doi.org/10.36721/PJPS.2025.38.3.REG.13980.1

Analysis of the therapeutic efficacy of tinidazole combined with compound chlorhexidine gargle in the treatment of oral infections caused by *Candida albicans* following orthodontics

Haibao Sun[1], Wei Feng[1], Xiaoling Wang[1], Peng Wang[2] and Dan Zheng[3]*

[1]Department of Orthodontics, Shaoxing Stomatological Hospital, Shaoxing City, Zhejiang Province, China
[2]Department of Periodontology, Shaoxing Stomatological Hospital, Shaoxing City, Zhejiang Province, China
[3]Department of Prosthodontics, Shaoxing Stomatological Hospital, Shaoxing City, Zhejiang Province, China

Abstract: This study evaluated the efficacy of tinidazole combined with compound chlorhexidine gargle in treating oral *Candida albicans* infections after orthodontic procedures. Sixty patients (July 2021–December 2023) were divided into an observation group (tinidazole + chlorhexidine, n=30) and a control group (chlorhexidine alone, n=30). After 7 days, the observation group showed significantly lower oral *Candida albicans* colony counts (t=6.536, P<0.05), a higher total clinical efficacy rate (96.67% vs. 80.00%, χ^2=4.043, P<0.05), reduced oral pain scores (t=12.420, P<0.05), lower periodontal indices (PLI and GI, t=18.884, 18.689, P<0.05), decreased levels of inflammatory markers (IL-1β, IL-6, TNF-α, and hs-CRP, t=5.859, 5.868, 3.436, 16.303, P<0.05), and improved oral health-related quality of life scores (t=8.693, 11.198, 5.086, 5.549, P<0.05). The incidence of adverse reactions was lower in the observation group (χ^2=0.577, P>0.05), with no recurrence observed. The combination therapy demonstrated superior clinical efficacy and safety compared to monotherapy. The combined application can effectively reduce the number of *Candida albicans* colonies in the oral cavity, alleviate oral pain, decrease the levels of inflammatory markers, improve periodontal health and oral health-related quality of life and exhibit good safety.

Keywords: Tinidazole; compound chlorhexidine gargle; oral *Candida albicans* infection, orthodontics; therapeutic efficacy

Submitted on 08-01-2025 – Revised on 27-01-2025 – Accepted on 04-04-2025

INTRODUCTION

Oral infections caused by fungi belonging to the *Candida* genus, notably *Candida albicans* (Lopes and Lionakis, 2022), typically present with clinical symptoms including white pseudomembrane, erythematous plaques, erosion, or ulceration on the oral mucosa (Ponde *et al.*, 2021). *Candida* fungi adhere to and invade oral mucosal epithelial cells by producing adhesins and invasive enzymes, leading to cellular damage and inflammatory responses (d'Enfert *et al.*, 2021). They can even form biofilms in the oral cavity, increasing their resistance to antimicrobial drugs and making treatment more difficult (Lin *et al.*, 2021). Oral *Candida albicans* infections are more prevalent in children and immunocompromised individuals, especially newborns and patients undergoing orthodontic treatment (de Arruda *et al.*, 2024). Orthodontic treatment improves oral function by adjusting the position of teeth and jaws and enhances aesthetics (Coppola *et al.*, 2023), but the disruption of the oral microenvironment during treatment significantly increases the risk of infection (Shimada *et al.*, 2022). The presence of orthodontic appliances such as brackets and archwires increases the difficulty of cleaning, making plaque more prone to accumulation and providing favorable conditions for the growth and overproliferation of *Candida* (Hosseini *et al.*, 2024; Robbins *et al.*, 2023). Additionally, oral mucosal damage that may result from orthodontic treatment provides an opportunity for *Candida* invasion (Freitas, 2022; Han *et al.*, 2024). Oral *Candida albicans* infection not only causes oral discomfort but may also affect the progress and efficacy of orthodontic treatment. Effective prevention and treatment of oral *Candida albicans* infection after orthodontic treatment have now become important topics in clinical research.

Compound chlorhexidine gargle consists of chlorhexidine gluconate and metronidazole. Chlorhexidine gluconate, a broad-spectrum antibacterial agent, disrupts the membrane permeability barrier of pathogenic microorganisms, causing the leakage of cellular contents and inhibiting bacterial-associated metabolic enzymes, thereby effectively exerting its antibacterial effect (Contaldo *et al.*, 2023; Maziere *et al.*, 2024; Wade *et al.*, 2021). Compound chlorhexidine gargle exhibits good inhibitory effects on a variety of bacteria and fungi and is currently commonly used in the clinical treatment of gingivitis, periodontitis and oral mucosal inflammation (Adam *et al.*, 2023). As a nitroimidazole derivative, tinidazole enters susceptible microbial cells and, in low-oxygen or anaerobic environments, is reduced by electron transport proteins to form cytotoxic amino radicals (Pereira Sousa and Kogawa, 2023). These radicals inhibit cellular DNA synthesis, promote DNA degradation and block bacterial DNA metabolism, thereby inhibiting the growth of anaerobic bacteria and achieving an antibacterial effect (Shao *et al.*, 2024), which in turn reduces the occurrence of periodontal

**Corresponding author:* e-mail: skzhengdan@hotmail.com

diseases and oral infections (Huang *et al.*, 2021). Previous research has demonstrated that the combination of tinidazole and compound chlorhexidine gargle is highly efficacious in treating periodontal diseases, the combined application of these two agents significantly decreases the periodontal attachment index, plaque index and levels of inflammatory cytokines in patients with periodontal diseases, with clinical efficacy superior to that of tinidazole monotherapy (Lei, 2021).

