

CLINICAL, RADIOLOGICAL AND HISTOLOGICAL EVALUATION OF PULPAL RESPONSE OF PRIMARY TOOTH FOLLOWING PULPOTOMY WITH LIVE YEAST CELL DERIVATIVES AND FORMOCRESOL

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Article Info	ABSTRACT
<p>Received 15/08/2016 Revised 27/08/2016 Accepted 12/09/2016</p> <p>Key words: Pulpotomy, Live Yeast Cell Derivative (LYCD), Formocresol.</p>	<p>Pulpotomy is the most common pulp treatment of primary molars, where surgical amputation of infere coronal pulp results in preserving the vitality and function of radicular pulp. The aim of this study is to evaluate the clinical and radiological and histological efficacy of live yeast cell derivative and formocresol as pulpotomy medicament. Pulpotomy was done in 96 teeth in 72 children, aged between 5-10 years. The radicular pulp were treated using live yeast cell derivative (LYCD) and formocresol. Following pulpotomy procedure, the teeth were evaluated clinically and radiographically and histologically at 3 month intervals. Clinical and Radiological results evaluated by Fishers exact test, showed no statistical significance between the two groups with two-tailed p-value 0.8629 (P>0.05). Histological picture of LYCD resembles normal pulp tissue cellularity while formocresol showed pulp fibrosis and viable pulp tissue. Results from our study showed that LYCD can be considered as alternative biocompatible pulpotomy agent. Further studies using larger samples and longer evaluation periods are recommended.</p>

INTRODUCTION

Pulpotomy still occupies majority of primary pulp therapies performed to save and restore the primary tooth until it can be normally exfoliated [1]. This procedure involves intentional removal of coronal pulp followed by placement of ideal medicament over radicular pulp to promote healing and preservation of more amount of vital radicular pulp [2,3]. There exists much controversy regarding ideal pulpotomy medicaments. Search for ideal pulpotomy medicament lead from formocresol, MTA,

electro surgery to laser [4-6] and is still on. Though re-evaluation on use of formaldehyde in pediatric dentistry prevails, still five-minute formocresol protocol is preferred pulp therapy for primary teeth since its introduction from 1904 [7]. Moderate to severe inflammation have been seen in about 50% of the cases treated with either formocresol or ferric sulfate, and pulp necrosis in 40 % cases of formocresol pulpotomy. Concept of wound healing is the most neglected segment in conventional pulpotomy



procedure. Recent studies have revealed that the pulp has a great capacity for healing, this has led us to investigate the possibilities of ideal pulpotomy material capable of compensating for the inconveniences mentioned in the above studies. Wound healing acceleration agents can be considered in pulpotomy as it directly results in acceleration of the normal rate of wound healing of radicular pulp. A biocompatible wound healing material like Live yeast cell derivative (LYCD) can be considered in pulpotomy that has previously shown to stimulate wound healing.

Secondly LYCD contains a substance capable of stimulating wound healing by increasing oxygen consumption, epithelialization, and collagen synthesis [8] which are responsible for the increased biological activity and accelerated wound healing which may result in more amount of vital radicular pulp, unlike conventional pulpotomy medicaments. LYCD, on various aspects of wound healing, showed invitro formation of collagen and new granulation tissue. These factors also increased the consumption of oxygen by cultured fibroblasts and the rate of epithelialization [9]. Subramanyam [10] observed a beneficial effect of a pharmaceutical preparation that contained healing factors on artificially induced ulcers. With all this information it could be hypothesized that this property of LYCD would also benefit remaining vital radicular pulp tissue in pulpotomy. The aim of this present in vivo study was, therefore to compare the clinical and radiographic and histological effectiveness of LYCD and formacresol as pulpotomy medicaments of primary molar teeth.

MATERIALS AND METHODS

Preparation of LYCD

Preparation was carried out in Apex labs as described by J. Peter Bentley

Step 1 - Where Fresh live baker's yeast (*Saccharomyces cerevisiae*) (480 g) was added to a vessel that contained 1/2 L of methanol-denatured alcohol.

