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CLINICAL AND RADIOGRAPHICAL EVALUATION OF ANKAFERD BLOOD STOPPER IN CONJUNCTION WITH LOW-LEVEL LASER THERAPY AS PULPOTOMY AGENT IN PRIMARY TEETH

Ramy Saieed[1 *], Samy Youssef El Bayoumy[2], Ibrahim Farouk Barakat[3], Mahmoud Taha el Destawy[4]

ABSTRACT

Objective: This study was performed to evaluate the clinical and radiographic success of Ankaferd blood stopper (ABS) alone or in conjunction with low-level laser therapy (LLLT), as a pulpotomy agent in primary teeth and compare it with the formocresol (FC) alone or in conjunction with LLLT. **Subjects and Methods:** Children aged between 3-9 years were included in the present study following specific inclusion and exclusion criteria. The enrolled children had a total of 64 carious primary teeth that are indicated for pulpotomy treatment. The involved teeth were equally categorized into four groups according to the type of pulpotomy agent: ABS alone (G I), ABS followed by LLLT (G II), FC (G III), and FC followed by LLLT (G IV). The clinical and radiographic evaluations were performed after 3 months, 6 months, 9 months, and 12 months following specific designed criteria. **Results:** The results of this study revealed that the use of ABS had better clinical and radiographic success rates when compared with the FC either alone or in conjunction with low-level laser therapy but without statistically significant. Also, the results showed that the use of LLLT improve the clinical and radiographic success outcomes of both of ABS and FC but without statistically significant. **Conclusion:** ABS could be used as successful alternative to FC during pulpotomy treatment. Low-Level Laser Therapy has positive effect on the clinical and radiographic outcomes of the ABS and FC pulpotomy medicaments.

KEYWORDS: Ankaferd Blood Stopper, Formocresol, Low-Level Laser Therapy, Pulpotomy, Primary Teeth

INTRODUCTION

Pulpotomy is a vital pulp therapy (VPT) of primary dentition and it is a commonly clinical conservative procedure which performed in the extensively carious primary molars, which involves the amputation of the coronal pulp with the preservation of the vitality of the remaining radicular pulp [(1)]. The rationale of the clinical success of this surgical procedure is based on the ability of the pulp tissue to heal following the amputation of the infected or inflamed coronal pulp [(2)].

After coronal pulp amputation the hemostasis was achieved, then the pulp stump was covered either with an agent that capable of to fix the underlying pulp tissues or with capping medicament that promotes the process of healing [(3)]. However,

1. Assistant Lecturer, Pedodontic and Oral Health Department, Faculty of Dental Medicine (Boys – Cairo), Al-Azhar University.
2. Professor, Pedodontics and Oral Health Department, Faculty of Dental Medicine (Boys – Cairo), Al-Azhar University.
3. Assistant Professor, Pedodontics and Oral Health Department, Faculty of Dental Medicine (Boys – Cairo), Al-Azhar University.
4. Professor, Oral Medicine, Periodontology, Diagnosis and Oral Radiology Department, Faculty of Dental Medicine (Boys – Cairo), Al-Azhar University.

• ***Corresponding author*:** ramykhairallah6@gmail.com

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the ideal pulp agent which used for covering the pulp stump or filled the pulp chamber should not interfere with the physiologic process of the root resorption [4].

Several pulp-capping materials have been introduced and clinically used including sodium hypochlorite, calcium hydroxide, formocresol (FC), and recently mineral trioxide aggregate (MTA), during the pulpotomy treatment in order to retain the vitality of the remaining pulp tissues or secure pulp healing [5]. Formocresol is one of the pulpotomy agents which has the ability to induce devitalization action and prevents the autolysis of the pulpal tissues [6]. Moreover, FC has the ability to disinfect the infected pulp tissues, and is considered as the gold standard pulpotomy agent in the primary teeth [6,7].

However, FC was classified as carcinogenic agent in previous researches and also considered as cytotoxic agent and concerned about its safety was increased lately[8]. Therefore, the need to natural, safe, and biocompatible alternative was resulted in the development of new agents such as ABS. Ankaferd blood stopper (ABS) was firstly introduced as an introduced hemostatic solution, and it was obtained from standardized mixtures of five different plants namely; glycyrrhiza glabra, urtica dioica, alpinia officinarum, vitis vinifera, and thymus vulgaris [9].

