth which results in significantly higher clinical and radiographic successes in all time periods up to exfoliation[16]. MTA has been shown to stimulate the propagation of human osteoblasts by offering a biologically active substrate for the cells[22], which may justify the cessation of bone resorption observed in the MTA group of this study. However, no repair was seen on the radiographic images of cases treated with MTA, while significant improvement was observed radiographically in the cases treated with 3Mixtatin. The superior results of 3Mixtatin compared to those of MTA in primary teeth are probably related to the antibacterial and sterilizing effects along with anti-inflammatory and bio-inductive properties of 3Mixtatin.

Although studies show MTA without matrix provides an effective seal of root perforations and clinical healing of the surrounding periodontal tissue[17,35], radiographic results in the MTA group of the

present study were not significantly improved in the follow-ups. Also, despite the evidence put forward by Oliveira et al.[36] for repair of iatrogenic and mechanical defects in primary molars with MTA, we were not able to replicate the repair with MTA in primary teeth with interradicular root resorption and/or perforation, most probably because the etiology of perforation and resorption was infection not iatrogenic causes. This may also indicate that MTA seal alone may not be able provide the required environment for tissue healing in these primary teeth with interradicular root resorption and/or perforation , and that an additional antibacterial effect is necessary to first eliminate the infection. In a similar line, a recent report on the treatment of three hopeless primary molars with root perforations and extensive root resorption has shown complete bone healing using calcium enriched mixture (CEM) cement[37]. This observed difference could be attributed to many possible factors including the effectiveness of antimicrobial properties and high pH (12.5) of MTA and CEM, all being the characteristic that promote growth of the cementum and formation of bone[17,37,38].

The findings of the present study may lead to a paradigm shift in the pulpal treatment of primary teeth in the future. However, such conclusive inference should be weighed against some limitations of the study such as a small sample size. Since MTA was used as an inert material in this study, further studies are warranted to compare the effect of the antibiotic mixture, 3Mixtatin and simvastatine alone, probably with a survival analysis. In addition, the results may support a conclusion that odontoblasts along with osteoblasts and possibly cementoblasts may be responsible for the observed healing, which requires further investigation on histological aspects.

CONCLUSION

The following conclusions can be made based on the findings from the present study. First, primary molar radiolucency in hopeless teeth with furcation root perforations from dental infections was successfully treated after 24 months with 3Mixtatin compared to MTA. Second, improvement of clinical outcomes in the follow-up period in such teeth with 3Mixtatin was superior to those with MTA.

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