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THE INFLUENCE OF MINIMALLY INVASIVE ACCESS CAVITIES ON THE CLEANING ABILITY OF PRIMARY INFECTED ROOT CANALS: AN IN-VITRO STUDY

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KEYWORDS

Conventional endodontic access cavities, Minimal endodontic access cavities, Sodium hypochlorite irrigation, ChloroExtra, Confocal Laser Scanning Microscope.

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ABSTRACT

Introduction: An endodontic treatment's success is contingent upon appropriate shaping, cleaning, as well as effective disinfection followed by obturation, which seals the complex anatomy completely. For the purpose of maintaining the maximum volume of dentin, a novel endodontic access cavity concept has been recently gained popularity. The preservation of the most important peri-cervical dentin is the primary focus of this types of coronal access. In contrast to traditional endodontic access cavity preparation (TEAC), conservative endodontic cavity (CEC) preparation is a minimally invasive procedure that can preserve tooth structures, such as pericervical dentin. Therefore, the current study might be of value. **Aim:** the current study was carried out to evaluate the influence of minimally invasive endodontic access cavities (truss, pointed) versus conventional ones on cleaning ability of primary infected root canals. **Materials and Methods:** the research was approved by Research Ethical Committee (REC) of the Faculty of Dentistry Suez Canal University, with appoval no. #201/2019. This study was carried on 36 intact freshly extracted human mandibular first molars. The selected teeth were initially infected with Fusobacterium nucleatum (ATCC 10953), and Streptococcus Intermedius (ATCC 27335) then were randomly allocated into three equal groups (n=12) based on the type of access cavity: Group (A0): the selected teeth will receive conventional access cavities, Group (A1): the selected teeth will receive truss access cavities, and Group (A2): the selected teeth will receive pointed access cavities. Biomechanical preparation was performed using TruNatomy and irrigated with NaOCl 2.5% + EDTA 17% and ChloraEXtra + EDTA 17%. Confocal Laser Scanning was performed. All records were collected, tabulated and statistically analyzed to evaluate the influence of minimally invasive endodontic access cavities versus conventional ones on cleaning ability of primary infected root canals. **Results:** The findings revealed that Conservative access with ChloroExtra irrigation had the statistically significantly lowest mean Bacterial Percentage reduction. While Conventional access cavity either using NaOCl or ChloroExtra irrigation showed the highest bacterial reduction, followed by Truss access cavity with NaOCl, Truss access cavity with ChloroExtra, and Conservative access cavity with NaOCl respectively, with no significant difference. Using ChloroExtra irrigation negatively affected the bacterial reduction in each group, regardless of the type of access used, with the least bacterial reduction in the conservative access design group. While NaOCl irrigation increased the bacterial reduction within each group, with comparable results among all groups. **Conclusion:** 1. Using both NaOCl and ChloroExtra lead to reduction in bacterial count. 2. Conventional endodontic access cavities are still considered the gold standard in root canal treatment. 3. In terms of bacteria reduction and cleaning capabilities, minimal endodontic access cavities did not outperform conventional endodontic access, truss endodontic access cavity can achieve equivalent outcomes to standard endodontic access cavities. 4. Sodium hypochlorite irrigation is more effective in microbial reduction regardless of endodontic access cavity design used in comparison to ChloroExtra.

INTRODUCTION

Root canal treatment is aimed to eradicate harmful pathogens from the root canal and to create an environment, in which any remaining organism cannot survive. A successful outcome of an endodontic treatment depends on proper cleaning and shaping, efficient irrigation and disinfection followed by three dimensional obturation to seal the root canal. To achieve all of the above-mentioned goals, effective endodontic treatment relies basically on accurate access cavity preparation.[1,2].

Straight line access preparation usually accompanied with loss of additional tooth structure which finally might decrease tooth fracture resistance. So, the most essential factor regarding the fracture resistance and survival of root-filled teeth is the amount of remaining dentin. Root canal treated teeth are more prone to fracture than healthy teeth, owing to the elimination of additional dentin during treatment [1].

The morphological, functional and esthetic rehabilitation of root canal treated teeth is quite problematic. Earlier studies displayed that by critically dropping the amount of dentin, coronal access cavity preparation decreased the fracture strength of teeth and amplified cuspal deflection during function [3,4].

To overcome such problem, the emergence of minimally invasive dentistry and the modern imaging devices (CBCTs), illumination and magnification have inspired the rise of recent conservative endodontic access cavity design. The trend is to preserve sound dentin by avoiding deroofing of the pulp chamber as well as avoiding aggressive dentin removal [5].