The mechanism of action for ABS is based on the formation of a network of an encapsulated blood protein. Additionally, it was reported that the ABS could be used effectively in individuals either with normal or deficient (primary and secondary) hemostatic parameters [10]. Some previous studies also reported that ABS can be used safely to control bleeding in various dental treatments including pulpotomy [10,11].

Low-level laser therapy was used in previous clinical studies as a medication which has a positive impact on the remaining pulp tissue during vital pulp therapy (pulpotomy) in order to retain the pulp vitality of the remining pulp stamps [11,12]. Furthermore, LLLT has been tested successfully as a safe tool that has the ability to promote the process of healing and stimulate the repairing in human pulp tissues [12].

Thus, the present study was directed to evaluate clinically and radiographically the efficacy of Ankaferd Blood Stopper alone or in conjunction with low-level laser therapy as pulpotomy agent in primary teeth and compare it with the gold standard FC.

SUBJECT AND METHODS

This randomized clinical study was conducted on children aged from 3-9 years after approval of Ethical Committee, Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo) with approval reference No (EC Ref No.238\292 PDS-2019). The enrolled children were elected from outpatients of the Pedodontics and Oral Health Department, Faculty of Dental Medicine, Al-Azhar University (Cairo, Boys).

This study involved 64 deeply carious primary molars in both jaws indicated for pulpotomy. The involved teeth were randomly divided into 4 equal main groups (n=16) according to the type of pulp capping medicaments as follow; ABS alone (G I), ABS followed by LLLT (G II), FC (G III), and FC followed by LLLT (G IV). The sample size calculation for this study was determined based on the results of the previous studies by Bagheri et al.[13].

Subject selection:

Before beginning of the current study, all chosen children and his/her parents were informed about the all used clinical procedures and then signed a consent form with detailed information. The inclusion of children in this present study was based on child age, presence of a deeply carious primary molar, with irreversible pulpitis in any quadrant require pulpotomy, hemorrhage control within time not exceed 5-minutes after pulp amputation, crowns of the involved teeth have at least 2/3 remaining structure to be restored with stainless-steel crown (SSC), no evidence of any pathological clinical mobility, swelling, or existence of sinus tract. Also, the in-

volved teeth should show no radiographic evidence of radiolucency or bone loss periapically, and absence of furcation radiolucency, no root resorption more than 2/3 of its radiographic length, no evidence of pathological periodontal ligament space widening, external or internal root resorption [13-15].

Intervention procedures:

Preoperative assessment:

Complete dental examination along with periapical radiographs for any painful carious primary molar with possible indication of pulpotomy were performed before the beginning of any operative procedures for each enrolled subject to assess the tooth condition and to ensure proper case selection with the following procedures; Pulpal diagnosis was determined by cold test and electric pulp test; Periapical diagnosis was based on percussion and palpation examination along with radiographic evaluation; A comprehensive clinical examination was performed to rule out intra/extraoral swelling, the presence of a sinus tract, or other major pathology [13,14]. All preoperative preapical radiographs were taken by paralleling technique with a beam guiding device.

Pulpectomy procedures:

The pulpotomy procedure was performed as follows: local anesthesia, followed by placement of a rubber dam. Then, caries was removed with a slow-speed handpiece and carbide bur. After that, access cavity preparation by a sterile fissure bur. The amputation of the pulp from the pulp chamber was carried out with a sharp spoon excavator. Then, the amputated pulp stamp was irrigated copiously with sterile solution of saline and then dried lightly with sterile cotton pellet. After that a sterile and moist cotton pellet was placed over the amputated pulp with slight pressure to achieve hemostasis for maximum of 5 minutes or the tooth was excluded [13-15]. Then, the teeth were treated depending on the group to which they were assigned.

Restoration procedures:

In group I; after coronal pulp removal and achieving hemostasis, a cotton pellet was dipped in ABS solution (Ankaferd Health Products Ltd., Istanbul, Turkey), squeezed dry and placed on the pulp tissues for 10-15 seconds. Then, the ABS was flushed away from the pulp chamber with sterile solution of saline [14-16]. In group III; after coronal pulp removal and achieving hemostasis, a cotton pellet was dipped in a 1:5 dilution of Buckley's FC (Sultan Healthcare, Englewood, NJ, USA), squeezed dry and placed on the pulp tissues for 5 minutes for complete hemostasis. Then, the FC was flushed away from the pulp chamber with sterile solution of physiologic saline [14,16].