The complexity of oral *Candida albicans* infections often makes it difficult to achieve ideal therapeutic effects with monotherapy (Parlatescu *et al.*, 2021). Tinidazole and compound chlorhexidine gargle, as two commonly used antibacterial agents, have demonstrated good efficacy when used individually. Tinidazole controls the growth and reproduction of *Candida albicans* at the source by inhibiting the synthesis and replication of bacterial DNA (Augostini *et al.*, 2023). Compound chlorhexidine gargle directly kills *Candida albicans* by disrupting cell membranes and inhibiting DNA metabolism (Rajendiran *et al.*, 2021).The simultaneous use of these two agents could potentially lead to a synergistic effect, improving treatment results. Additionally, the local application form of tinidazole allows the drug to directly act on the lesion site, improving drug utilization and bactericidal efficacy. The use of compound chlorhexidine gargle in the form of rinsing also enhances patients' self-care abilities and treatment adherence. The objective of this study is to explore the effectiveness of combining tinidazole with compound chlorhexidine gargle for treating oral *Candida albicans* infections post-orthodontic treatment, ultimately aiming to establish a more robust scientific foundation for clinical management of these infections and enhance oral hygiene protocols post-orthodontics.

MATERIALS AND METHODS

Sample size estimation and study design

This study is a retrospective clinical controlled study conducted in the clinical center of our hospital for diagnosis and treatment. The study employed an independent samples t-test for data analysis and utilized G-power software for sample size estimation. During the estimation process, we selected an effect size of 0.8, set the significance level (α) at 0.05 (for a two-tailed test) and the statistical power (1-β) at 0.8. Based on these parameters, the calculated sample size was 26 participants per group, i.e., N1=N2=26, with a total sample size of 52. The actual number of participants recruited for the experiment was 60, exceeding the planned sample size, thus meeting the statistical requirements of the study design. The designed operational procedure is illustrated in fig. 1.

Study population

Sixty patients with oral *Candida* infections after orthodontic treatment between July 2021 and December 2023 were selected. Based on different treatment protocols, they were categorized into observation group (30 patients) and control group (30 patients).

Inclusion criteria: (1) Clinical symptoms and signs were consistent with oral *Candida* infections, with positive results in pathogen examination; (2) Had undergone orthodontic treatment recently; (3) Age ≥ 18 years; (4) Complete clinical data; (5) Informed consent was obtained.

Exclusion criteria: (1) History of recent antifungal, antibiotic, or steroid treatment; (2) Pre-existing oral *Candida* infections or other oral mucosal diseases, such as leukoplakia, before orthodontic treatment; (3) Presence of systemic diseases such as diabetes, hyperthyroidism and hypoproteinemia; (4) Severe abnormalities in heart, liver, or kidney function or immune deficiency; (5) Mental illness or communication disorders; (6) Pregnant or lactating women.

Treatment methods

Control Group: Patients were treated with compound chlorhexidine gargle. After brushing teeth in the morning and evening, 10-20 mL of compound chlorhexidine gargle was placed in the mouth and thoroughly gargled for at least 2-5 minutes before being spat out. This treatment was continued for 7 consecutive days.

Observation Group: Patients were treated with tinidazole combined with compound chlorhexidine gargle. The usage of compound chlorhexidine gargle was the same as in the control group. Tinidazole was given with an initial dose of 2 g, followed by 1g daily, taken 0.5 hours after meals for a total duration of 3 consecutive days.

Observed indexes

(1) *Colony Count of Oral Candida*: Prior to sampling, participants were advised to avoid eating, drinking, smoking for 30 minutes and to rinse their mouths with drinking water. A sterile, disposable syringe was used to collect 0.5 mL of saliva. Mix the saliva sample thoroughly and evenly spread it onto the surface of CHROMagar Candida medium. Place the inoculated plates in a 37°C incubator and incubate for 24 hours. On CHROMagar Candida medium, *Candida albicans* colonies typically appear as green or emerald green, raised, circular colonies. Colony count was done using a digital colony counter.

(2) *Clinical Efficacy: Marked efficacy*: Disappearance or significant improvement of symptoms such as oral pain, white plaques, burning sensation and redness of oral mucosa, dry mouth, tongue pain and gingival bleeding; Effective: Some improvement in symptoms; Ineffective: The aforementioned criteria were not met. Total effective rate = marked efficacy cases + effective outcome cases / total cases × 100%.

(3) *Oral Pain Score*: the visual analog scale (VAS)

(Haapiainen *et al.*, 2023) was used for assessment. The score ranged from 0 to 10, which categorized into mild pain (0-3), moderate pain (4-6) and severe pain (7-10).

(4) *Periodontal Indices*: the plaque index (PLI) (Li *et al.*, 2023) and gingival index (GI) (Shen and Yu, 2021) was used for assessment. PLI scoring criteria: 0 for no plaque; 1 for fine plaque at the gingival margin; 2 for a narrow band of plaque ≤1mm wide; 3 for plaque covering ≥1mm but <1/3 of the tooth surface; 4 for plaque covering 1/3 to 2/3 of the tooth surface; and 5 for plaque covering ≥2/3 of the tooth surface. GI scoring criteria: 0 for healthy gingiva; 1 for mild gingival color change with mild edema and no bleeding on probing; 2 for red, shiny gingiva with bleeding on probing; and 3 for markedly red and swollen gingiva or ulceration with a tendency for spontaneous bleeding.

(5) *Inflammatory Markers*: The levels of IL-1β, IL-6, TNF-α and hs-CRP were measured using ELISA. Fasting venous blood samples were collected, centrifuged and the supernatants were assayed according to the kit's instructions to determine the levels of these inflammatory markers.