A novel endodontic access cavity design for dentin conservation has been suggested. Its primary focus on preservation of the peri- cervical dentin and a portion of the pulp chamber roof (Soffit)[1]. In contrast to traditional endodontic access cavity preparation (TEAC), conservative endodontic cavity (CEC) preparation is a minimally invasive procedure that can preserve tooth structures, such as pericervical dentin [6].

Thus, the objectives of the present study was to evaluate the influence of minimally invasive endodontic access cavities designs (truss, pointed) versus conventional ones on cleaning ability of primary infected root canals.

The null hypothesis of this study was that there is no significant difference in the cleaning ability using minimally invasive endodontic access cavities and the conventional ones.

METHODOLOGY

I. 1 Study design

The current study was an in vitro study. This study was approved by the Ethical committee of the Faculty of Dentistry, Suez Canal University with approval **no. #283/2020**. It was carried out on a total of 36 unidentified intact freshly human mandibular first molars extracted due to periodontal problems.

I. 2. Sample size calculation

Power analysis was used to calculate the whole sample size for a Chi-square test comparing three groups and two subgroups. The effect size (w) was 0.75, with an alpha () level of 0.05 (5%) and a beta () level of 0.10 (10%), i.e. power = 90%; the minimum projected sample size was 36 participants. The calculation was based on the findings of earlier investigations 2. As a result, the total sample size is 36, with 12 participants in each group. G*Power Version 3.1.9.2 was used to calculate sample size.

I.3. Sample selection

The teeth included in this study, had the following criteria:

- Sound lower first molars, with mature apices.
- No cracks
- No caries
- Moderate root curvature (10^{o}-20^{o}) according to Schneider method [7].

I.4 Grouping of samples

The selected samples were divided into three equal groups (n=12) according to the type of access cavity:

Group (A0): the selected teeth received conventional access cavities

Group (A1): the selected teeth received truss access cavities

Group (A2): the selected teeth received pointed access cavities

The samples of each group were randomly subdivided into two equal subgroups (n=6) according to the type of irrigant:

Subgroup (B0): irrigation was performed using Sodium Hypochlorite (2.5%) + EDTA 17%

Subgroup (B1): irrigation was performed using ChloraEXtra + EDTA 17%

II. 1. Preparation of samples

The selected samples were cleaned of calculus and soft tissue remnants using a hand curette. The teeth were disinfected using 5.25% sodium hypochlorite for 10 minutes and then rinsed with distilled water. Then teeth were examined clinically to exclude the ones with caries, also using magnification by magnifying loupes 2.5X to exclude the ones with cracks. The selected teeth were mounted in standardized acrylic resin blocks using a mold with a dimension of 1 × 1 × 2.5cm. The teeth were then stored in normal saline solution at room temperature until the time of use.

Thereafter, the teeth were classified into three groups (n=12) and prepared according to each specified group, as following;

Group A0: Conventional access cavity

The cavity was opened by using a diamond round bur perpendicularly at the deepest point of the occlusal surface. After reaching the dentin, the pulp was reached using a #4 steel round burs. Then the coronal access was prepared by complete de-roofing of the pulp chamber with exposure of all pulp horns and straight-line access into the canals.

Group A1: Truss Access Cavity (TAC)

The principle of TAC is to maintain part of the roof chamber to achieve a more conservative opening. So it was necessary to measure on X-rays with a periodontal probe the distance between marginal ridge and canal orifices, in order to try to locate, on the occlusal surface, the correct position and direction where to use the bur. Thereafter, a single access to mesial canals was created with bucco- lingual direction and another one, circular, distally to reach the distal root canal. So, the two accesses on the same occlusal surface were separated by an enamel/dentin bridge. Two separate mesial and distal rounded cavities were prepared up to the mesial and the distal canals. The coronal access to the pulp chamber was gained from the occlusal surface to the roof of the pulp chamber by using a diamond round bur oriented in the way of the long axis of the tooth. Coronal pulp roof was intact between the mesial and distal access cavities in the truss of the tooth structure [8].

Group A2: Pointed Access Cavity

The initial access cavity was performed by using a diamond round bur perpendicularly at the deepest point of the occlusal surface. After reaching the dentin, the pulp was reached using a #4 steel round burs. Then, when the pulp chamber is reached, the cavity was slightly expanded buccolingually using a fissure bur. The mesiodistal length of the cavity was set to 2 mm; meanwhile, the buccolingual length of the cavity was 3 mm. Steel round bur was adjusted as 45– 50-degree angle to the axial walls. The #4 steel round bur was used obliquely to straighten the inner walls. This preparation normally begins at the occlusal surface's central fossa, only away to identify the canal orifices while maintaining part of the tooth pulp chamber ceiling. Furthermore, the distance between the central fossa and the roof of the pulp chamber had to be measured on X-rays with a periodontal probe in order to try to determine the right position and direction to apply the bur on the occlusal surface[9].

