Antioxidant mix: A novel pulpotomy medicament: A scanning electron microscopy evaluation

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Abstract

Aim: This study aims to evaluate the clinical, radiographic, and histological success rate of antioxidant mix as a new pulpotomy agent for primary teeth. Settings and Design: Commercially available antioxidants, namely Antioxidants plus trace elements (OXin-Xt®, India) were used. Materials and Methods: This prospective study was carried out on 36 primary molar teeth in 32 children, with age that ranged from 6 to 9 years. Regular conventional pulpotomy procedure followed by placement of antioxidant mix over the radicular orifice was done. Recall was scheduled for 3, 6, and 9 months, respectively, after treatment. Results: Thirty-six pulpotomized primary molars were available for follow-up evaluations. Scanning electron microscopy analysis of samples showing convex shaped hard tissue barrier formation may be proof of the role of antioxidant material in localization and direction and morphology of the hard tissue barrier. One tooth which presented with pain was assessed as unsuccessful. Conclusion: Quite promising clinical, radiographic, and histological results of antioxidants in the present study shows their potential to be an ideal pulpotomy agent.

Keywords: Antioxidants, pulpotomy, reactive oxygen species

Introduction

Pulp therapy helps in the retention of pulpally involved deciduous teeth in a healthy state until the time of normal exfoliation. Of all pulp therapies, pulpotomy still occupies the major portion.[1] Outer core of pulpotomy involves radical section of infected coronal pulp leaving behind the vital radicular pulp tissue while in inner stream wound healing of the radicular pulp should be proper and more amount of radicular pulp vitality should be maintained by placement of the pulpotomy agent. As major scientific safety concerns have been raised about popular pulpotomy medicaments regarding toxicity and potential carcinogenicity[2,3] search for an alternative ideal pulpotomy agent is on till date! Very few studies have focused on wound healing agents in pulpotomy. Appropriate method, of wound healing, is essential for the restoration of anatomical continuity of damaged tissue and disturbed functional status of the radicular tissue. Wound healing processes are well-organized, biochemical and cellular events, leading to the growth and regeneration of injured radicular tissue in a special manner.[4,5] Healing of wounds involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured radicular tissue.[6] Antioxidants counter the excess proteases and reactive oxygen species (ROS) often formed by neutrophil accumulation in the wounded area and protect protease inhibitors from oxidative damage.[7] Fibroblasts and other cells may be killed by excess ROS and tissues will be made less flexible, so antioxidant substances will reduce the possibility of these adverse events occurring. All these facts and overall antioxidant effects appear to be important in the successful treatment of wound healing. This study aims to evaluate the clinical and radiographic success rate of antioxidants as a pulpotomy agent and scanning electron microscopy (SEM) evaluation of antioxidant biochemical role as coenzyme precursors in radicular pulp healing processes.

Hypothesis

Wound healing agents proving more efficient in local rather than systemic application.

Materials and Methods

The biochemical role of antioxidants is to function as coenzyme precursors in healing processes as shown in Table 1. The trace elements essentially act as cofactors for antioxidant enzymes [Table 2] involved in the destruction of toxic free radicals produced in the body as a normal consequence of the healing processes.
In addition, some of the vitamins may take part in the chain of antioxidative reactions by donating and accepting electrons from ROS including free radical acts as wound healing accelerators.

This study was carried out on thirty-six primary molar teeth of 32 children, with a mean age that ranged from 6 to 9 years indicated for an orthodontic extraction were selected for study. The children were selected from the Pediatric Dental Department, Mamata Dental College, Khammam, Telangana. Each child had maxillary or mandibular primary molars indicated for pulpotomy. Discomfort of the procedure and their benefits were fully explained to the parents of the participating children and written consent obtained.

The teeth selected for pulpotomy were evaluated by clinical assessment and radiographic intraoral periapical radiograph.

The criteria for selection of the teeth were as proposed by American Academy of Pediatric Dentistry guidelines.[8]
- Tooth with no spontaneous pain
- At least two-thirds of the root length is still present
- No sign of internal or other kind root resorption
- Hemorrhage from the amputation site is easy to control.

**Preparation of antioxidant mix**
Commercially available antioxidants, namely Antioxidants plus trace elements (OXIn-Xt™, India) were used [Tables 1 and 2].