(6) *Oral Health-Related Quality of Life*: The Oral Health Impact Profile-14 (OHIP-14) (Yuwanati *et al.*, 2021) was used for assessment. The scale covers four dimensions: pain and discomfort, functional limitation, psychological disability and physical disability. The overall score ranges from 0 to 56, with higher scores reflecting poorer oral health.

(7) *Adverse Reactions and Recurrence*: Adverse reactions including mucosal irritation, taste alteration, intraoral pigmentation, diarrhea, nausea and vomiting, were monitored. Follow-up was conducted for 3 months to record recurrences of oral *Candida* infections and assess the durability of treatment efficacy.

Ethical approval

This study was approved by the Ethics Committee of the Shaoxing Stomatological Hospital (2025-8-1).

STATISTICAL ANALYSIS

Quantitative data was analyzed using mean ± standard deviation (SD) depending on the data distribution while qualitative data was described by frequencies and percentages. Hypothesis testing was conducted at a significance level of $p<0.05$ using SPSS 25.

RESULTS

Baseline characteristics

Comparison of baseline characteristics revealed no statistical differences between the observation group (n=30) and the control group (n=30), minimizing the risk of confounding variables that may affect the study results (table 1).

Number of oral candida colonies

Table 2 compares the oral *Candida* colony counts between the two groups. Pre-treatment, there was no significant difference in oral *Candida* colony counts between the two groups (t=0.347, P>0.05). Post-treatment, the observation group exhibited fewer oral *Candida* colonies than the control group (t=6.536, P<0.05), indicating that the combined use of tinidazole and chlorhexidine gluconate oral rinse is more effective in inhibiting *Candida* growth than chlorhexidine gluconate oral rinse alone.

Clinical efficacy

The data in table 3 compares the clinical efficacy between the two groups. Following treatment, the observation group demonstrated a significantly higher total effective rate of clinical efficacy, at 96.67%, compared to 80.00% in the control group (χ^2=4.043, P<0.05).

Oral pain score

The data in table 4 compares oral pain intensity between the two groups. Post-treatment, both groups exhibited decreased VAS scores, the observation group had a lower VAS score compared to the control group (t=12.420, P<0.05). This suggests that compared to monotherapy, combination therapy is more effective in alleviating oral pain.

Periodontal index

The data in table 5 compares periodontal conditions between the two groups. Following treatment, both groups experienced reductions in PLI and GI indices. Notably, the observation group had lower PLI and GI indices compared to the control group (t=18.884, 18.689, P<0.05).

Inflammatory markers

The data in table 6 compares the levels of inflammatory cytokines between the two groups. Post-treatment, both groups exhibited significant reductions in the levels of IL-1β, IL-6, TNF-α and hs-CRP, however, the observation group had lower levels compared to the control group (t=5.859, 5.868, 3.436, 16.303, P<0.05).

Oral health quality

After treatment, pain and discomfort, functional limitations, psychological disability and physical disability scores were lower in the observation group than in the control group (t=8.693, 11.198, 5.086, 5.549, P<0.05) (table 7).

Adverse reactions and recurrence

Specifically, one case of recurrence was noted in the control group, whereas no recurrences were reported in the observation group. Additionally, the observation group had an adverse reaction incidence of 10.00%, which was numerically lower than the 16.67% observed in the control group (χ^2=0.577, P>0.05) (table 8).

Table 1: Baseline Characteristics

Parameter	Observation Group (n=30)	Control group (n=30)	χ^2/t value	*p*-value
Gender				
Male	16 (53.33)	17 (56.67)	0.067	0.795
Female	14 (46.67)	13 (43.33)		
Age (Years)	24.93±4.77	25.33±4.45	0.336	0.738
Education level				
College and below	18 (60.00)	20 (66.67)	0.287	0.592
Bachelor's degree and above	12 (40.00)	10 (35.33)		
Economic status				
Good	13 (43.33)	14 (46.67)	0.929	0.628
Medium	9 (30.00)	11 (36.67)		
Poor	8 (26.67)	5 (16.67)		
Orthodontic treatment type				
Orthodontic treatment	19 (63.33)	16 (53.33)	0.617	0.432
Fixed orthodontics	11 (36.67)	14 (46.67)		
Infection time (Days)	4.53±1.48	4.77±1.28	0.654	0.516
Infection site				
Oral mucosa	23 (76.67)	25 (83.33)	0.417	0.519
Lips	6 (20.00)	7 (23.33)	0.098	0.754
Tongue	9 (30.00)	8 (26.67)	0.082	0.774
Oral *Candida* colonization ($\times10^3$ CFU/mL)	76.59±11.21	77.68±12.95	0.347	0.730
Smoking status				
Never	11 (36.67)	9 (30.00)	0.300	0.584
Current / Former	19 (63.33)	21 (70.00)		
Alcohol consumption status				
Never	5 (16.67)	6 (20.00)	0.111	0.739
Current / Former	25 (83.33)	24(80.00)		
Brushing frequency				
once per day	9 (30.00)	7 (23.33)	0.476	0.788
Twice per day	19 (63.33)	20 (66.67)		
Three or more times per day	2 (6.67)	3 (10.00)		
History of oral disease				
Periodontal disease	6 (16.67)	8 (26.67)	0.373	0.542
Dental caries	17 (56.67)	19 (63.33)	0.278	0.598
History of prior antifungal therapy				
Yes	9 (30.00)	6 (20.00)	0.800	0.371
Three or more times per day	2 (6.67)	3 (10.00)		
No	21 (70.00)	24 (80.00)		

Table 2: Number of oral *candida* colonies ($\times10^3$ CFU/mL, $\bar{x}\pm s$)

Group	Pre-treatment	Post-treatment
Observation group (n=30)	76.59±11.21	31.44±7.75*
Control group (n=30)	77.68±12.95	44.20±7.37*
t-value	0.347	6.536
p-value	0.730	<0.001

Note: Compared with the same group before treatment, *P<0.05. The same applies hereinafter.

