Differences in the effectiveness of chlorhexidine mouthwash with and without cetylpyridinium chloride in the healing of minor recurrent aphthous stomatitis: an experimental study

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ABSTRACT

Introduction: Minor recurrent aphthous stomatitis (RAS) is the most common type of oral ulcer. Topical antiseptics, such as chlorhexidine, are commonly used as first-line therapy to promote ulcer healing. However, the use of chlorhexidine in the oral cavity may be associated with adverse effects, leading to the development of combinations with other antiseptics, such as cetylpyridinium chloride (CPC). This study aimed to analyze the differences in the effectiveness of chlorhexidine with and without CPC in reducing ulcer size and pain among patients with minor RAS. **Methods:** This study employed a quasi-experimental method with a randomized controlled trial (RCT) design and a double-blind approach involving 32 subjects, divided into two groups. Group I received a mouthwash containing a combination of chlorhexidine and CPC, while Group II received chlorhexidine mouthwash alone. Ulcer size was measured using a periodontal probe, and pain levels were assessed using a Visual Analog Scale (VAS) at baseline (Day 1) and on Days 3 and 7 after the intervention. **Results:** There was a decrease in ulcer size and pain intensity in both groups from day 1 to day 7, with no significant difference between groups ($p>0.05$). However, the chlorhexidine + CPC group showed a lower mean ulcer size on day 7 compared to the chlorhexidine group. **Conclusion:** The use of chlorhexidine mouthwash, with or without CPC demonstrated comparable effectiveness in reducing ulcer size and pain in patients with minor RAS.

Keywords

Recurrent aphthous stomatitis, chlorhexidine, cetylpyridinium chloride, mouthwash, topical antiseptic

Perbedaan efektivitas obat kumur klorheksidin dengan dan tanpa cetylpyridinium chloride dalam penyembuhan stomatitis aftosa rekuren tipe minor: studi eksperimental

ABSTRAK

Pendahuluan: *Stomatitis aftosa rekuren (SAR) tipe minor merupakan ulser pada mulut yang paling sering dijumpai. Terapi awal yang dapat digunakan adalah antiseptik topikal seperti klorheksidin yang dapat membantu proses penyembuhan. Penggunaan klorheksidin di rongga mulut dapat menimbulkan efek samping, sehingga dikembangkan kombinasi dengan antiseptik lain seperti cetylpyridinium chloride (CPC). Penelitian ini bertujuan menganalisis perbedaan efektivitas klorheksidin dengan dan tanpa CPC dalam mengurangi ukuran dan nyeri ulser pada pasien SAR tipe minor.* ***Metode:*** *Penelitian ini menggunakan metode eksperimental dengan desain randomized controlled trial (RCT) dan double blind pada 32 subjek yang dibagi menjadi dua kelompok. Kelompok I menerima obat kumur kombinasi klorheksidin dan CPC, sedangkan kelompok II menerima obat kumur klorheksidin. Ukuran ulser diukur menggunakan periodontal probe dan tingkat nyeri dinilai dengan visual analog scale (VAS) pada hari ke-1, serta hari ke-3 dan ke-7 pasca intervensi.* ***Hasil:*** *Terdapat penurunan ukuran ulser dan tingkat rasa nyeri pada kedua kelompok dari hari ke-1 hingga hari ke-7 tanpa perbedaan yang signifikan antar kelompok ($p>0,05$). Namun, kelompok kombinasi klorheksidin dan CPC menunjukkan rata-rata ukuran ulser yang lebih rendah pada hari ke-7 dibandingkan kelompok klorheksidin.* ***Simpulan:*** *Penggunaan obat kumur klorheksidin dengan dan tanpa CPC memiliki efektivitas yang serupa dalam mengurangi ukuran ulser dan rasa nyeri pada pasien SAR tipe minor.*

Kata kunci

Stomatitis aftosa rekuren, klorheksidin, cetylpyridinium chloride, obat kumur, antiseptik topikal

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is one of the most common oral ulcerative diseases. It is characterized by recurrent, shallow, round or oval ulcers with well-defined erythematous margins and a yellowish-gray center.[1] RAS commonly occurs on the labial and buccal mucosa, as well as the floor of the mouth.[2] The initial symptoms of RAS include pain and discomfort, particularly during movements involving the affected area, such as eating, speaking, and swallowing.[3] Approximately 25% of the global population is estimated to have experienced RAS at least once.[1,4]

According to the 2018 Indonesian Basic Health Research (Riskesdas), the prevalence of RAS in Indonesia was reported to be 2.8%.[5] Based on its clinical characteristics, RAS is classified into three subtypes: minor, major, and herpetiform.[2,6,7] Minor RAS is the predominant subtype, accounting for approximately 70–85% of all reported cases.[4,8] Major RAS represents 7–20% of cases, whereas herpetiform RAS is relatively uncommon, with a reported prevalence of 5–10%.[8]

The exact etiology and pathogenesis of RAS remain unclear. However, several predisposing factors, including bacterial infection, genetic susceptibility, hematologic deficiencies, local trauma, psychological stress, allergies, and hormonal influences, have been implicated, highlighting the multifactorial nature of the disease.[1,2,6,9,10] Consequently, current treatment strategies are not disease-specific and primarily aim to relieve pain, accelerate ulcer healing, reduce the frequency and severity of recurrences, and prevent ulcer recurrence.[2,11,12] For patients with minor RAS, topical therapy is considered the first-line treatment and includes topical glucocorticoids, local anesthetics, antibiotics, and topical antiseptics.[2,7,11]