Step 2 -The mixture was gently agitated and heated for 2 hours at 60°C. The hot slurry was filtered.

Step 3 -The filtrate was treated with 2.4 g of charcoal, stirred for 1 hour at an ambient temperature and filtered.

Step 4 -The alcohol was removed under pressure, and the filtrate was further concentrated to give 30 mL of aqueous solution.

Step 5 -To this acquired, radiopaque gelling agent base was added so that LYCD concentration of 2,000 units/g will be maintained uniformly.

Step 6 - The concentrated LYCD gel was then stored in 5ml syringe for ease of application on radicular pulp (Fig 1)

Sample selection and grouping:

This study was conducted on children who had attended the outpatient Department of Pedodontics and Preventive dentistry, Mamata Dental College and Hospital, Khammam with good general health and no history of systemic illness or hospitalization. The

protocol was approved by institutional human ethical committee. 96 primary teeth requiring pulpotomy treatment which met the clinical criteria (clinical and radiographic) were selected randomly from 72 children aged between 5 to 10 years. 48 teeth were assigned to be treated with formocresol, and an equal number with LYCD pulpotomies for clinical and radiological evaluation. In this clinical study off 96 teeth, 24 orthodontically indicated extracted teeth were included equally in both groups and then to be subjected to the histopathological evaluation of pulpal response. Prior to the participation in this investigation informed consent was signed by all subjects and their parents.

The following criteria were considered for case selection [11,12].

Clinical criteria

- 1) Exposure of vital pulp by caries
- 2) No swelling, fistula in relation to carious tooth
- 3) No tenderness on percussion
- 4) No mobility and possibility of proper crown restoration of the teeth.

Radiological criteria involves

No widening of periodontal ligament space
No loss of lamina dura
No periapical or furcation involvement.

Histological criteria involves

Extraction indicated teeth for orthodontic purpose

Procedure

Tooth was anaesthetized (2% lidocaine & 1:80,000 adrenaline), rubber dam isolation was done and caries was removed with an excavator. Pulp chamber was opened with slow speed round bur. On pulpal exposure, roof of the pulp chamber was removed and coronal pulp tissue is amputated with hand instruments. Drying and bleeding control were obtained using slight pressure with sterile moist cotton pellet for a period of 3-5min.

The teeth were then randomly divided into two groups for the following procedures:

- **Group 1 (Experimental):** 0.5ml LYCD gel was gently applied on the pulp stump for 5 minutes
- **Group 2 (Control):** Blotted cotton pellet moistened with Formocresol (S.S. White, USA) was placed in contact with the pulp orifices for 5 minutes for fixation.

In both groups, the remaining part of the cavity in the crown of every tooth was filled with a layer of reinforced Zinc oxide Eugenol (IRM, DENTSPLY) followed by miracle mix placement. (Fig 2)

Treatment follow-up

Clinical evaluations were carried out at Three and Six months, and inspected for any sign or symptom including report of pain, swelling, mobility, tenderness to percussion, and draining fistula.



Radiographic success was considered as absence of pathologic resorption (both internal and external), furcation or periapical radiolucency, calcific metamorphosis and periodontal ligament widening. Histopathological evaluations were carried out at 3 and 6 months for the teeth indicated for extraction as part of orthodontic treatment (24 teeth), these teeth were divided into two groups of 6 teeth for each type of pulpotomy treatment from prespective period of 3 and 6 months. Then sections were stained with hematoxylin-Eosin (HE) according to the method of Brown & Brenn. Sections of the pulpal tissue were evaluated by light microscopy for pulpal edema, vascular changes, inflammation and extent, tissue necrosis and extent. Statistical analysis was performed using Fishers exact test. Statistical significant was defined as $P > 0.05$.

RESULTS

All 96 pulpotomized primary molars, were available for follow-up evaluations.