II.2. Observations

II.2.a Microbiological evaluation

Upon completing of the endodontic access cavity, the teeth were sterilized by autoclaving at 121°C. A pure culture of Fusobacterium nucleatum (ATCC 10953), and Streptococcus Intermedius (ATCC 27335) were inoculated into the root canal of the selected teeth. To guarantee that the amount of bacteria was around 1.5 x 108 colony forming units, the cell solution was adjusted to 1.0 McFarland Standard. These strains were grown in anaerobic jars for 24 hours at body temperature on brain heart infusion broth and agar plates. Each specimen was cultured with 100 l of the bacterial solution using sterile pipettes. The samples were incubated for 21 days at 37 degrees Celsius.

In all groups, cleaning and shaping of the buccal and lingual root canals was performed using TruNatomy (TRN) using Endo Micromotor with 16:1 handpiece (NSK Endo-Mate TC2) with a speed 500 rpm and torque 1.5 Ncm. The canals were first instrumented using TRN Orifice Modifier OM (20/.08 taper), then TRN Glider (17/.02), followed by the shaping file TRN Prime (26/.04), then the shaping file TRN Medium (36/.03) to the full working length in a reaming motion, according to the manufacturer's protocol.

Subgroup (B0): canals were intermittently irrigated throughout the procedure using Sodium Hypochlorite (2.5%) + EDTA 17% (total 10 mL) between each file using Endo irrigation needle (30G*25mm) 2-3 mm short of the working length alternatingly.

Subgroup (B1): canals were intermittently irrigated throughout the procedure using ChloraEXtra + EDTA 17% between each file.

II.2.b Confocal laser scanning microscope

Horizontal cross sections of all specimens were performed at 2, 4, and 6 mm from the apex using a 0.3 mm Isomet saw running at 200 rpm with constant water spray. The sectioned specimens were stained with BacLight stain Live/Dead (Invitrogen, Carlsbad, CA), then assessed for adherence of bacteria using confocal laser scanning microscopy established at specifications of the excitation/ emission wavelengths of 480/500 nm with the application of fluorescein diacetate dye, using a 40X magnification oil lens. Three sequential images were taken; one of the 2, 4-, and 6-mm sectioned specimens from the apex. In total, 36 images were taken from each group. The images were acquired and fragmented into layers using the Leica Application Suite-Advanced Fluorescence software

These images were exported to the bioImageL TM v21 software in order to quantify the amounts of live (green) and dead (red) bacteria. The image layers were used to reconstruct the sections of the contaminated tubules in 3D form for an evaluation of the volume and distribution of the contamination. Each layer was evaluated individually with the "Surface and Volume Distribution" function. Bacterial viability and biovolume data were obtained from the analysis of each layer. The recorded red/green fluorescence intensities were used to analyze the percentage of dead bacteria over both dead and live bacteria [10].

III. Statistical analysis

All data was collected, tabulated and statistically analyzed. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests. All data showed parametric (normal) distribution. Data were represented as mean, standard deviation (SD) and 95% Confidence interval for the mean (95% CI) values.

Independent Sample t-test was used to compare between each group. The Two-way Analysis of Variance (ANOVA) test was used to compare between percentage reductions in-between groups. When the ANOVA test was significant, Tukey's post-hoc test was utilized for pair-wise comparison.

The significance threshold was chosen at P 0.05. The statistical analysis was carried out using IBM® SPSS® Statistics Version 20.

RESULTS

IV.1. Intragroup comparison

IV.1.a. Results of Bacterial percentage reduction mean values in conventional access group:

Data in [Table (1) & Figure 1] showed CLSM images and bacterial reduction mean values of conventional access group. Bacterial reduction mean values of conventional access cavity using NaOCl or ChloroExtra are 87% and 83% respectively. Using NaOCl or ChloroExtra irrigation showed no statistically significant difference in bacterial reduction with conventional access cavity.

Table (1) *Descriptive statistics of Bacterial percentage reduction mean values in conventional access group*

Group Access type	Irrigant	Mean	SD	95% CI		P-value
				Lower bound	Upper bound	
Conventional	NaOCl	87.00	1.000	84.52	89.48	0.993
	ChloroExtra	83.00	8.888	60.92	105.08	

