ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 12 | December - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

COMPARISON AND EVALUATION OF PERIAPICAL HEALING OBTAINED BY USING ANTI-FUNGAL AS AN ADJUNCT INTRACANAL IRRIGATION TECHNIQUE TO STANDARD DISINFECTION PROTOCOL USED IN TYPE 2 DIABETES MELLITUS PATIENTS FOR REDUCING CANDIDA *ALBICANS* COUNT.

Endodontics

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ABSTRACT

Diabetes mellitus significantly increases the risk and severity of endodontic infections, with *Candida albicans* being highly prevalent in type 2 diabetic patients. Its dentinophilic behavior and ability to invade dentinal tubules contribute to persistent infections and treatment failure. Because routine irrigants show limited antifungal activity, the need for an effective adjunctive antifungal protocol is evident. This study compares an antifungal irrigation technique with the standard protocol to enhance disinfection and periapical healing. **Method And Materials:** Fifty type 2 diabetic patients requiring endodontic therapy in mandibular premolars were assigned to two groups: Group 1 (n=25) received 2% sodium hypochlorite and 17% EDTA, while Group 2 (n=25) received 1% clotrimazole. Microbial samples were collected before and after irrigation using paper points and cultured for *Candida albicans* CFU analysis. **Results:** Mann–Whitney U test revealed a significant reduction in CFU counts, with complete inhibition in Group 2. Periapical healing based on Strindberg criteria was superior in Group 2.**Conclusion:** Clotrimazole (1%) can serve as an effective adjunctive irrigant to improve endodontic outcomes in diabetic patients.

KEYWORDS

Sodium hypochlorite, Clotrimazole, CFU, Strindberg criteria.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia due to impaired insulin secretion or insulin resistance. Type 2 DM (T2DM), the most common form, affects over 9% of adults globally and is rapidly increasing, particularly in India—the "diabetes capital of the world" [1–4]. DM impairs immunity, delays healing, and predisposes individuals to oral complications such as periodontal disease, hypo salivation, and increased bacterial and fungal infections [5]. Diabetic patients exhibit greater severity of gingivitis and periodontitis due to altered host response and microvascular changes [6–8].

The association between DM and endodontic disease is increasingly evident. Apical periodontitis (AP) shares systemic risk factors with periodontal disease [9], and fungi-especially *Candida albicans*-are now recognized contributors to persistent endodontic infections [10]. *C. albicans* is the most frequently isolated fungus from infected canals, with prevalence ranging from 1–17% [11–12]. Diabetics demonstrate higher oral carriage (up to 77%) [13], facilitated by its dentinophilic, thigmotropic behaviour and enhanced adhesion in the presence of smear layer or after EDTA/NaOCl treatment [14].

Although irrigants like NaOCl and chlorhexidine possess antifungal activity, no standardized protocol effectively eliminates *C. albicans*. Clotrimazole has shown promising antifungal potential [15]. Since diabetics exhibit larger periapical lesions, delayed healing, and higher failure rates [16–17], and limited Indian data exist [18], this study aimed to compare periapical healing in T2DM patients using an antifungal adjunct irrigation technique versus standard protocols by assessing reduction in *C. albicans* counts.

MATERIAL AND METHOD:

The study was conducted in the outpatient department of Conservative Dentistry and Endodontics, Bhabha College of Dental Sciences , Bhopal from May 2023-May 2025 after approval from the Institutional Ethics Committee Reference no.BCDS/DEAN/2023/3452.

The study included 50 patients aged 40–65 years with type 2 diabetes mellitus and controlled HbA1c levels (>6.5 to <7). Eligible patients had irreversible pulpitis and apical periodontitis in mandibular premolars with lesions <5 mm. Exclusion criteria included age >65 years, poorly controlled diabetes (HbA1c >7.5), other systemic diseases, pregnancy or lactation, medical compromise, grossly decayed teeth, aggressive periodontitis, lesions >5 mm, or fewer than 14 remaining teeth.