In group II and group IV; after medicament application, flushing, and draying of the pulp champers, The medicaments in access cavity were activated with LLLT (Diode laser, Lasotronic, Poland) applied in continuous mode with total energy of 4.0 J/cm2 at 135 seconds exposure with all need precautions (Figure 1). After that, the pulp champers in all groups were then covered with freshly thick mix of reinforced zinc oxide and eugenol (ZOE) paste (DENTSUPLY Detrey GmbH, Konstanz, Germany) [14,16]. Then, the pulp chamber of each tooth in all groups were filled with glass ionomer cement (Fuji IX GP, GC Corporation, Tokyo, Japan) and finally restored with SSCs (3M ESPE, USA) [17].

FIG (1) LLLT after application of medicament over the amputated pulp stumps.

Follow-up protocol and assessments of the outcomes:

The evaluations of the involved primary molars in both tested groups comprised clinical and radiographic examinations at three-month intervals for the following 12 months at 4 different follow-up periods; 3-month, 6-months, 9-months, and 12 months. Standardized forms were used to record the following signs and symptoms: [15]

The clinical assessment of the involved pulpotomized primary molar teeth were considered as clinically successful if they met the following criteria; absence of pain or tenderness upon percussion or palpation. Absence of pathological tooth mobility, swelling, sinus tract or fistula [13,18].

The radiographic assessment was considered follows European guidelines for using dental radiographs in children. All postoperative follow-up preapical radiographs were taken by paralleling technique with a beam guiding device. All radiographic films were exposed and processed conventionally under similar conditions and analyzed under standardized conditions (darkened room, magnification) [15,17]. The involved pulpotomised primary molars were judged as radiographically successful if they met recorded absence of periodontal ligament space widening, periapical and furcal radiolucency, pathologic internal or external root resorption, or lamina dura loss [13,18].

Statistical analysis:

The collected data were tabulated and analyzed statistically using SPSS statistical version 21 (Statistics Statistical Procedures Companion, Chicago, IL, USA). Chi-square test was used for comparison of all binary outcome data at different times points. One-way ANOVA test was used to compare the mean age in all examined groups. The results were considered significant at $p<0.05$.

RESULTS

The demographic results of the present study showed no statistically significant difference regard to age and gender among the all studied groups (Table 1 and 2). However, the clinical follow-up results showed non-statistically significant difference between the all studied groups at different follow-up periods. Also, the clinical results revealed that the use of the two tested medicaments showed better but non-statistically significant results when used in conjunctions with the LLLT when compared with the use of each medicament alone. Moreover, the results revealed that the use of ABS has better clinical follow-up results when compared to the FC as pulp medicament (Table 3).

However, the radiographic follow-up results also showed non-statistically significant difference between the all studied groups at different follow-up periods. Moreover, the radiographic results revealed that the use of the two tested medicaments when used in conjunctions with the LLLT showed better but non-statistically significant results when compared with the use of each medicament alone. Furthermore, the results revealed that the use of ABS has better radiographic follow-up results when compared to the FC as pulp medicament (Table 4).

TABLE (1): Age distribution of the enrolled children along the study:

Variables		Mean ± SD	f-ratio	P-value
Age range	**Group**			
3-9 years	G I (ABS)	6.71±0.69	0.7036	0.5535 ns
	G II (ABS/laser)	6.82±0.94		
	G III (FC)	6.40±0.91		
	G IV (FC/laser)	6.58±0.89		

**; significant at p < 0.05.*

; non-significant at p >0.05. ns= non-significant.

TABLE (2) Gender distribution of the enrolled children along the study:

Variables	Male	Female	Chi-square	P-value
Group	**No.; (%)**	**No.; (%)**		
G I (ABS)	11(68.75%)	5 (31.25%)	0.7465	0.8622ns
G II (ABS/laser)	10 (62.5%)	6 (37.5%)		
G III (FC)	11(68.75%)	5 (31.25%)		
G IV (FC/laser)	9 (56.25%)	7 (43.75%)		

**; significant at p < 0.05.*

; non-significant at p >0.05. ns= non-significant.