Results from clinical and radiological evaluation (Table: 1)

Fishers exact test showed that there were no statistically significant differences between the success rates of the 2 groups with two-tailed p-value 0.8629 ($P > 0.05$). Figure 3 shows the clinical and radiographic status of teeth in study groups after 3 and 6 months.

Two tooth in group 1 and three teeth in group 2 showed spontaneous pain during the two intervals. Clinical success rates in groups 1 and 2 were 94.4%, and 91.6% respectively (Table 1).

Radiographically at 3 to 6months interval four teeth in the group 1 and two tooth in the group 2 demonstrated furcation and periapical radiolucency. Radiographic success rates were 88.8%, 94.4% for groups 1 and group2 respectively (Table 1).

After 3 months

Group 1

All specimens showed signs of vitality with the presence of a chronic lymphocytes, intact odontoblasts along with decrease inflammatory infiltrate from cervical to along the entire root (fig 4: a).

Group 2

Most specimens showed vital pulpal tissue with moderate inflammation consisting of lymphocytes and plasmocytes within two-thirds of the radicular root. Two of the samples showed pulp hyperemia along the lymphocytic infiltration in the cervical region (fig4 : b).

After 6 months

Group 1

All specimens demonstrated inflammation status varied from mild to moderate in nature predominately with chronic lymphocytes. Intact odontoblastic cell layer

in the pulp with normal arrangement and few degenerated cells were seen at the cervical third of crown, along the entire root (fig4 :c,d). Normal mesenchymal connective tissue with no inflammatory reaction was seen. Tissue necrosis was not found in any of the specimen. The cellularity of the pulp and its fibrous elements resembles normal pulp tissue (fig4 :e)

Group 2

Three specimen showed intact irregular odontoblastic layer with moderate inflammatory infiltrate in the middle third of radicular pulp tissue. In one specimen, the pulp showed slight hypervascularization with new blood vessels formation with engorged RBCs. Two specimens revealed fibrosis in deeper pulp tissue in the middle zone to viable tissue in the apical region. (fig4 :f).

DISCUSSION

The ideal material for vital pulp treatment should accelerate the remaining pulp tissue to return to a healthy state. LYCD (Kalpan JZ 1984) is viscous fluid, with many peptide active components which can accelerate wound healing. The main source of success assessment of pulpotomy agents comes from clinical and histological observations and experience. This clinical investigation was conducted to compare the potential effectiveness of LYCD and formocresol as pulpotomy medicaments in primary teeth. Though Fishers exact test showed no statistically significance based on the clinical and radiographic results achieved, with 95% clinical success, LYCD showed slight superior over formocresol. Success rate of Formocresol pulpotomies in present study is in par with previous studies success ranges from 55% to 98% [13].

Possible explanation for the superior clinical success rates of LYCD recognises it as classic growth factors that act through cell surface receptors and a tyrosine kinase mechanism which are necessary to bring about a biological response (fibroblast growth factor) in wound healing. Secondly stimulating wound healing by increasing oxygen consumption

Though strict parameters are followed in this study, there is no means of precisely determining clinical status of the pulp tissue in the radicular part and is one of the major causes of unpredictability of pulpotomy outcome. Most probably 5% and 8% clinical failure of lycd and formocresol in this study may also be the result of wrong determination of status of remaining pulpal tissue.¹⁴

Ideal pulpotomy agents should leave the radicular pulp vital, healthy and completely enclosed within an odontoblasts lined dentin chamber. Histological picture of LYCD in present study showed intact odontoblasts cell layer in the pulp with normal arrangement with few degenerated cells were seen at the cervical third of crown, along the entire root indicating vital radicular pulp, in contrast to irregular odontoblastic layer and apical viable



tissue found in radicular pulp tissues, some loss of continuity, inflammatory cells and areas of partial formocresol.

