

# Treatment of non-vital primary molar using lesion sterilization and tissue repair (LSTR 3Mix-MP)

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## Treatment of non-vital primary molar using lesion sterilization and tissue repair (LSTR 3Mix-MP)

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### ABSTRACT

**Background:** Root canal preparation and anatomic variations of deciduous teeth often cause the child patient uncooperative and sometimes the treatment failure. The non-threatening treatment and non-invasive approaches is needed to obtain a good cooperation from child patient. **Purpose:** The study was aimed to clinically evaluate the use of 3Mix-MP- a combination of antibacterial drugs, i.e. metronidazole, minocycline and ciprofloxacin (3Mix), and macrogol and propylene glycol (MP) - as pulp medicament on a necroses primary molar. **Methods:** Subject were the children patients of Pediatric Dental Clinic Universitas Airlangga Dental Hospital. Eight primary molars with pulp necroses due to dental caries were selected as samples. The treatment was done based on the concept of lesion sterilization and tissue repair (LSTR) therapy. A slice of 3 Mix-MP pastes was placed in the cavity and then sealed with glass-ionomer cement. Subjects were asked for recall visit in 1, 3 and 6 months post treatment, for clinical and radiographic evaluation. The antibacterial effect of 3 Mix-MP was compared with tempophore on mixed bacteria of pulp cavity which was isolated prior to therapy. The antibacterial effect was determined by measuring the inhibition zone after 24 hours anaerobe incubation. **Results:** Seven out of 8 subjects on recall visit showed no acute or chronic clinical symptoms, such as fistulae, abscess, purulent exudates, swelling or feel any pain during mastication. Microbiological test result showed LSTR 3Mix-MP had antibacterial effect higher than tempophore ( $p < 0.001$ ). **Conclusion:** The study revealed that 3Mix-MP treatment showed clinical and radiographic positive response on necrose primary molar.

**Key words:** LSTR 3Mix-MP, pulp necroses, primary teeth

### ABSTRAK

**Latar belakang:** Preparasi saluran akar dan variasi anatomi gigi sulung seringkali menyebabkan pasien anak tidak kooperatif dan kadang menyebabkan kegagalan perawatan. Perawatan yang tidak menakutkan dan non-invasif diperlukan untuk mendapatkan kerjasama yang baik dari pasien anak. **Tujuan:** Penelitian ini bertujuan untuk evaluasi klinis penggunaan 3Mix - MP- kombinasi obat antibakteri, yaitu metronidazole, minocycline dan ciprofloxacin (3Mix), dan makrogol dan propilen glikol (MP) – sebagai pengobatan pulpa pada gigi molar sulung yang nekrose. **Metode:** Subjek adalah pasien anak dari Klinik Kedokteran Gigi Anak Rumah Sakit Gigi & Mulut Universitas Airlangga. Delapan gigi molar sulung dengan nekrosis pulpa akibat karies gigi dipilih sebagai sampel. Pengobatan dilakukan berdasarkan konsep

*lesion sterilization and tissue repair (LSTR). Selapis pasta 3 Mix - MP ditempatkan di kavitas gigi dan kemudian ditumpat dengan semen glass-ionomer. Subjek diminta untuk kontrol untuk evaluasi klinis dan radiografi 1, 3 dan 6 bulan pasca perawatan. Efek antibakteri 3 Mix - MP terhadap bakteri campuran rongga pulpa yang diisolasi sebelum terapi dibandingkan dengan tempophore . Efek antibakteri ditentukan dengan mengukur zona hambat setelah 24 jam inkubasi anaerob. Hasil: Tujuh dari 8 subjek pada saat kontrol tidak menunjukkan gejala klinis akut atau kronis, seperti fistula, abses, eksudat purulen, bengkak atau merasa sakit selama pengunyahan. Hasil uji mikrobiologi menunjukkan LSTR 3Mix - MP memiliki efek antibakteri yang lebih tinggi daripada tempophore ( $p < 0,001$ ). Kesimpulan: Hasil penelitian menunjukkan bahwa penggunaan 3Mix - MP memberikan respon positif pada perawatan molar suhng yang nekrotik secara klinis dan radiografis.*