Table 3: Clinical efficacy [*n*(%)]

Group	Markedly	Effective	Ineffective	Total effective rate
Observation group (n=30)	20 (66.67)	9 (30.00)	1 (3.33)	29 (96.67)
Control group (n=30)	11 (36.67)	13 (43.33)	6 (20.00)	24 (80.00)
χ^2-value				4.043
p-value				0.044

Table 4: Periodontal index (score, $\bar{x}$±s)

Group	VAS	
	Pre-treatment	Post-treatment
Observation group (n=30)	5.22±1.16	1.90±0.47*
Control group (n=30)	5.15±1.49	3.92±0.76*
t-value	0.225	12.420
p-value	0.823	<0.001

Table 5: Periodontal index (score, $\bar{x}$±s)

Group	PLI		GI	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Observation group (n=30)	1.83±0.30	0.37±0.09*	2.88±0.43	0.47±0.15*
Control group (n=30)	1.89±0.37	0.88±0.12*	2.98±0.37	1.28±0.18*
t-value	0.697	18.884	0.940	18.689
p-value	0.489	<0.001	0.351	<0.001

Table 6: Inflammatory markers ($\bar{x}$±s)

Time	Group	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	hs-CRP (mg/dL)
Pre-treatment	Observation group (n=30)	28.18±7.00	25.11±7.67	39.95±8.78	0.90±0.34
	Control group (n=30)	28.28±5.73	26.63±7.80	40.05±10.09	0.74±0.38
	t-value	0.057	0.765	0.041	1.711
	p-value	0.955	0.447	0.967	0.092
Time	Group	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	hs-CRP (mg/dL)
Post-treatment	Observation group (n=30)	17.18±4.49*	17.33±2.04*	24.76±5.93*	0.12±0.34*
	Control group (n=30)	24.25±4.85*	21.45±3.25*	29.83±5.49*	0.27±0.39*
	t-value	5.859	5.868	3.436	16.303
	p-value	<0.001	<0.001	0.001	<0.001

Table 7: Oral health quality (score, $\bar{x}$±s)

Time	Group	Pain and discomfort	Functional limitations	Restricted abilities	Physical and mental impairments
Pre-treatment	Observation group (n=30)	7.43±1.14	6.87±1.53	9.60±2.34	5.13±0.86
	Control group (n=30)	7.23±1.28	6.23±1.38	9.23±2.27	5.00±0.87
	t-value	0.641	1.686	0.616	0.597
	p-value	0.524	0.097	0.541	0.553
Time	Group	Pain and discomfort	Functional limitations	Restricted abilities	Physical and mental impairments
Post-treatment	Observation group (n=30)	3.07±0.69*	3.03±0.67*	5.90±1.21*	3.10±0.84*
	Control group (n=30)	4.60±0.67*	4.97±0.67*	7.97±1.87*	4.13±0.57*
	t-value	8.693	11.198	5.086	5.549
	p-value	<0.001	<0.001	<0.001	<0.001

Table 8: Adverse reaction and recurrence [*n*(%)]

Group	Mucosal irritation	Altered taste	Intraoral pigmentation	Diarrhea	Nausea	Recurrence	Total rate
Observation group (n=30)	0 (0.00)	1 (3.33)	0 (0.00)	1 (3.33)	1 (3.33)	0 (0.00)	3 (10.00)
Control group (n=30)	1 (3.33)	2 (6.67)	1 (3.33)	0 (0.00)	0 (0.00)	1 (3.33)	5 (16.67)
χ^2-value							0.577
p-value							0.448