TABLE (3) Clinical evaluation of the all studied groups after follow-up periods:

Variables	Group	Success	Failure	P-value	Chi-square	P-value
		No.; (%)	No.; (%)			
Clinical evaluation (3-months)	G I (ABS)	15 (93.75%)	1 (6.25%)	0.309 ns	2.064	0.559 ns
	G II (ABS/laser)	16 (100%)	0 (0%)			
	G III (FC)	15 (93.75%)	1 (6.25%)	0.309 ns		
	G IV (FC/laser)	16 (100%)	0 (0%)			
Clinical evaluation (6-months)	G I (ABS)	14 (87.5%)	2 (12.5%)	0.544 ns	1.142	0.766 ns
	G II (ABS/laser)	15 (93.75%)	1 (6.25%)			
	G III (FC)	13 (81.25%)	3 (18.75%)	0.626 ns		
	G IV (FC/laser)	14 (87.5%)	2 (12.5%)			
Clinical evaluation (9-months)	G I (ABS)	14 (87.5%)	2 (12.5%)	0.544 ns	4.102	0.250 ns
	G II (ABS/laser)	15 (93.75%)	1 (6.25%)			
	G III (FC)	11 (68.75%)	5 (31.25%)	0.693 ns		
	G IV (FC/laser)	12 (75%)	4 (25%)			
Clinical evaluation (12-months)	G I (ABS)	13 (81.25%)	3 (18.75%)	0.626 ns	1.828	0.608 ns
	G II (ABS/laser)	14 (87.5%)	2 (12.5%)			
	G III (FC)	11 (68.75%)	5 (31.25%)	0.693 ns		
	G IV (FC/laser)	12 (75%)	4 (25%)			

**; significant at p < 0.05. ; non-significant at p >0.05. ns= non-significant.*

TABLE (4) Radiographic evaluation of the all studied groups after follow-up periods:

Variables	Group	Success	Failure	P-value	Chi-square	P-value
		No.; (%)	No.; (%)			
Radiographic evaluation (3-months)	G I (ABS)	16 (100%)	0 (0%)	1 ns	Nan	Nan
	G II (ABS/laser)	16 (100%)	0 (0%)			
	G III (FC)	16 (100%)	0 (0%)	1 ns		
	G IV (FC/laser)	16 (100%)	0 (0%)			
Radiographic evaluation (6-months)	G I (ABS)	15 (93.75%)	1 (6.25%)	0.309 ns	2.133	0.545 ns
	G II (ABS/laser)	16 (100%)	0 (0%)			
	G III (FC)	14 (87.5%)	2 (12.5%)	0.544 ns		
	G IV (FC/laser)	15 (93.75%)	1 (6.25%)			
Radiographic evaluation (9-months)	G I (ABS)	15 (93.75%)	1 (6.25%)	1 ns	3.490	0.321 ns
	G II (ABS/laser)	15 (93.75%)	1 (6.25%)			
	G III (FC)	12 (75%)	4 (25%)	0.668 ns		
	G IV (FC/laser)	13 (81.25%)	3 (18.75%)			
Radiographic evaluation (12-months)	G I (ABS)	14 (87.5%)	2 (12.5%)	0.544 ns	2.370	0.499 ns
	G II (ABS/laser)	15 (93.75%)	1 (6.25%)			
	G III (FC)	12 (75%)	4 (25%)	0.668 ns		
	G IV (FC/laser)	13 (81.25%)	3 (18.75%)			

*; significant at p < 0.05. ; non-significant at p >0.05. ns= non-significant.

DISCUSSION

Pulpotomy studies in primary teeth focus typically on the effect of the medicament which placed over the pulp stumps of the amputated pulp tissues [17,19]. However, studies rarely designed to investigate the effect of these medicaments in combination with lasers to determine the enhancing healing effect and the long-term outcomes of these materials with the aid of laser [20,21]. Therefore, this study was conducted to evaluate clinically and radiographically the effect of ABS and FC alone or in conjunction with LLLT in the pulpotomy of primary molar teeth with irreversible pulpitis.

Formocresol considered the popular and gold standard pulp-capping medicament which used since its introduction in the primary dentition pulpotomy [14]. However, its reported cytotoxicity, the FC is still used in pulp therapy of primary dentition due to its clinically documented high success rates after one- to two-years of follow-ups [14,22]. Hence, in the present study formocresol was selected as a control group to evaluate the long-term success rate of pulpotomy using ABS in conjunction with LLLT as a medication on the amputated pulp.