**: Significant at P ≤ 0.05*

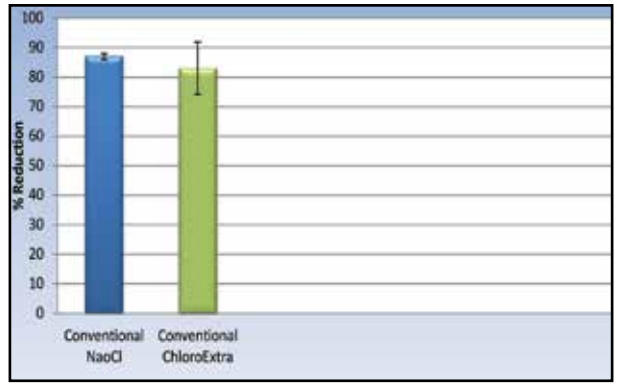

Fig. (1) Bar chart representing mean and standard deviation values for bacterial percentage reduction in conventional access group

IV.1.b. Results of Bacterial percentage reduction mean values in conservative access (pointed) group:

Data in [Table 2 & Figure 2] showed CLSM images and bacterial reduction mean values of conservative access group. Bacterial reduction mean

values of conservative access cavity using NaOCl or ChloroExtra are 67.3% and 57.3% respectively. Conservative access cavity either using NaOCl or ChloroExtra irrigation showed also no statistically significant difference.

Table (2) *Descriptive statistics of Bacterial percentage reduction mean values in conservative access group*

Group Access type	Irrigant	Mean	SD	95% CI		P-value
				Lower bound	Upper bound	
Pointed	NaOCl	67.33	8.737	45.63	89.04	0.761
	ChloroExtra	57.33	5.859	42.78	71.89	

**: Significant at P ≤ 0.05*

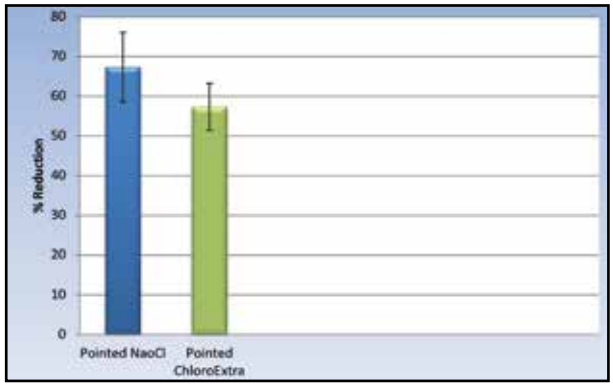

Fig. (2) Bar chart representing mean and standard deviation values for bacterial percentage reduction in conservative access group

IV.1.c. Results of Bacterial percentage reduction mean values in truss access group:

Data in [Table 3 & Figure 3] showed CLSM images and bacterial reduction mean values of truss access group. Bacterial reduction mean values of truss access cavity using NaOCl or ChloroExtra revealed 82.6% and 74.6% respectively, without significant variance.

Regardless of the type of access, using ChloroExtra irrigation negatively affected the bacterial reduction in comparison to NaOCl, but with no statistically significant difference.

Table (3) *Descriptive statistics of Bacterial percentage reduction mean values in truss access group*

Group Access type	Irrigant	Mean	SD	95% CI		P-value
				Lower bound	Upper bound	
Truss	NaOCl	82.67	14.189	47.42	117.91	0.885
	ChloroExtra	74.67	10.599	48.34	101.00	

**: Significant at P ≤ 0.05*

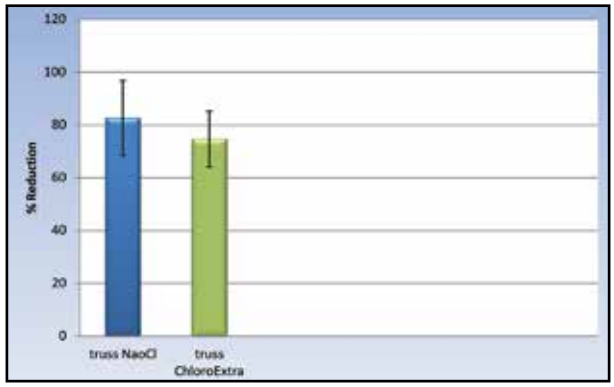

Fig. (3) Bar chart representing mean and standard deviation values for bacterial percentage reduction in truss access group

IV.2. Intergroup comparison

Descriptive statistics and results of intergroup comparison between Bacterial percentage reduction are presented in [Table 4 and Figures 4].

Two-way ANOVA test showed that there was statistically significant difference between the groups (P-value <0.05). Pair-wise comparisons between the groups using Tukey's test revealed that conservative access (pointed) with ChloroExtra irrigation showed the statistically significantly lowest mean bacterial Percentage reduction. While

conventional access cavity either using NaOCl or ChloroExtra irrigation showed the highest bacterial reduction, followed by Truss access cavity with NaOCl, Truss access cavity with ChloroExtra, and conservative access cavity with NaOCl.