Pre-operative evaluation included clinical examination, recording of height, weight, and HbA1c, and assessment of pain, tenderness, sinus tract, and mobility. Periapical status was assessed using digital radiovisiography, and each tooth was assigned a PAI score, with ≥3 indicating pathology.

Four blinded endodontists (two postgraduate students and two specialists) evaluated the radiographs using reference images. In doubtful cases, the higher score was selected. The average of the four scores was rounded to the nearest whole number, with mid-values adjusted based on the most experienced observer.

Table 1: PAI Score Radiographic Findings

PAI Score	Description of Radiographic Findings
1	Normal periapical structure
2	Small changes in bony structures
3	Change in bony structures with mineral loss
4	Apical periodontitis with well defines radiolucent area
5	Severe apical periodontitis with and exacerbating features.

All of the subjects were equally and randomly divided into two groups (n=25) Group1- Sodium hypochlorite and EDTA , Group 2- Irrigation with 2 ml of 1% Clotrimazole (Candid Mouth paint, Glen mark, India). Before root canal treatment, local anesthesia (2% lignocaine with 1:200000 adrenaline) was given as required. Caries and weakened tooth structure were removed, and the tooth was isolated with a rubber dam. An access cavity was prepared using a BR-41 bur under water coolant, and canals were located with a DG-16 explorer. The first sample (S1) was collected with a sterile No. 15 paper point, placed in 2 mL phosphate-buffered saline, and vortexed. Initial canal negotiation was performed with a No. 15 K-file, and working length was determined using an apex locator.Coronal enlargement was done with a Hyflex CM Orifice Opener (25/0.08) at 250–400 rpm and 2.5 Ncm torque, followed by complete canal preparation using Hyflex CM rotary files (20/0.04, 20/0.06, 25/0.06, 30/0.04) at 300-500 rpm and 2.5 Ncm torque.

After cleaning and shaping, the smear layer was removed with 17% EDTA, followed by disinfection with the assigned irrigant and activation using the EndoActivator. The second sample (S2) was collected, canals were flushed with saline, and obturation was completed. Samples were cultured on Sabouraud Dextrose Agar, incubated, and Candida CFU counts were recorded. Subjects were recalled for follow-up, where two blinded endodontists assessed periapical healing using the PAI Index and Strindberg criteria (Table 2).

Table 2: Strindberg Criteria.

Criteria	Clinical symptoms	Radiographic Findings
Success	No symptoms	Contours and width of the periodontal ligament (PDL) are normal PDL contours are widened mainly around excess root filling Lamina dura is intact

Failure	Symptoms Present	Unchanged periradicular rarefaction Decrease in periradicular rarefaction, but no resolution Appearance of new rarefaction or an increase in the size of initial rarefaction Discontinuous or poorly defined lamina dura.

Figure1: Access cavity preparation of lower mandibular premolar under rubber dam.

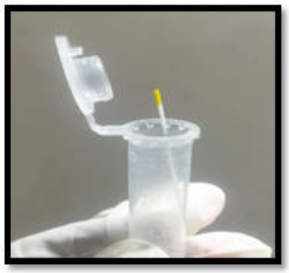


Figure 2: Sample collection in Eppendorf tube.

Microbiology Procedures:

A 0.5 McFarland standard suspension of *Candida albicans* was prepared, and 0.3 mL was inoculated into each of the 50 paper-point samples. The samples were incubated at 36°C and 91% humidity for 72 hours. At 48 hours, aliquots were plated on 4% Sabouraud Dextrose Agar to confirm fungal growth. After 72 hours, samples were rinsed three times with sterile PBS, and aliquots were plated again onto fresh SDA using a 1-µm inoculation loop. These plates were incubated for 48 hours, after which *C. albicans* colony-forming units (CFUs) were counted under 400× magnification to assess antifungal activity. (Figure 3-4)