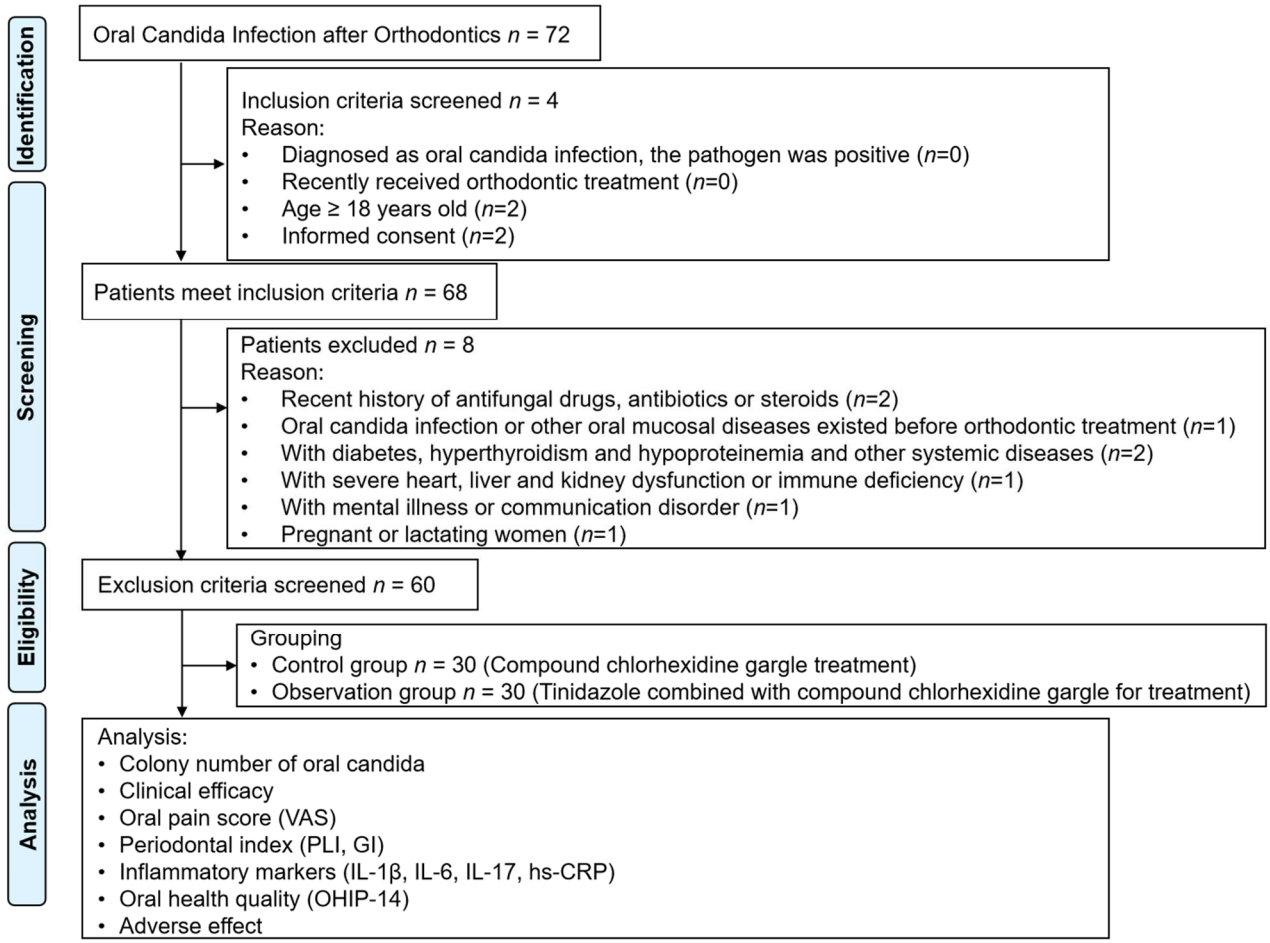

Fig. 1: Design operation process

DISCUSSION

Orthodontic treatment, through tooth movement and the application of corrective appliances, has effectively improved occlusion and oral aesthetics, gaining widespread popularity in recent years. Nonetheless, orthodontic treatment alters the oral microecological environment and appliances increase the challenge of maintaining oral hygiene, rendering patients highly susceptible to oral *Candida* infections (Kirschen *et al.*, 2021). As a common fungal infection, oral candidiasis not only causes oral pain and discomfort but may also affect the orthodontic treatment outcome and daily life quality of patients. Chlorhexidine gluconate oral rinse, a broad-spectrum antibacterial agent, finds extensive use in the management of oral diseases including gingivitis and pericoronitis. Its active ingredient, chlorhexidine gluconate and metronidazole, can effectively inhibit the growth of various bacteria and fungi, reducing inflammatory reactions. However, in some cases, the use of chlorhexidine gluconate oral rinse alone may be difficult to fully control the infection, especially in the complex and susceptible environment after orthodontic surgery (Abuhajar *et al.*, 2023; Ashrafizadeh, 2024; Ren, 2024). Tinidazole is a nitroimidazole derivative with broad-spectrum antibacterial and bactericidal properties.

Research indicates that tinidazole can suppress the growth of anaerobic bacteria in the oral cavity, alleviate oral inflammatory responses and assist in managing oral *Candida* infections. The combined use of tinidazole and chlorhexidine gluconate oral rinse may enhance the antibacterial effect through synergistic action, thereby improving the treatment outcome of oral *Candida* infections post-orthodontics. The objective of this study is to assess the effectiveness of tinidazole in combination with chlorhexidine gluconate oral rinse in treating oral *Candida* infections post-orthodontic treatment, aiming to offer a more potent combined treatment approach for clinical diagnosis and management.

The results indicate that after treatment, the observation group exhibited a reduced number of oral *Candida* colonies, superior clinical effectiveness and lower PLI and GI scores compared to the control group. These findings suggest that the combined application of tinidazole and chlorhexidine gluconate oral rinse demonstrates positive clinical outcomes in the treatment of oral *Candida* infections following orthodontic treatment. Compared to the sole use of chlorhexidine gluconate oral rinse, the combined therapy demonstrated a more significant reduction in the number of *Candida* colonies in the oral cavity and an improvement in periodontal health. The main components of chlorhexidine gluconate oral rinse are chlorhexidine gluconate and metronidazole. Chlorhexidine gluconate, as a broad-spectrum antibacterial agent, adsorbs onto the surface of oral mucosa, modifying bacterial cell membrane permeability and leading to the leakage of bacterial contents. This process inhibits bacterial growth and eliminates bacteria (Pettas *et al.*, 2021). Metronidazole has an anti-anaerobic effect, effectively inhibiting the reproduction of anaerobic bacteria. When the oral rinse is used for mouthwash, the medication adsorbs onto the negatively charged surfaces of teeth, dental plaque and oral mucosa, followed by diffusion and gradual release, producing a sustained antibacterial effect (Karajacob *et al.*, 2023). Therefore, chlorhexidine gluconate oral rinse can improve the oral microenvironment and reduce inflammation through its broad-spectrum antibacterial activity. Tinidazole, as a nitroimidazole antibacterial agent, primarily targets most anaerobes and certain aerobes. It exerts its bactericidal effect by inhibiting dihydrofolate reductase in bacterial DNA synthesis, thereby blocking bacterial DNA replication and repair (Phan *et al.*, 2023). Research indicates that tinidazole exhibits a favorable supplementary therapeutic effect in the treatment of periodontal and oral infectious diseases. Due to its long half-life, tinidazole is more effective in treating chronic infectious oral diseases (Korbecka-Paczkowska and Karpiński, 2024). The combined application of tinidazole and chlorhexidine gluconate oral rinse maintains oral cleanliness and smooth tooth surfaces by rinsing away residual food debris, soft debris, dental plaque and free bacteria in gingival sulcus or the oral cavity. This reduces the number of bacteria in the oral cavity. In addition, the administration of tinidazole further inhibits bacterial growth, prevents pathogenic bacteria from residing on tooth surfaces and in periodontal pockets and decreases supragingival plaque accumulation (Salehi *et al.*, 2022). In summary, the combination of tinidazole and chlorhexidine gluconate oral rinse can effectively reduce the number of *Candida* colonies in the oral cavity and enhance periodontal and oral health.