For access cavity designs, regardless of the irrigant used, conventional access cavities have shown the highest bacterial reduction. However, the least bacterial reduction was found with conservative access cavities.

Table (4) *Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups*

Irrigant / Access type	NaOCL		ChloroExtra		P-value
	Mean	SD	Mean	SD	
Conventional	87.00	1.000	83.00 ^{A}	8.888	0.015*
Pointed	67.33	8.737	57.33 ^{B}	5.859	
Truss	82.67	14.189	74.67 ^{AB}	10.599	
P-value	0.684		0.035*		

**: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant differences according to Tukey's test*

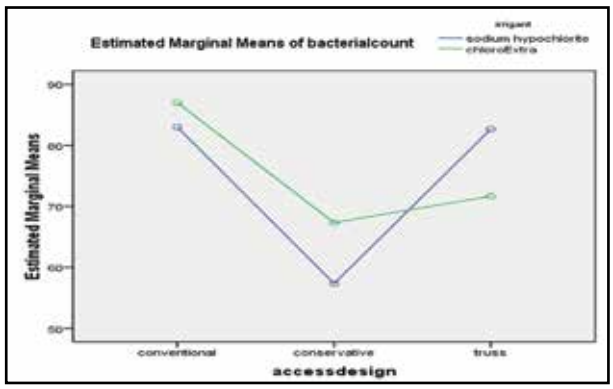

Fig. (4) Box Plot showed bacterial percentage reduction in all groups

II.2.a. Results of Bacterial percentage reduction mean values of using NaOCl irrigant:

Data in [Table 5 & Figure 5] showed bacterial reduction mean values of using NaOCl in conventional, conservative, and truss access cavity groups of 87%, 67.33% and 82.67% respectively. NaOCl irrigation increased the bacterial reduction within each group, with comparable results among all groups. The highest bacterial reduction was seen in the conventional access design group, and the least bacterial reduction with the conservative (pointed) access cavity design group, without significant difference

Table (5) *Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups (NaOCl irrigant) regardless of the access design used.*

Irrigant	Access type	Mean	SD	95% CI		P-value
				Lower bound	Upper bound	
NaOCl	Conventional	87.00	1.000	84.52	89.48	0.684
	Pointed	67.33	8.737	45.63	89.04	
	Truss	82.67	14.189	47.42	117.91	

**: Significant at P ≤ 0.05*

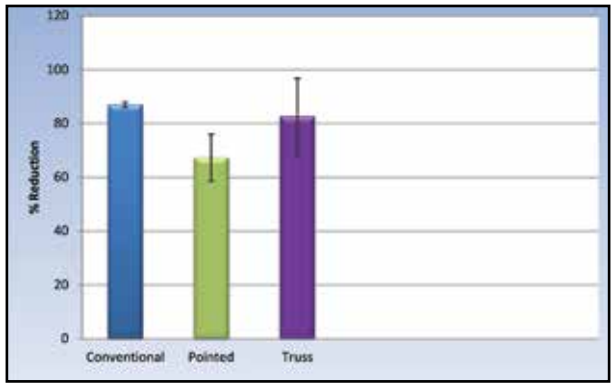

Fig. (5) Bar chart representing mean and standard deviation values for bacterial percentage reduction in NaOCl irrigants used

II.2.b. Results of Bacterial percentage reduction mean values of using ChloroExtra irrigant:

Data in [Table 6 & Figure 6] showed bacterial reduction mean values of using ChloroExtra in conventional, conservative, and truss access cavity groups of 83%, 57.33% and 74.67% respectively. The least statistically significant bacterial reduction was recorded in the conservative access design group in comparison to the conventional access design group. However, this difference is statistically insignificant with the truss access design group.

Table (6) Descriptive statistics and results of Two-way ANOVA and Tukey's tests comparison between Bacterial percentage reduction in the different groups (ChloroExtra irrigant) regardless of the access design used.

Irrigant	Access type	Mean	SD	95% CI		P-value
				Lower bound	Upper bound	
ChloroExtra	Conventional	83.00 ^{A}	8.888	60.92	105.08	0.035*
	Pointed	57.33 ^{B}	5.859	42.78	71.89	
	Truss	74.67^{AB}	10.599	48.34	101.00	

**: Significant at P ≤ 0.05, Different superscripts in the same column indicate statistically significant differences according to Tukey's test*