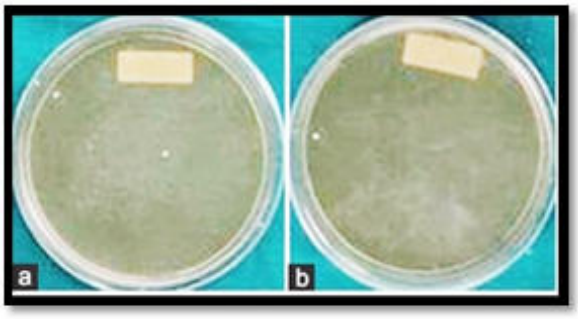


Figure 3: Candida albicans count on sabaouraud dextrose agar-(a) Preoperative count in group 1; (b) Postoperative count in group 1

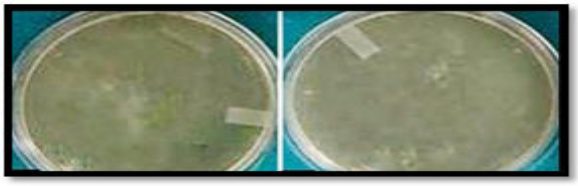


Figure 4: Candida albicans count on Sabaouraud dextrose agar-(L) Preoperative count in group 2; (R) Postoperative count in group 2

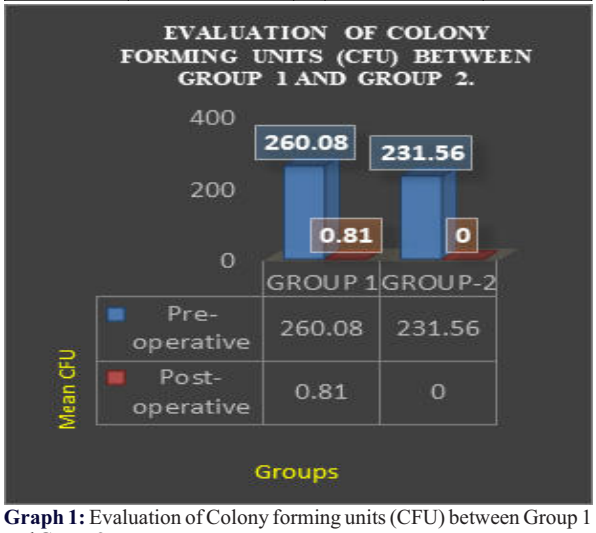
Statistical tests were performed using the SPSS software (SPSS 16 Inc., Chicago, IL, USA). Data were analyzed statistically using the, Mann–Whitney U-test.The level of statistical significance was set at $P < 0.05$.

RESULT:

The parameters studied for root canal irrigation protocols were the Pre and Postoperative CFU of C. albicans. The mean preoperative and postoperative fungal CFU values are shown in Table 1 and graphically depicted in Graph 1.

Table No. 1: Comparison Of Colony Forming Units Between Group 1 And Group 2.

Groups	CFU	N	Mean Rank	P-Value
Group-1	Pre-op CFU	25	31.82	0.000
	Post-op CFU	25	19.18	
Group-2	Pre-op CFU	25	33.5	0.000
	Post-op CFU	25	17.5	



Graph 1: Evaluation of Colony forming units (CFU) between Group 1 and Group 2.

Table 1 and Graph 1 show a highly significant difference in CFU counts ($p = 0.000$). Group 2 (antifungal irrigant) achieved complete postoperative CFU elimination, while Group 1 (NaOCl) showed a mean CFU of 0.816. This demonstrates the superior antimicrobial efficacy of the antifungal irrigant, with other variables remaining comparable.

Factors	Group	N	Mean	Std. Deviation	P-Value
CFU-1 pre-op	1	25	260.08	29.624	0.002
	2	25	231.56	28.771	
CFU-2 postop	1	25	0.816	0.7335	0.000
	2	25	0	0	

Table 2 shows a significant difference between pre- and post-operative CFU in both groups ($p = 0.000$), indicating a statistically meaningful reduction in CFU levels.