**Kata kunci:** LSTR 3Mix-MP, nekrose pulpa, gigi suhng

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## INTRODUCTION

Approximately 75% of the teeth with deep caries have been found from clinical observation to have pulp exposure. Over 90% of the asymptomatic teeth with deep carious lesions could be successfully treated without pulp exposure using indirect pulp therapy techniques, so the premature extraction can be prevented.<sup>1</sup> Primary teeth play an integral role in the development of occlusion. Premature loss of a primary tooth through infection has the potential to destabilize the developing occlusion with space loss, arch collapse, and premature, delayed or ectopic eruption of the permanent successor teeth. Effective pulp therapy in the primary dentition must not only stabilize the affected primary tooth, but also create a favorable environment for normal exfoliation of the primary tooth. It does not harm the developing enamel or disturb normal eruption of its permanent successor. The primary teeth preserved, are consider to be the best space maintenance treatment until natural exfoliation.<sup>2</sup>

However, before treatment plan of primary molar with deep caries was made, it is important to consider some factors, e.g. age of children, the general health, the period of permanent dental eruption, the severity of dental caries, the cooperation of children, and the cost of treatment.<sup>3</sup> These examples, in any combination, illustrate many treatment considerations that

could be important in an individual patient with pulp necrose.<sup>1</sup> The general treatment for primary molar teeth with diagnose of necroses pulp actually consists of some root canal treatment techniques. However, complete elimination of bacteria is not always achieved in clinical practice due to the anatomical complexities of root canals and consequent limitations of access by instruments and irrigators.<sup>4</sup> Furthermore, root canal treatment procedure in children is more challenging and difficult than in adult due to the following reasons: behavior management problems in children, limited mouth opening, complexities of the root canal system, and danger of injuring the permanent tooth bud. The variation and eventual alterations in the number and size of the primary root canals made it inaccessible even to the smallest endodontic instruments.<sup>1</sup> In general, the least invasive intervention that is predictably associated with a healthy, adaptive healing response in the affected primary or permanent tooth, therefore, is needed to be done.<sup>2</sup> All inflammatory periapical lesions should be initially treated with conservative nonsurgical procedures.<sup>5</sup>

Thus, in order to anticipate those problems, nowadays the Cariology Research Unit of Niigata University School of Dentistry in Japan has developed the concept of lesion sterilization and tissue repair (LSTR) by using combination of metronidazole, ciprofloxacin, and minocycline (3Mix), and combination of carrier materials, macrogol and propylene glycol (MP) for disinfecting the lesion in oral mouth, including dentinal, pulp, and periapical lesion. The recovery of the infected tissue then is expected to occur if the lesion is disinfected.<sup>6-8</sup>

In addition, in vitro and in situ researches are done extensively for analyzing the affectivity of mixing medicines (3Mix) towards bacteria in oral mouth, including endodontic lesion in permanent teeth. The research of LSTR 3Mix-MP on permanent dental actually has been done before by Hoshino,<sup>9</sup> Kuswandari,<sup>10</sup> and Khalil,<sup>11</sup> nevertheless, other supporting researches are still needed in different places in order to examine whether this treatment is also effective for primary molar teeth considering with the morphological difference between the crown of primary molar teeth and that of permanent teeth with many difficulties in maintaining root canal. The wider dentin tubules of primary molar teeth and the thinner enamel of primary molar teeth than those of the permanent teeth can make the spreading of bacteria into root canal easier. Furthermore, in child patients treatment sometimes is difficult to be achieved since the flat anatomy of root canal in the primary molar teeth, and since it also have many branches that can make the conventional root canal treatment difficult.

<sup>1</sup> Due to the polymicrobial nature of the infected root canal, combination of antibacterial drug is required. The treatment with LSTR is considered to be useful to cure the pulp necroses since it can exterminate bacteria in root canal. Clinical procedures of LSTR therapy is also simple and does not need long and <sup>29</sup> continual visiting, thus it makes dentists easier to treat pulp necroses of primary molar teeth.<sup>6</sup> The aim of study was to evaluate the success of LSTR 3Mix-MP treatment subjectively, clinically, and radiographically in primary molar teeth with diagnosis of pulp necroses in 1, 3, and 6 months after the treatment. The study could give an alternative treatment of pulp necroses in primary molar teeth that is easily, simple, effective, cheap acceptable for children.