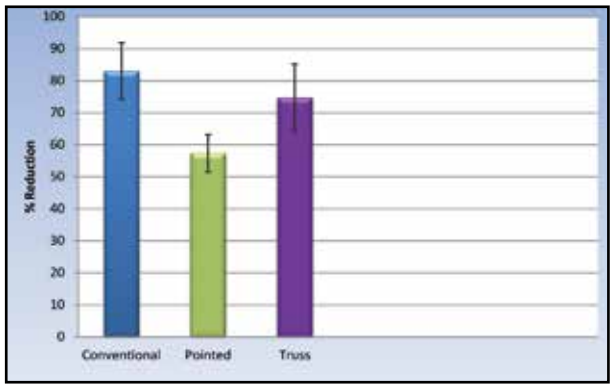

Fig. (6) Bar chart representing mean and standard deviation values for bacterial percentage reduction in ChloroExtra irrigants used

DISCUSSION

Preparing access to the pulp chamber and root canal system is a critical step in effective endodontic therapy[11]. Procedures like as localization, chemo-mechanical preparation, and obturation could be done properly only with a suitable access cavity.[12]. The traditional endodontic access cavity (TEC) design focuses on the inclusion of all pulp horns and removal of the roof of the pulp chamber so that the coronal portion of the root canal system is sufficiently debrided [13].

The fracture resistance of root canal teethed treath appears to be significantly influenced by the designs of the endodontic access cavity and the cumulative loss of tooth structure. The endodontic access cavity preparation may have an impact on the amount of remaining dental material. So, alternative approach to TEC; minimally invasive access cavities have recently been proposed [14].

As an alternative to conventional endodontic access cavities (TEC), minimal access cavities have been introduced to save the maximum amount of tooth structure, consequently enhancing the fracture resistance of teeth treated with root canal therapy[15,16]. Two distinct methods of implementing the minimally invasive endodontic access idea are truss access cavities (TAC) and conservative endodontic access cavities (CEC). CEC aims to preserve the pulp chamber roof to some extent by removing the least amount of tooth structure possible. Although TAC entails creating distinct cavities in teeth with multiple roots (mandibular molars, for example, one cavity above the mesial and one above the distal root canals) in order to enhance dentin preservation[13, 15].

Canal detection, canal cleaning and shaping procedures are more challenging via these types of minimal cavities [13, 17]. The limitations of this type

of access is caused by restriction, which leads to inappropriate cleaning and shaping processes and may jeopardize the proper removal of root canal microbiota. The fact that the number of studies currently covering these topics is limited, it is clear that evaluating microbial reduction during root canal instrumentation subjected to different endodontic access cavity design is a topic that requires investigation.

Thus, the current research was carried out to evaluate the influence of minimally invasive access cavity designs (truss, pointed) versus conventional ones on cleaning ability of primary infected root canals, using different irrigants.

Human mandibular first molars, were used in this study, as they are mostly available and usually extracted due to periodontal problems [18].

Considering the anatomical intricacies of the canal system, mechanical canal preparation may lower the bacterial burden inside the root canal system by 100-1000 times.[19]. Employing an antimicrobial root canal irrigant is essential to increase root canal system disinfection because 35 to 50 percent of the canal system's surface area is mostly left uninterrupted by endodontic tools [20].

Irrigants should possess antimicrobial activity, tissue-dissolution activity, lubrication, and it should optimally have the capacity to remove smear layer and debris in their optimal state (Zhender et al, 2006). Owing to its high antibacterial effect and tissue solubility, NaOCl is the most often utilized irrigation solution in endodontics. NaOCl is accessible in aqueous solutions ranging from 1 to 15% with an alkaline pH. (around 11). One significant shortcoming of NaOCl is its inability to eradicate the inorganic part of the smear layer [21].

To enhance the wettability and diffusion of NaOCl, a detergent was added to 5.85 percent NaOCl to lower its surface tension in the Chlor-XTRA irrigating solution. It has a bright, green, golden look. It has an odour similar to chlorine and is fully soluble in water. It has 2.6 times the digestive power of ordinary NaOCl. Moreover, its wetting capacity is 2.5 times higher than that of ordinary NaOCl.[22]. Chlor-XTRA has been shown by Mohammadi et al. (2012) to be more efficient against residual bacteria than NaOCl, Chlorhexidine, Tetraclean, and Hypoclean in the agar diffusion antibacterial test[23]. Chlor-XTRA dissolved much more tissue than other solutions in every concentration, according to research by Stojicic et al. (2010). Consequently, it was employed in this investigation to compare with NaOCl irrigant[24].

Recently, TruNatomy files, a new generation of rotary, designed to shape root canal systems to a continuously tapering preparation with maximum preservation of peri-cervical dentin. Compared to earlier generations of rotatory instruments, this new file system has more safety, enhanced cutting efficiency, increased cleaning capabilities, and mechanical qualities[25].