**Preparation procedure**
- Removal of the coating material that enclose the tablet
- On the day of treatment, each tablet was grinded to a fine powder using porcelain mortar and pestle
- Combination ratio of 1:6 of normal saline and antioxidant powder was used for obtaining standard consistency using clean spatula and mixing pad.

**Technique**
Each tooth was anesthetized, isolated, and then access to the pulp chamber was obtained with 330 carbide bur followed by extirpation and amputation of the coronal pulp with sharp spoon excavator. Stasis at the orifice of the radicular pulp was achieved with a moist cotton pellet placed on orifices under pressure for 3 min. After stasis, standard consistency antioxidant mix was placed over the orifice followed by a layer of reinforced Zinc Oxide Eugenol (IRM DENTSPLY) and miracle mix placement [Figure 1]. Postoperative periapical radiographs of the treated teeth were taken and considered as baseline.

![Figure 1: (a) Preoperative photograph. (b) Preoperative radiograph. (c) After access opening and coronal pulp amputation. (d) After placement of antioxidant. (e) After final restoration with miracle mix. (f) Immediate postoperative radiograph](image-url)
Re-examination
After treatment, regular clinical re-examination was carried out for every 3 months for a period of 9 months. Intraoral periapical radiographs of each treated tooth were taken at intervals of 3, 6, and 9 months, respectively [Figure 2]. Success rate was considered according to the following criteria.[9,10]

Normal tooth mobility, lack of sensitivity to percussion, lack of patient complaints (discomfort), and normal appearance of surrounding soft tissue without pathological clinical and radiographic changes.

Cases with clinical evidence of pain on eating and/or tenderness on percussion and/or persistence or appearance of sinus tract and/or presence of intraoral or extraoral swellings were considered a failure. Finally collected data were subjected to analysis.

Scanning electron microscopy–examination
Of 36 teeth treated, 12 teeth were extracted at intervals of 3, 6, and 9 months for SEM evaluation. For SEM analysis, the samples were fixed (10% formalin, 24 h, and 26°C) and dehydrated (25-100% ethanol). The specimens were mounted on aluminum studs and desiccated followed by gold sputter coating, and examined in a scanning electron microscope (LEO, model #435VP, Cambridge, UK) under different magnifications. The observed changes were photographed and analyzed.

Results
Thirty-six pulpotomized primary molars were available for follow-up evaluations. The results are divided into clinical, radiographic, and SEM findings.

Clinical findings
Figure 3 shows the clinical findings during the follow-up period.

![Figure 2: I (a and b) 3rd month follow-up – the clinical picture and radiographic picture, II (a and b) 6th month follow-up, III (a and b) 9th month follow-up – clinical picture and radiographic picture](image)
Thirty-six teeth observed after 3 months showed no clinical signs or symptoms of failure.
- After 6 months follow-up, one case complaining of pain and dislodged restoration was seen clinically
- After 9 months, no signs of failure were observed in remaining 12 teeth
- At the end of the study, one case was considered to be clinically failure of treated 36 teeth.

**Radiographic findings**
Figure 3 shows the radiographic findings during the follow-up period.
- After 3 months, normal radiographic findings were observed in all 36 teeth
- After 6 months follow-up one tooth showed widening of lamia dura and the presence of periapical and furcal radiolucency
- After 9 months, no signs of failure were observed in the remaining 12 teeth
- At the end of the study, one tooth with dislodged restoration, pain and widened lamina dura and the presence of periapical and furcal radiolucency were considered as clinical and radiographic failure.

**Six months**
Of 11 specimens, nine specimens presented with a characteristic tubular dentin barrier [Figure 5]. Other two specimens presented with an amorphous hard tissue dentin barrier.

Centroperipheral localization of the hard tissue barrier is seen in most of the samples, characterizing the complete hard tissue barrier formation.

**Nine months**
Seven specimens showed predominance of dentinal tubules. Mixed hard tissue barrier was seen in three specimens. Mineralized tissue barriers in centroperipheral area of nine samples show the existence of a direct relationship between the antioxidant material, localization, and direction and morphology of the hard tissue barrier [Figure 6].

**Discussion**
The ideal pulpotomy agent should accelerate the recovery of remaining radicular pulp tissue to a healthy state so that involved tooth attains normal physiological state. The proof of success of pulpotomy agents comes from clinical observation and experience. This clinical investigation aims to determine the biopotential effectiveness of antioxidants as pulpotomy agents.