## MATERIAL AND METHODS

<sup>4</sup> This study was a clinical research study with the pre test-post test control group design. The locations of study were Clinic of Pediatric Dentistry Universitas Airlangga Dental Hospital and Ikhlas Kindergarten at Jalan Sekolahan 22, Surabaya. The targeted population in this research were the students of Ikhlas Kindergarten (51 children) who meet the criteria of sample. The samples were the maxillary or mandibular primary molar teeth with diagnosis of pulp necroses due to dental caries, based on the clinical and radiologic examination. The teeth must also met criteria of the following root canal treatment indication: the tooth should be restorable after the treatment, the root was in full length or 1/3 of root length resorbed based on periapical radiographic. Patients were cooperative, had no any systemic abnormality or not under medical therapy relating with their systemic condition, had no allergic history with any chemical materials and antibiotics, must be permitted by their parents who then signs informed consent. Finally, the method of sample collecting in the study was random sampling.

The criteria of treatment success were subjective, clinical and radiographic evaluations.<sup>12</sup> The treatment was subjectively considered to be success if the early symptoms or spontaneous pain in percussion decreased or disappeared in period of observation (1, 3, and 6 months). The treatment was clinically considered to be success if the patients with or without early symptoms or spontaneous pain in percussion and fistula, inflammation of intra oral, and subjective complains at the first visit had decreased, or had no pain at all during observation period. The treatment was considered to be radiographically success if the area of periapical radiolucency decreased and had no new periapical radiolucency at the end of observation period (6 months).

Clinical examination conducted on primary molar teeth with pulp necroses. This clinical examination was aimed to examine the presence of gingival inflammation; abscess or fistula; abnormal dental mobility; pain after light pressure on tooth; and any spontaneous pain.<sup>2</sup> Moreover, radiographic examination before and after the treatment was also needed to obtain some following information; the existence of external root resorption of treated primary molar tooth, the existence of internal root resorption of treated primary molar tooth, the existence of bifurcation involvement, and the existence of periapical radiolucency.<sup>2</sup> For children who met the criteria of subject, their parents were explained about the aims, the expected results, the advantages and disadvantages of LSTR 3Mix-MP treatment, and the stages of the research as well as the schedule of recall visit. After the parents approved it, they signed the informed consent.

Tools and hand instruments were sterilized prior the examination. Enteric coating and capsule covering the medicine were opened. Each of the medicines then was smoothed by using porcelain mortar and pestle. The powder was stored in covered container, and saved in refrigerator at the temperature of 16° C. Next, the powder was mixed with the ratio of metronidazole 500 mg: ciprofloxacin 500 mg: minocycline 500 mg (3Mix) = 1:3:3. And, the carrier materials then were mixed with macrogol and propylene glycol (MP) = 1:1. Finally, those mixing medical powder were mixed with the carrier materials for forming pasta 3 Mix-MP. The isolation of working area must be conducted in children by using cotton roll. The wall of dental cavity was cleaned chemically by using 35% of phosphate acid. The cavity then was half-filled with 3Mix-MP and covered with glassionomer cement Fuji IX with liquid consistency (the ratio 1 powder: 2 liquid) without pressure. At recall visit (1, 3, and 6 months post-treatment) anamnesis was conducted towards the patients' parents. In intra oral examination on treated teeth of the children, moreover, palpation, percussion, and pressure examinations were conducted. Then, radiology examination was conducted. The obtained data, finally, were noted in the prepared form.

Sterile paper point was put into the dental root canal for 1 minute to obtain mixed bacteria in the necrose pulp. Paper point then was put into the medium tube containing BHI Broth, and aerobically incubated for 24 hours. The blood agar was divided into 4 zones. Bacteria culture in the BHI Broth was taken by micropipette. Each of zones of blood agar was given one drop (10 µliter), and leveled with spreader. Afterwards, LSTR 3Mix-MP and Tempophore, about

half of measuring spoon, were applied in each of absorbed papers. Those absorbed papers then were put on Blood Agar and anaerobically incubated in for 48 hours. The antibacterial effect determined by the inhibition zone. The bigger zone of inhibition the stronger its antibacterial effect.