It is important to emphasize that pulpal necrosis was not found in any of the histological sample of LYCD while pulp fibrosis was reported in two of samples in formocresol, this is in agreement with previous studies by where histological studies on formocresol pulpotomy showed necrosis of the radicular pulp. Over all clinical,

necrosis in

radiological and histological picture LYCD clearly represents its biocompatible nature to preserve the radicular pulp vitality over formocresol. Most importantly unlike formocresol, LYCD has been used in several preparations without reports of deleterious effects for approximately 45 years [14-16].

Fig 1. Prepared LYCD gel in 5ml syringe



Fig 2. (a)Preoperative Radiograph (b) After access opening and amputation of coronal pulp (c): After placement of LYCD GEL (d) Post-operative radiograph

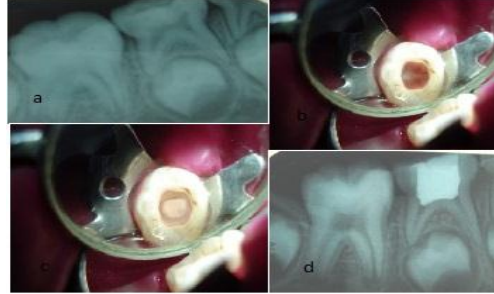


Fig 3. i (a),(b) 3rd month follow up - Clinical picture and Radiographic picture ii (a),(b) 6th month follow up - Clinical picture and Radiographic picture

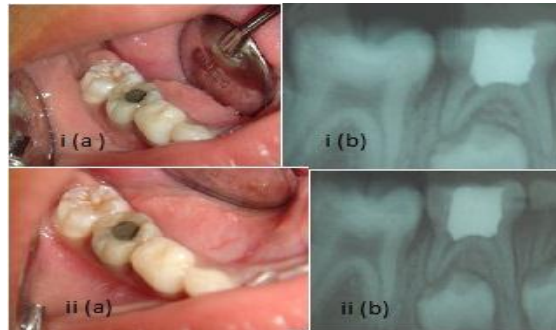


Fig 4. 3months

Group 1 – (a)Histological specimen shows Chronic lymphocytes with intact odontoblasts
Group2 - (b) Histological specimen shows pulp hyperemia & lymphocytic infiltration

6 months

Group1-(c,d) Histological specimen shows intact odontoblastic layer & Few degenerated cells seen along entire root
(e)Histological specimen shows pulp tissue appears normal without inflammatory infiltrate & intact odontoblastic layer
Group2 -(f) Histological specimen shows Fibrosis in middle third of root.

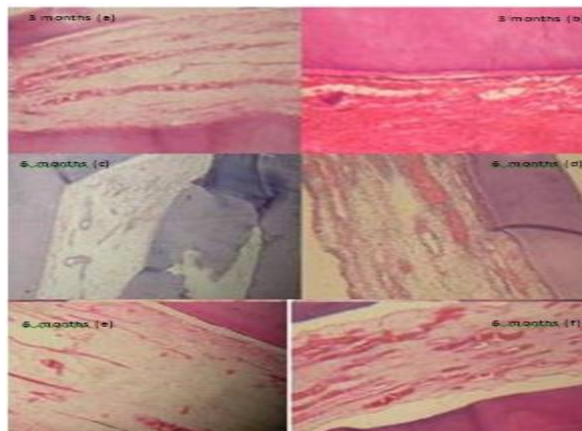


Table1. Clinical and radiographic success rate in different groups after 3 and 6 months

Groups	Success Rate	
	Clinical	Radiological
LYCD	94.4%	88.8%
FC	91.6%	94.4%
P>0.05		

CONCLUSION

In the light of present findings, clinically and radio graphically the success rate of LYCD gel is quite promising and histological picture shows a promising potential to be an ideal pulpotomy agent over formocresol in this short term evaluation period. Definite conclusions could not be done, because of the small number of examined teeth, as well as relatively short follow-up period. Authors suggest a large clinical trial study along

with histological evaluation and long term follow-up, before recommending this reliable biological method for pulp therapy of primary and young permanent teeth in the clinical practice.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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