Remaining persistent bacteria lies either under the pulp chamber dentin that was kept by those conservative accesses in parts of the root canal remained untouched. These regions consist of isthmuses, dentin tubules, lateral root canals, unprepared spaces inside the root canal walls, and apical ramifications. [26, 27, 28]. In the present research, confocal laser microscope was used for the microbiological examination, which is considered a qualitative assessment [29, 30].

Scanning electron microscopy (SEM) was also incorporated in previous to examine them, although CLSM has grown in popularity because of its benefits over SEM. First of all, sample preparation for SEM necessitates certain actions like applying a gold coating, which may harm the samples. In

contrast, sample preparation for CLSM requires no such special actions, allowing imaging without endangering the materials. [31]. Second, because it can be difficult to discern between the bacteria in the canals and the dentin, interpreting SEM images in studies assessing canal bacterial penetration into dentinal tubules can be difficult. In contrast, because fluorescent dyes are added, CLSM pictures may be clearly distinguishable. [18]. Thirdly, the final picture in CLSM may be produced by combining images taken at different depths[32]. On the other hand, SEM has a larger magnification than CLSM, which makes it difficult to image and assess the full surface area. With the reduced CLSM magnification, it is possible to evaluate greater regions thanks to the use of fluorescent materials for increased image clarity[33-36].

Microorganisms that initially invade and colonize the necrotic pulp tissue cause primary intraradicular infection. Fusobacterium (e.g., F. nucleatum), gram-negative bacteria appear to be the most common microorganisms in primary endodontic infections [37]. Even though anaerobic gram-negative bacteria are reported to be the most common microorganisms in primary infections, several gram-positive bacteria have also been frequently detected in the endodontic mixed consortium, some of them in prevalence values as high as the most commonly found gram-negative species. The genera of gram-positive bacteria often found in primary infections include Streptococcus (e.g., S. intermedius group) [38]. Thus, these two types of bacteria (F. nucleatum and S. intermedius) were used in this study for microbial evaluation.

Principal aim was to compare the intracanal microbial reduction linked to three distinct endodontic access cavity types: truss access canals, conservative endodontic access cavities, and conventional endodontic access cavities (TEC and CEC) (TAC). A substantial percentage microbiological reduction was seen in all groups when TEC and CEC groups were compared, with a greater percentage reduction in the TEC group.

I. Intragroup comparison:

Results of the current study regarding the intragroup comparisons showed bacterial reduction in all groups either using NaOCl or ChloroExtra, with no significant difference. Since irrigation of the root canal is considered to be the key element in eradication of bacteria. Studies have showed that copious irrigation with an antimicrobial solution during mechanical root canal preparation has an essential effect on the reduction of intraradicular microorganisms [28]. This was in accordance with previous studies that reported that efficient irrigation plays crucial role in increasing bacterial reduction [39-41]. This reinforces the thought that using thorough irrigation allowing proper root canal disinfection, making the role of the access cavity limited. This could be also attributed to the instrumentation of the root canals, that contributes in increasing microbial reduction, as it provides a room for efficient irrigation in the canal [6, 42].

Regardless of access type, the results of bacterial percentage reduction using irrigants, have shown that NaOCl irrigation increased the bacterial reduction within each group. On the other hand, using ChloroExtra irrigation negatively affected (insignificantly) the bacterial reduction in each group. This may be related to the concentration of NaOCl and the quantity of surfactant in the mixtures, which may have an impact on NaOCl's stability and capacity to permeate abnormalities in the main canal [43]. This was consistent with another study that showed that surfactant by itself did not seem to enhance NaOCl's ability to disintegrate tooth pulp tissue [44].

This was in contrast to earlier research that found ChloroExtra to be more effective than

NaOCl, CHX, Tetraclean, and Hypoclean at killing bacteria (Wang et al, 2012 and Mohammadi et al. 2012). According to their claims, the combination of NaOCl and surfactant exhibited lower contact angle with dentin and is more efficient in dissolving soft tissue[24, 45, 46]. The divergent outcomes seen in our investigation may be attributed to the varying concentration and quantity of surfactants included in the mixtures. Studies on the impact of surfactants on NaOCl's tissue solubility are still debatable[47].

II. Intergroup Analysis:

Regarding the results of the intergroup microbial counts of the current study, conservative access (pointed) with ChloroExtra irrigation offered lowest mean values of bacterial percentage reduction. While conventional access cavity either using NaOCl or ChloroExtra irrigation showed the highest bacterial reduction with mean values 87 and 83 respectively, followed by Truss access cavity with NaOCl, Truss access cavity with ChloroExtra, and conservative access cavity with NaOCl, with no significant difference. This because of CEC significantly increases coronal interferences, making it more difficult to properly instrument and clean the root canals. The TAC provides more direct and unrestricted access to the root canals than CEC does, even though it is a less invasive procedure, which may account for the improved outcomes, recording mean values of 82.67 for NaOCl irrigant and 74.67 for ChloroExtra irrigants. This supports the idea that, especially when compared to the TEC access, less invasive endodontic accesses may compromise the overall efficacy of lowering the microbial load during root canal preparation. This came in contact with the thought of less invasive endodontic accesses may compromise the overall efficacy of lowering the microbial load during root canal preparation [48-50].