## RESULTS

Based on the observation of LSTR 3Mix-MP treatment on the 4-6 year old children of TK Ikhlas in Surabaya, 7 of 8 children were successfully treated with LSTR 3Mix-MP. There were 5 children who were successfully treated without any complains and 2 children were successfully treated though they got pain in the first and third months, but they recovered in the sixth month. There was one child who was not successfully treated. Moreover, during the examination of subjective symptom one month after LSTR 3Mix-MP treatment, there were 3 students got pain. One student relieved the pain at the third month, yet the pain emerged again at the sixth month. The two children recovered their pain from the third month to the sixth month. However, there was one student who had pain during mastication at the sixth month. Meanwhile, five children had no pain from the start and had stable condition until the end of the treatment.

The radiographic examination result, however, was difficult to measured since from the beginning of treatment there was no periapical radiolucency or resorbtion of alveolar bone in seven of eight children. In general, LSTR 3Mix-MP treatment radiographically did not show differences between before and after the treatment. Nevertheless, there was permanent radiolucency in one child and physiologic resorbtion in one of the primary molar root (data not shown). Based on the result, it showed that average of the inhibition zone of LSTR 3Mix-MP was bigger than that of Tempophore on bacteria culture of root canal (Table 1).

Before taking the test for measuring the difference of inhibition zone between LSTR 3Mix-MP group and Tempophore group, statistic test had been taken on each group for analyzing the distribution of the data. Kolmogorov Smirnov test on LSTR 3Mix-MP group and Tempophore groups were more than 0.05 which means that either LSTR 3Mix-MP group or Tempophore group has normal distribution of the data.

Moreover, in order to **determined** the difference of resistance zone between LSTR 3Mix-MP and Tempophore Independent t-test conducted. Based on the result of the statistic test, it was

known that  $p=0.001$  ( $p<0.05$ ). It means that the difference of the inhibition zone was significant between LSTR 3Mix-MP and Tempophore on bacteria culture of root canal.

## DISCUSSION

Based on the result it was known that the treatment was successfully conducted in seven of eight children. Similarly, the research conducted by Takushige<sup>6</sup> also showed successful LSTR 3Mix-MP treatment, about 79% of his cases. Besides that, the research conducted by Kuswandari<sup>10</sup> and Khalil<sup>11</sup> also pointed out that LSTR 3Mix-MP treatment on primary molar teeth with diagnosis of pulp necrose is considered to be a successful procedure, which the level of success reached 100% in two months of treatment. Furthermore, Kuswandari<sup>10</sup> also stated that the early condition of primary molar teeth before LSTR 3Mix-MP treatment determined the level of clinical recovery. It means that primary molar teeth without any clinical symptoms in initial condition will recover faster than those with periapical inflammation.

The failure in one child was due to broken restoration of GIC at the first month and at the sixth month of the treatment, so that the elimination of bacteria could not be reached, and the child had pain during mastication. According to Chugal *et al.*,<sup>14</sup> eradication of microorganisms from an infected root canal system is the key to successful root canal treatment since there are millions of bacteria in root canal with pulp necrose. Thus many bacteria infected root canal, especially anaerob bacteria. The virulency of endodontic flora is generally low, but the pathogenic character and intraradicular defense are affected by the combination of many factors, e.g. interaction with other microorganisms in root canal, the ability of bacteria in disturbing the defense system of the host, the secretion of lipopolysakarida (LPS) and other bacterial products, and the enzyme synthesis breaking the tissue of the host. It is also supported by Narayanan & Vaishnavi,<sup>15</sup> that bacteria can infect through many pathways, such as 1) through dental crown or root after the broken pulp due to trauma; 2) through periodontal tissue into broken dentin tubuli, lateral cavity, and accessory cavity or apical and lateral foramen; 3) through lymphatic route or hematogenous (anakoeresis). Thus, in order to eliminate those bacteria more than one antibiotic is needed.