Groups	PAI	N	Mean Rank	P-Value
Group-1	Pre-op	25	37.48	0.000
	Post-op	25	13.52	
Group-2	Pre-op	25	37.4	0.000
	Post-op	25	13.6	

Table 3 shows a significant difference in pre- and post-operative PAI scores between the groups ($p = 0.000$). Both groups showed improvement, with Group 2 demonstrating slightly better periapical healing than Group 1.

Groups	Failure	Success	Total	P-Value
Group-1	2	23	25	0.153
	8.00%	92.00%	100.00%	
Group-2	0	25	25	
	0.00%	100.00%	100.00%	

Table 4 shows the distribution of Strindberg Criteria for treatment outcomes. Group 1 demonstrated a 92% success rate, while Group 2 achieved 100% success. Failure rates were 8% in Group 1 and 0% in Group 2, indicating a higher overall success rate in Group 2.

DISCUSSION

Endodontic and periodontal infections are widespread globally. While the association between periodontal disease and diabetes is well established, fewer studies have evaluated the effect of diabetes on

endodontic conditions. Given the limited evidence on diabetes as a modifying factor, this cross-sectional study aimed to assess the prevalence of apical periodontitis (AP) and the incidence of root canal treatment (RCT) in type 2 diabetes mellitus (T2DM) patients.

Diabetes mellitus includes metabolic disorders marked by hyperglycemia caused by impaired insulin secretion or insulin resistance and affects over 9% of adults worldwide. India has one of the largest diabetic populations, projected to rise from 40.9 million in 2006 to 69.9 million by 2025 (ICMR 2006). HbA1c is the gold standard for glycemic monitoring, with ≤6.5% recommended for optimal control (AACE 2013).

Microorganisms such as *Enterococcus faecalis* and *Candida albicans* can persist in the root canal system despite treatment (Peciuliene et al. 2001; Molander et al. 1998) [18–19]. *C. albicans*, although a commensal, becomes pathogenic in compromised hosts due to dentin adhesion and tubule penetration. Its involvement in persistent infections—6/24 cases (Sundqvist et al.) [20] and 2/22 cases (Siqueira et al. 2004) [21]—highlights the need for targeted antifungal strategies.

NaOCl is widely used, but may require up to 30 minutes for full antifungal action (Vianna et al. 2004) [22]. Saurabh et al. (2010) showed enhanced efficacy with a final rinse of 1% Clotrimazole [23]. Guided by this, the present study compared NaOCl alone versus NaOCl followed by Clotrimazole, consistent with Maciel Filho et al. (2018) and Srinivasan et al. (2020) who reported superior antifungal outcomes with Clotrimazole [24–25].

Periapical status was assessed using the Periapical Index (PAI) by Ørstavik et al. (1986) [26], adopting broader criteria than earlier studies such as Britto et al. (2003) [27].

Given the strong association of *C. albicans* with persistent infections, irrigants with antifungal activity are essential. Although both groups showed reduced biofilms, complete elimination was not achieved. Residual fungi and age-related dentin changes highlight the need for adequate irrigant volume and contact time. Findings support using Clotrimazole as a final rinse to enhance antifungal action and improve periapical healing in T2DM patients.

CONCLUSIONS

Clotrimazole-based irrigation demonstrated superior antifungal efficacy, showing complete inhibition of *Candida albicans* CFU in diabetic patients' root canals. Although postoperative CFU difference was not statistically significant ($P > 0.05$), Clotrimazole consistently performed better than standard irrigants as an adjunct.

The findings support the use of 1% Clotrimazole as a cost-effective adjunct to standard irrigation protocols to improve endodontic outcomes in diabetic patients. Larger, long-term studies are recommended to validate its role in intracanal disinfection and periapical healing.

Further, extensive studies on large number of samples are required to establish more reliable results forming a conclusive recommendation to irrigation protocols in the infected root canals of diabetic patients.

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