Furthermore, few studies reported that access cavity design also could compromise the instrumentation of the canals, leaving a higher proportion of untouched canal areas, and a higher percentage of pulpal tissue remnants, which could potentially affect thorough disinfection and hindering efficient bacterial reduction and elimination [2, 13].

In contradiction, a recent study assessed the influence of contracted access cavities as (CEC) and truss access cavities (TAC) during RCT on mandibular molars in terms cleaning and shaping ability , they came to the conclusion that there was no benefit to access cavities and that effective irrigation and canal cleaning were the key factors in bacteria decrease [51]. This could be contributed to using rotary files with larger diameter and tapering, allowing enlarged prepared canals with sufficient root canal irrigation.

The TAC access, which offers a more direct route to the mesial and distal canals through two distinct cavities, may have contributed to the statistical similarity of the results between the TEC and TAC groups. In contrast, the CEC access causes significantly more coronal interferences during instrumentation [51]. Since the constricted access cavity design affects mechanical instrumentation and would negatively affect the irrigant's cleaning ability, these coronal interferences could be to blame for the inadequate elimination of bacterial count that recorded in samples of the CEC group, especially when using ChloroExtra irrigant [13].

Our study's findings were consistent with those of Krishan et al. (2014), who found that the TEC group's distal canal mandibular molar bacterial reduction values were greater than those of the CEC group[2]. Furthermore, the results of Rover et al. (2017) and Moore et al. (2016) indicated that these access methods in maxillary molars decreased the identification of root canals, compromised the canals' capacity to be cleaned, and did not improve fracture resistance [52, 53].

In order to understand the in vitro effects of various endodontic file systems utilizing various endodontic access cavity designs on the eradication of Enterococcus faecalis from the root canal systems, another study was conducted recently. In order to understand the in vitro effects of various endodontic file systems utilizing various endodontic access cavity designs on the removal of Enterococcus faecalis from the root canal systems, another study was conducted recently [54].

It is crucial to emphasize that this study evaluated the decrease of microorganisms in contracted access cavities. As long as eradication of microorganisms is the final goal of root canal therapy, and here too no proof to suggest that using less aggressive access cavities is advantageous [26, 28].

It has been documented that root canals prepared using a variety of tools and methods nevertheless contain unprepared portions. Biofilm colonization in these regions might result in the persistence of infection in the root canals.[30, 31].

Nearly studies have found similar outcomes concerning unprepared area when dissimilar access cavities were compared to other studies, the use of these conservative access designs is undoubtedly more difficult compared with conventional one [6, 52]

The almost of these contracted accesses are recognized to be extremely difficult to execute, even in lab settings when the majority of the difficulties encountered during patient treatment are absent. In order to justify the incorporation of these approaches into practitioners' daily routines, there must be a solid proof of the substantial benefits these types access[13].

Although these approaches have been touted for their potential advantages, there is currently little data to back up their usage, which makes it difficult to justify or substantiate the advice given to practitioners to include these techniques into their routine endodontic processes. The current study's results led to null hypothesis refusal. To assess the viability of employing conservative access cavities in terms of enhancing the teeth's resistance to fracture and the instrumentation's capacity for cleaning, more research is advised.

VI. Limitations

1. Collecting the freshly extracted teeth for periodontal reasons.
2. Limited number of samples used.
3. Minimal endodontic accesscavities (Trussand Pointedaccess Cavities) are difficult to perform and require special tools.
4. Confocal laser scanning electron microscope is not considered to be the best method for evaluation, as it doesn't differ between live and dead bacteria, unlike molecular investigation.
5. Inoculation and culture of bacteria in extracted teeth is a very sensitive technique.

CONCLUSIONS

Within the limitations of the current study, the following can be concluded:

1. Using both NaOCl and ChloroExtra lead to reduction in bacterial count.
2. Conventional endodontic access cavities are still considered the gold standard in root canal treatment.
3. In terms of bacteria reduction and cleaning capabilities, minimal access cavities did not provide any advantages over traditional endodontic access canals, omparable outcomes to standard endodontic access cavities can be achieved using truss endodontic access canals in terms of microbiological reduction.

4. Sodium hypochlorite irrigation is more effective in microbial reduction regardless of endodontic access cavity design used in comparison to ChloroExtra.

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