LSTR 3Mix-MP is considered as the combination of metronidazole, minocycline, and ciprofloxacin (3Mix), and those three antibiotics mixed with macrogol and propylene glycol (MP) were proven to be able to penetrate efficiently through root canal and to disinfect the lesion of caries. Laboratory test showed that LSTR 3 Mix-MP could kill mixed bacteria in root canal more potent than that of Tempohore. This result inline with the clinical result. Since LSTR 3Mix-MP was bactericidal in aerob bacteria and in obligate anaerob bacteria that are resistant, LSTR 3Mix-MP could eliminate bacteria from dental tissue infected in primary or permanent molar teeth. This assumption is that by eliminating bacteria, the infection, inflammation, and pain are also eliminated.<sup>6</sup>

The success of LSTR 3 Mix-MP treatment on those 5 samples was marked by the fact that it did not cause any pain, or any clinical and radiological abnormality that indicates the successful disinfection process of bacteria. It is evident that in primary endodontic cases root canal environment provides better nutritional supply, rich with peptides and amino acids for bacterial inhabitants of root canal system, so that elimination of microorganisms from infected root canals is a difficult task.<sup>16</sup> Due to the polymicrobial nature of infected root canal, single empirical antibiotic is insufficient in disinfection of the root canal. Non specific antibiotic suppress most of the microbial flora and allow residual virulent micro-organisms to repopulate the root canal. Therefore it is essential to use combination of antibiotics to act against all endodontic pathogens and to prevent resistance.<sup>17</sup>

Besides the main root canal considering as capillary blood vessel that can be cleaned, there are also many additional root canal in primary molar teeth that cannot be cleaned with mechanic tools.<sup>17</sup> Thus, good material are needed to deliver three-antibiotic mixture (3Mix) and create asepsis condition either in the main or in the additional root canal. Propylene glycol is solvent commonly used in industries, food, and daily goods. The penetration effect of propylene glycol into root dentin was investigated by Cruz *et al.*<sup>18</sup> The area and the depth of penetration of Safranin-O dye in propylene glycol were reported to be significantly greater than dye with distilled water into root dentine. Propylene glycol delivered dye through the root canal system rapidly and more effectively indicating its potential use in delivering intracanal medicaments. Lesion sterilization and tissue repair (LSTR), involves the use of antibacterials combined with a mixture of macrogol and propylene glycol (MP) as the efficient vehicle to carry the antibacterials within the tooth.

Like the procedure of indirect pulp capping, the application of 3Mix-MP topically on dental lesion caries in vivo could also be done by taking the tissue that cannot be re-mineralized, and by leaving the tissue of caries in the deepest area of cavity. Studies have shown that dentin matrices contain reservoirs of bioactive molecules and odontoblast capable of directing tissue repair. By sterilizing the infection and not taking all dentin in pulp, it means that dentin matrix is maintained and preserved as the reservoir source of growth factor secreted by functional odontoblast and pulp fibroblast.<sup>9,10,19</sup> Growth factor then gives molecular signal for the surviving post-mitotic odontoblast cells to proliferate and differentiate to stimulates the secretion of reactionary type of tertiary dentin matrix.<sup>21</sup>

LSTR treatment consisting of three-antibiotic mixture could eliminate bacteria taken from carious lesions, pulp necrose, infected root dentine and endodontic lesions of permanent teeth<sup>20</sup> and deciduous teeth<sup>6</sup> that disturbing the process of odontoblast proliferation and differentiation. By using propylene glycol and macrogol considering as carrier of materials (solvent), the growth factor in dentin matrix was possibly penetrated into dentin tubuli, so it could stimulate the form of tertiary dentin matrix. Based on this advantage, the treatment of root canal with 3Mix-MP on primary molar teeth with pulp infection could give positive effect in this treatment.

Finally, LSTR 3Mix-MP treatment could be considered to be successful if the tooth did not have any clinical symptoms until that tooth was physiologically extracted and then substituted by the permanent one. Therefore, the evaluation was supposed to be done continually until the tooth is physiologically extracted with the various numbers of samples. The result of this research clinically and radiographically showed that LSTR 3Mix-MP could be used to treat primary molar teeth with pulp necrose diagnosis. LSTR 3Mix-MP treatment can be used as an alternative therapy for conventional root canal treatment in primary molar teeth with pulp necrose diagnosis. Nevertheless, in order to ensure the success rate and to examine other factors determining the success of this treatment, it is needed to take further research with the bigger number of samples in the longer period, and by comparing with the conventional treatment of root canal.

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**Table 1.** The average of inhibition zone (mm)

<b>Group</b>	<b>N</b>	<b><math>\bar{x}</math></b>	<b>SD</b>
LSTR 3Mix-MP	10	38.0250	3.2789
Tempophore	10	19.9300	3.3067

Note: n= the number of research sample, SD= Standard Deviation

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