The findings further suggest that post-treatment, the observation group exhibited lower VAS scores, reduced levels of IL-1β, IL-6, TNF-α and hs-CRP, lower scores for pain, discomfort, functional and capacity limitations and psychophysical impairments, as well as a decreased incidence of adverse reactions compared to the control group. The results indicate that the use of tinidazole in combination with chlorhexidine gluconate oral rinse can efficiently diminish oral pain, decrease inflammatory marker levels, enhance oral health quality and demonstrate favorable safety in patients with oral *Candida* infections post-orthodontic treatment. The active ingredients of chlorhexidine gluconate oral rinse include strong peppermint extract, which possesses a potent cooling effect, providing a refreshing sensation to the skin and mucosa (Poyil *et al.*, 2024). Additionally, it promotes effective vasoconstriction on the internal surfaces of the oral cavity, alleviating pain and discomfort in the oral and pharyngeal regions and accelerating oral blood circulation and recovery (Siwach and Verma, 2021). The use of chlorhexidine gluconate oral rinse through rinsing enhances patients' self-care abilities and treatment adherence, further accelerating the improvement of oral health quality (Wood *et al.*, 2023). The local application form of tinidazole allows the drug to directly act on the lesion site, significantly enhancing drug utilization and bactericidal efficacy. Research has demonstrated that the use of tinidazole combined with chlorhexidine gluconate oral rinse in periodontal disease patients leads to a notable decrease in the concentrations of inflammatory cytokines (Li *et al.*, 2021). The decrease in inflammatory cytokine levels helps to reduce the inflammatory response of oral mucosa, further alleviating pain (Galler *et al.*, 2021; Thomas *et al.*, 2022). Chlorhexidine gluconate oral rinse exerts a broad-spectrum antibacterial effect against various bacteria and fungi, thereby decreasing the population of harmful oral bacteria and creating a more conducive environment for tinidazole to exert its bactericidal action (Nomura *et al.*, 2020). In turn, tinidazole targets specific anaerobes and aerobes for eradication, further inhibiting the growth of *Candida*, alleviating inflammatory responses and relieving oral pain (Zhao *et al.*, 2024). The combination of the two agents can more comprehensively cover the major pathogenic bacteria in the oral cavity, reducing the potential for drug resistance that may arise with single-drug therapy, enhancing the safety of the treatment regimen and lowering the recurrence rate of

infections, thereby facilitating the improvement of oral health quality (Harnan *et al.*, 2024).

This study has certain limitations, primarily including the lack of a positive control group using standard antifungal drugs (such as fluconazole or nystatin) in the design of the control group and the failure to conduct stratified analysis on confounding factors such as orthodontic appliance materials or patient oral hygiene habits, which may have affected the comprehensiveness and accuracy of the results. Additionally, although a 3-month follow-up period was set, the study duration was still relatively short, making it impossible to fully determine whether infections would recur after treatment. Lastly, the study did not discuss the risk of tinidazole resistance, which is an important issue that cannot be ignored in clinical applications. To address these limitations, future studies will consider adding a positive control group using standard antifungal drugs to more accurately assess the antifungal effect of tinidazole. Furthermore, the sample size will be expanded and stratified analysis will be conducted, taking into full consideration the impact of confounding factors such as the type of orthodontic appliance and patient oral hygiene habits on the results. The follow-up period will also be extended to more comprehensively assess the treatment effect and the risk of infection recurrence. At the same time, an assessment of the risk of tinidazole resistance will be included to gain a more comprehensive understanding of the potential issues associated with the clinical use of this drug.

CONCLUSION

The combined application of tinidazole and chlorhexidine gluconate oral rinse has demonstrated favorable clinical outcomes in the treatment of oral *Candida* infections following orthodontic procedures. Compared to the use of chlorhexidine gluconate oral rinse alone, the combined therapy exhibits a more potent effect in decreasing the *Candida* colony count in the oral cavity, thus effectively managing the progression of infection. Additionally, it significantly alleviates oral pain, decreases serum levels of inflammatory markers, mitigates inflammatory responses and promotes oral tissue repair and healing. The combined treatment can also effectively enhance periodontal health and improve oral health quality with good safety, rendering it a viable option for clinical promotion and implementation.

Conflict of interest statement

All authors declare no conflict of interest.

REFERENCES

Abuhajar E, Ali K, Zulfiqar G, Al Ansari K, Raja HZ, Bishti S and Anweigi L (2023). Management of chronic atrophic candidiasis (denture stomatitis)-A narrative review. *Int. J. Environ. Res. Public Health*, **20**(4): 3029.

Adam FA, Mohd N, Rani H, Mohd Yusof MYP and Baharin B (2023). A systematic review and meta-analysis on the comparative effectiveness of Salvadora persica - extract mouthwash with chlorhexidine gluconate in periodontal health. *J. Ethnopharmacol*., **302**(Pt A): 115863.