Recently, studies were directed to change the clinical practice toward reducing the need for fixation of the remining pulp tissue and designed to evaluate the effectiveness of safe biocompatible, and natural materials such as ABS [14]. Therefore, this study was conducted to evaluate the long-term clinical and radiographic success of ABS as a safe medication over the amputated pulp during pulpotomy treatment of the primary teeth.

In this study diode laser was selected because the diode laser of infrared light emits beam was used in contact with soft tissues in order to produces well-localized ablation by converting laser energy into heat. Moreover, the LLLT was selected in the present study in order to avoid the thermal damage of the remaining pulp tissue by heat exerted due to laser application which may affect the treatment outcome [23].

Prabhakar et al. [24] reported that thermal pulp injury was depends on the laser exposure time rather than the output power from the laser device. Thus, the current study was designed to stick to the standardized time of exposure of 135 seconds, so as to ultimately deliver a fluence of 4.0 J/cm^2.

In the current study upper and lower primary molar were included, this because it was reported that there was no difference in terms of treatment success between the lower and upper jaws during vital pulp treatment [16]. Also, in the current study the rubber dam used prior to beginning of any pulpotomy procedure to insure adequate isolation and avoiding any negative outcomes bias due to improper treatment procedures [5]. Additionally, in this study applying mechanical pressure via placement of a sterile cotton pellet with slight pressure over the amputated pulp tissues was selected to control bleeding; in order to avoid the bias of using hemostatic agent which could affect the outcomes of this study [13] Moreover, SSCs were used as final restoration in this study based on the assumption that there is less microleakage in teeth restored with SSCs than those restored with amalgam [13].

In this study, the demographic results of the all studied groups regarding to age, and gender exhibited non-statistically significant differences between the studied groups. This could be related to the narrow selection of age categories as well as the absence of gender bias during selection of subjects to be involved in this study.

The results of the present study showed that the use of ABS has the higher clinical and radiographic success rates when compared with FC at the 4 different follow-up periods. This could be related to ABS is an effective hemostatic and antibacterial agent in dentistry when used as a pulpotomy medicament in primary teeth [25].

Moreover, the higher success rates of ABS in the present study in comparison with the FC could

be attributed to the histologic finding of the study of Koyuturk et al. [26] which reported that the FC when used as pulpotomy agent it produces a higher inflammatory cells density when compared to ABS. These are in accordance with previous studies that have reported severe reaction of the dental pulp to FC, and emphasizes the fact that the fixation of the pulpal tissues using FC is never complete [24,27].

Moreover, the result of the study by Agamy et al. [28], reported that the response of pulpal tissues to FC showed islands of inflammatory cells which dispersed in the pulp and were almost completely necrotic. This also could explain the relative lower results of FC in this study in comparison with the ABS during pulpotomy treatment due to the biocompatibility and anti-inflammatory effect of ABS.

Moreover, the clinical and radiographic success rates of ABS after 12 months in the present study agreed with the results of Cantekin and GümüG. [15] On the contrary, the results of Ozmen and Bayra [16], showed that both of the ABS and FC have the similar success rate after 24 months of follow-up during vital pulp treatment in the primary molars.

Also, the results of the present study showed that the use of laser in conjunction with all tested medication resulted in higher clinical and radiographic results, however, this improvement was insignificant at all follow-up periods. This could be attributed to that the application of LLL wavelengths have potent anti-inflammatory, faster healing, and diminishes the postoperative infection [23,24]. These findings play a positive role in preserving the vitality of the remaining pulpal tissues and hence implicate the positive healing outcomes in the present study.

This agreed with the results of Prabhakar et al.[24], which reported that the intervention with LLLT affirmed that LLLT reduces the pulpal inflammation. Also, the previous researcher of Sun and Tunér [29], have reported that LLLT has positive postoperative outcomes like regeneration, accelerated wound healing, relief of pain, and enhancement of local immunity. This could explain the superior but insignificant clinical and radiographic results of laser in conjunction with the different tested medication in the present study during the different follow-up periods.

CONCLUSION

From the results of this current study, it could be concluded that the use of ABS as a pulp-capping agent has better clinical outcomes during vital pulpal treatment of primary molars either alone or in conjunction with LLLT when compared with the gold standard FC. The ABS can be used as successful alternative to FC as pulp medicament during pulpotomy of primary molars

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