Both clinically and radiographically only one tooth of 36 pulpotomized teeth was a failure. Possible explanation for the success rate observed in this study may be because antioxidants counter the excess proteases and free radicals.
The present study. Several studies on antioxidants were found to improve healing ability.[16,17] Success in the present study shows that a combination of antioxidants comprising Vitamin A, B, and C along with trace elements like zinc are capable in radicular tissue repair and regeneration.

One tooth reporting with pain was considered as failure, the reasons may owe to that dislodged restoration, but radiograph still reveals plug of antioxidant over the radicular orifice indicating no means of precisely determining clinically status of the pulp tissue in the radicular part and is one of the major causes of success unpredictability in pulpotomy. However, several clinically significant factors are known to impede wound healing including hypoxia, infection, the presence of debris and necrotic tissue.

Conclusion

Quite promising clinical, radiographic, and histological results of antioxidants in the present study shows their potential to be an ideal pulpotomy agent. It becomes necessary to understand the characteristics, tissue uptake metabolism, biochemical interactions, and the biological activities of various antioxidants on pulpal healing. Understanding these biological interactions may provide reliable biological method for vital pulp therapy of primary teeth and young permanent teeth in the clinical practice.

References

1. Fuks AB. Vital pulp therapy with new materials for primary teeth: New directions and treatment perspectives. J Endod 2008;34:S18-24.
2. Ranly DM, Horn D, Hubbard GB. Assessment of the systemic distribution and toxicity of glutaraldehyde as a pulpotomy agent. Pediatr Dent 1989;11:8-13.
3. Patchett CL, Srinivasan V, Waterhouse PJ. Is there life after Buckley’s formocresol? Part II-Development of a protocol for the management of extensive caries in the primary molar. Int J Paediatr Dent 2006;16:199-206.
4. Stadelmann WK, Digenis AG, Tobin GR. Impediments to wound healing. Am J Surg 1998;176:39S-47.
5. Barbul A. Immune aspects of wound repair. Clin Plast Surg 1990;17:433-42.
6. Tauler P, Aguiló A, Cases N, Sureda A, Gimenez F, Villa G, et al. Acute phase immune response to exercise coexists with decreased neutrophil antioxidant enzyme defences. Free Radic Res 2002;36:1101-7.
7. Sen CK, Khanna S, Gordillo G, Bagchi D, Bagchi M, Roy S. Oxygen, oxidants, and antioxidants in wound healing: An emerging paradigm. Ann N Y Acad Sci 2002;957:239-49.
8. American Academy of Pediatric Dentistry. Guide line on pulp therapy for primary and immature permanent teeth. Int J Pediatr Dent 2012-2013;34:179-86.
9. Caicedo R, Abbott PV, Alongi DJ, Alarcon MY. Clinical, radiographic and histological analysis of the effects of mineral trioxide aggregate used in direct pulp capping and pulpotomies of primary teeth. Aust Dent J 2006;51:297-305.
10. Yildiz E, Tosun G. Evaluation of formocresol, calcium hydroxide, ferric sulfate, and MTA primary molar pulpotomies. Eur J Dent 2014;8:234-40.
11. Seale NS, Coll JA. Vital pulp therapy for the primary dentition. Gen Dent 2010;58:194-200.
12. Ehrlich HP, Tarver H, Hunt TK. Effects of Vitamin A and glucocorticoids upon inflammation and collagen synthesis. Ann Surg 1973;177:222-7.
13. Tanzer F, Ozalp I. Leucocyte ascorbic acid concentration and plasma ascorbic acid levels in children with various infections. Mater Med Pol 1993;25:5-8.
14. DiSilvestro RA, Cousins RJ. Mediation of endotoxin-induced changes in zinc metabolism in rats. Am J Physiol 1984;247:E436-41.
15. Banfi G, Iorio EL, Corsi MM. Oxidative stress, free radicals and bone remodeling. Clin Chem Lab Med 2008;46:1550-5.
16. Porto da Rocha R, Lucio DP, Souza Tde L, Pereira ST, Fernandes GJ. Effects of a vitamin pool (Vitamins A, E, and C) on the tissue necrosis process: Experimental study on rats. Aesthetic Plast Surg 2002;26:197-202.
17. Rasik AM, Shukla A. Antioxidant status in delayed healing type of wounds. Int J Exp Pathol 2000;81:257-63.

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