Ashrafizadeh M (2024). Cell death mechanisms in human cancers: molecular pathways, therapy resistance and therapeutic perspective. *J Can Biomol Therap*, **1**(1): 17-40.

Augostini P, Bradley ELP, Raphael BH and Secor WE (2023). *In vitro* testing of trichomonas vaginalis drug susceptibility: Evaluation of minimal lethal concentrations for metronidazole and tinidazole that correspond with treatment failure. *Sex Transm. Dis*., **50**(6): 370-373.

Contaldo M, Di Stasio D, Romano A, Fiori F, Della Vella F, Rupe C, Lajolo C, Petruzzi M, Serpico R and Lucchese A (2023). Oral candidiasis and novel therapeutic strategies: Antifungals, phytotherapy, probiotics and photodynamic therapy. *Curr. Drug Deliv*., **20**(5): 441-456.

Coppola G, Christopoulou I, Gkantidis N, Verna C, Pandis N and Kanavakis G (2023). The effect of orthodontic treatment on smile attractiveness: A systematic review. *Prog. Orthod*., **24**(1): 4.

d'Enfert C, Kaune AK, Alaban LR, Chakraborty S, Cole N, Delavy M, Kosmala D, Marsaux B, Fróis-Martins R, Morelli M, Rosati D, Valentine M, Xie Z, Emritloll Y, Warn PA, Bequet F, Bougnoux ME, Bornes S, Gresnigt MS, Hube B, Jacobsen ID, Legrand M, Leibundgut-Landmann S, Manichanh C, Munro CA, Netea MG, Queiroz K, Roget K, Thomas V, Thoral C, Van den Abbeele P, Walker AW and Brown AJP (2021). The impact of the Fungus-Host-Microbiota interplay upon *Candida albicans* infections: Current knowledge and new perspectives. *FEMS Microbiol Rev.*, **45**(3): fuaa060.

De Arruda JAA, Oliveira SR, Heimlich FV, de Amorim-Santos BM, Schneider AH, de Sena A, Rodrigues KES, Macari S, Souza DG, Travassos DV, Abreu LG, Silva TA and Mesquita RA (2024). Kinetics of neutrophil extracellular traps and cytokines in oral mucositis and Candida infection. *Oral Dis*., **30**(7): 4751-4761.

Freitas MPM (2022). Aligners, environmental contamination and the role of orthodontics. *Angle Orthod*., **92**(1): 148-149.

Galler KM, Weber M, Korkmaz Y, Widbiller M and Feuerer M (2021). Inflammatory response mechanisms of the dentine-pulp complex and the periapical tissues. *Int. J. Mol. Sci*., **22**(3): 1480.

Haapiainen H, Valli J, Murtola T, Huhtala H, Kaipia A and Raitanen M (2023). Visual analogue scale (VAS) in the evaluation of functional outcomes after three-dimensional laparoscopic prostatectomy. *Urology*, **172**: 121-125.

Han SH, Ko Y, Ham LK, Park JH and Kim Y (2024). Precautions and possibilities in orthodontic treatment of

periodontally compromised patients: Current recommendations. *J. Esthet. Restor. Dent*., **36**(4): 595-605.

Harnan S, Kearns B, Scope A, Schmitt L, Jankovic D, Hamilton J, Srivastava T, Hill H, Ku CC, Ren S, Rothery C, Bojke L, Sculpher M and Woods B (2024). Ceftazidime with avibactam for treating severe aerobic Gram-negative bacterial infections: Technology evaluation to inform a novel subscription-style payment model. *Health Technol. Assess*., **28**(73): 1-230.

Hosseini HR, Ngan P, Tai SK, Andrews LJ, 2nd and Xiang J (2024). A comparison of skeletal and dental changes in patients with a Class II relationship treated with clear aligner mandibular advancement and Herbst appliance followed by comprehensive orthodontic treatment. *Am J. Orthod. Dentofacial. Orthop*., **165**(2): 205-219.

Huang J, Xue J and Gu J (2021). Effects Of Minocycline Combined With Tinidazole For Treatment Of Chronic Periodontitis. *Clin. Invest. Med*., **44**(3): doi: 10.25011/cim.v44i3.36477.

Karajacob AS, Azizan NB, Al-Maleki ARM, Goh JPE, Loke MF, Khor HM, Ho GF, Ponnampalavanar S and Tay ST (2023). Candida species and oral mycobiota of patients clinically diagnosed with oral thrush. *PLoS One*, **18**(4): e0284043.

Kirschen R, Littlewood SJ, Blazewska-Amin A and Fleming PS (2021). Bonded orthodontic retention: A practical guide. *Br. Dent. J*, **230**(11): 709-716.

Korbecka-Paczkowska M and Karpiński TM (2024). *In vitro* assessment of antifungal and antibiofilm efficacy of commercial mouthwashes against *Candida albicans*. *Antibiotics*, **13**(2): 117.

Lei L (2021). Curative effect of compound chlorhexidine gargle combined with tinidazole on periodontal disease. *Chin J Mod Med*, **33**(29): 149-152.

Li B, Lu C, Yao X, Wu X, Wu G and Zeng X (2023). Effects of three orthodontic retainers on periodontal pathogens and periodontal parameters. *Sci. Rep*., **13**(1): 20709.

Li Z, Pan W, Shi E, Bai L, Liu H, Li C, Wang Y, Deng J and Wang Y (2021). A multifunctional nanosystem based on bacterial cell-penetrating photosensitizer for fighting periodontitis via combining photodynamic and antibiotic therapies. *ACS Biomater Sci Eng*, **7**(2): 772-786.

Lin D, Yang L, Wen L, Lu H, Chen Q and Wang Z (2021). Crosstalk between the oral microbiota, mucosal immunity and the epithelial barrier regulates oral mucosal disease pathogenesis. *Mucosal. Immunol*., **14**(6): 1247-1258.

Lopes JP and Lionakis MS (2022). Pathogenesis and virulence of *Candida albicans*. Virulence, **13**(1): 89-121.

Maziere M, Rompante P, Andrade JC and Rodrigues CF (2024). Are mouthwashes really effective against Candida Spp. *J Fungi*, **10**(8): 528.

Nomura R, Inaba H, Matayoshi S, Yoshida S, Matsumi Y, Matsumoto-Nakano M and Nakano K (2020). Inhibitory effect of a mouth rinse formulated with chlorhexidine gluconate, ethanol and green tea extract against major oral bacterial species. *J. Oral. Sci*., **62**(2): 206-211.

Parlatescu I, Nicolae C, Tovaru S, Radu L, Penes O and Varlas V (2021). The implication of candida infection in oral lichen planus lesions. *Maedica (Bucur)*, **16**(4): 585-589.

Pereira Sousa JC and Kogawa AC (2023). Overview of analytical methods for evaluating tinidazole. *J AOAC Int*, **106**(2): 309-315.

Pettas E, Savva V, Theofilou VI, Georgaki M and Nikitakis NG (2021). Oral candida infection in psoriatic patients treated with IL17A inhibitors: Report of 3 cases and a comprehensive review of the literature. *Diagnostics*, **12**(1): 3.

Phan QT, Solis NV, Cravener MV, Swidergall M, Lin J, Huang MY, Liu H, Singh S, Ibrahim AS, Mazzone M, Mitchell AP and Filler SG (2023). *Candida albicans* stimulates formation of a multi-receptor complex that mediates epithelial cell invasion during oropharyngeal infection. *PLoS Pathog*, **19**(8): e1011579.

Ponde NO, Lortal L, Ramage G, Naglik JR and Richardson JP (2021). *Candida albicans* biofilms and polymicrobial interactions. *Crit. Rev. Microbiol*., **47**(1): 91-111.

Poyil N, Abdul Razak P, Mohamed Ali Kp A, Venugopalan D, Jassim A and Krishna KA (2024). Influence of five different commercially available mouthwashes on the growth of candida albicans adhered to customized prefabricated heat-cured denture base acrylic resin sheets: An *In vitro* study. *Cureus*, **16**(4): e58301.

Rajendiran M, Trivedi HM, Chen D, Gajendrareddy P and Chen L (2021). Recent development of active ingredients in mouthwashes and toothpastes for periodontal diseases. *Molecules*, **26**(7): 2001.

Ren J (2024). Advances in combination therapy for gastric cancer: integrating targeted agents and immunotherapy. *Adv. Clin. Pharmacol. Ther*., **1**(1): 1-15.

Robbins JW, Alvarez MG, Beckel BT, Norris RT and Caesar RR (2023). Restoratively guided orthodontic treatment: The pre-orthodontic bonding concept. *J. Esthet. Restor. Dent*., **35**(1): 270-278.

Salehi M, Malekzadeh Shafaroudi A, Daryani M, Khalilian A, Ahangarkani F and Molania T (2022). *In vitro* interactions of nystatin and micafungin combined with chlorhexidine against Candida albicans isolates. *Curr. Med. Mycol*., **8**(1): 7-11.

Shao R, Wang Z, Yang C, Pan L, Chen X and Du G (2024). Tinidazole mouth rinse for the treatment of oral lichen planus: an observational pilot study. *BMC Oral Health*, **24**(1): 1145.

Shen X and Yu Z (2021). The effects of bracketless invisible orthodontics on the PLI, SBI, SPD and GI and on the satisfaction levels in children with malocclusions. *Am J. Transl. Res*., **13**(5): 5066-5072.

Shimada Y, Yoshida Y, Isogai R and Maki K (2022). Visualization of orthodontic forces generated by aligner-type appliances. *Dent. Mater J.,* **41**(4): 608-615.

Siwach A and Verma PK (2021). Synthesis and therapeutic potential of imidazole containing compounds. *BMC Chem*., **15**(1): 12.

Thomas SC, Xu F, Pushalkar S, Lin Z, Thakor N, Vardhan M, Flaminio Z, Khodadadi-Jamayran A, Vasconcelos R, Akapo A, Queiroz E, Bederoff M, Janal MN, Guo Y, Aguallo D, Gordon T, Corby PM, Kamer AR, Li X and Saxena D (2022). Electronic cigarette use promotes a unique periodontal microbiome. *mBio*, **13**(1): e0007522.

Wade RG, Burr NE, McCauley G, Bourke G and Efthimiou O (2021). The comparative efficacy of chlorhexidine gluconate and povidone-iodine antiseptics for the prevention of infection in clean surgery: A systematic review and network meta-analysis. *Ann. Surg*., **274**(6): e481-e488.

Wood GE, Kim CM, Aguila LKT and Cichewicz RH (2023). *In vitro* susceptibility and resistance of *Mycoplasma genitalium* to Nitroimidazoles. *Antimicrob Agents Chemother*, **67**(4): e0000623.

Yuwanati M, Gondivkar S, Sarode SC, Gadbail A, Desai A, Mhaske S, Pathak SK and M NK (2021). Oral health-related quality of life in oral cancer patients: Systematic review and meta-analysis. *Future Oncol*, **17**(8): 979-990.

Zhao LL, Yuan Q, Shen BL and Wang Q (2024). Clinical efficacy and safety of tinidazole combined with minocycline in treating peri-implantitis. *Am J. Transl. Res*., **16**(12): 7405-7415.