Chlorhexidine is a bisbiguanide topical antiseptic widely regarded as the gold standard for controlling oral microorganisms.[13–16] It has been shown to reduce the frequency and severity of ulcer episodes while promoting ulcer healing.[13,17] In addition to its antimicrobial activity, chlorhexidine also exhibits anti-inflammatory properties that help reduce inflammation and alleviate ulcer-associated pain.[18] However, its clinical use is frequently associated with undesirable adverse effects, including tooth discoloration, a burning sensation, altered taste perception, and irritation of the oral soft tissues.[19,20] These adverse effects may be minimized by the addition of other antiseptic agents, such as cetylpyridinium chloride (CPC), without compromising its effectiveness.[19–21]

CPC is a monocationic quaternary ammonium compound that is widely used as an antiseptic ingredient in various oral healthcare products, including mouthwashes.[22,23] CPC exerts its antimicrobial activity by disrupting microbial cell membranes and interfering with intracellular ionic balance, ultimately leading to cell death.[23] Furthermore, CPC has demonstrated superior penetration into microbial biofilms, which are often difficult for antiseptics such as chlorhexidine to access effectively.[22]

The combination of chlorhexidine + CPC has the potential to synergistically enhance its antiseptic effect by improving the inhibition and elimination of oral microorganisms.[21] However, studies comparing the effectiveness of chlorhexidine mouthwash with and without CPC in the management of minor RAS remain limited, particularly regarding their effects on ulcer size and pain intensity. The novelty of the present study lies in its direct comparison of these two mouthwash formulations in patients with minor RAS, which has received limited investigation to date. Therefore, this study aimed to compare the effectiveness of chlorhexidine mouthwash with and without CPC by evaluating ulcer size and pain intensity, thereby providing more specific clinical evidence to support the selection of a more effective topical therapy.

METHODS

This study was an experimental study employing a randomized controlled trial (RCT) design. Participants were allocated to two intervention groups. A double-blind design was implemented using a purposive sampling design. The study was conducted at the Universitas Padjadjaran Dental Hospital (RSGM Unpad) over a two-month period, from March to April 2025.

Participants were recruited using consecutive sampling based on predefined inclusion and exclusion criteria. Eligibility was determined through subjective assessment, clinical examination, and medical history taking performed by dental residents or clinical dental students from the Faculty of Dentistry, Universitas Padjadjaran (operators). Eligible participants were then randomly allocated to one of the two intervention groups using a simple randomization method.

Before distribution to the participants, the two mouthwash formulations were labeled as A and B. The coding and randomization procedures were performed by an operator who was not involved in outcome assessment. The treatment allocation was concealed from both the participants and the principal investigator responsible for data collection and evaluation. Consequently, neither the participants nor the investigator was aware of the identity of the assigned mouthwash throughout the intervention period, thereby maintaining the double-blind design.

The sample size was determined using Federer's formula, resulting in 16 participants in each mouthwash group, for a total sample size of 32 participants. The inclusion criteria were male or female participants aged 18–28 years with minor recurrent aphthous stomatitis (RAS) diagnosed by the operator (ulcer onset ≤72 hours), a moderate-to-poor OHI-S score, good general health, and willingness to provide written informed consent. Exclusion criteria included the use of mouthwash or other medications containing chlorhexidine or cetylpyridinium chloride (CPC), as well as recurrent oral manifestations associated with Behçet syndrome, herpes simplex virus (HSV) infection, or erythema multiforme.[9]

Participants underwent medical history taking and clinical examination by the operator. Pain intensity was evaluated using a 10-cm Visual Analog Scale (VAS), where a score of 0 indicated no pain and a score of 10 indicated unbearable pain. Oral hygiene status was assessed using the OHI-S to confirm a moderate-to-poor OHI-S score according to the inclusion criteria. Ulcer size was measured in millimeters using a periodontal probe by determining the greatest distance between two opposing ulcer margins.[12] When multiple ulcers were present, only the most recently developed ulcer (onset ≤72 hours) was selected for evaluation. Clinical assessments were performed on Day 1 before the intervention and repeated on Days 3 and 7 after the intervention, consistent with the methodology adopted in previous studies investigating similar interventions.[12]

Participants were instructed to rinse with 15 mL of the assigned mouthwash for 30 seconds, twice daily for 7 consecutive days. Treatment adherence was monitored using a daily checklist completed by each participant. Participants also completed a questionnaire evaluating subjective oral sensations associated with mouthwash use. The questionnaire was developed by the investigators based on previously published instruments used by Toonen et al., Mathurasai et al., and Guerra et al. to assess subjective oral sensations following mouthwash use. All participants completed the study protocol, with no dropouts recorded, and no stopping guidelines were applied during the trial. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro–Wilk test. Depending on the distribution of the data, between-group comparisons were performed using either the independent-samples t-test or the Mann–Whitney U test. Normally distributed continuous variables are presented as mean ± standard deviation (SD), and statistical significance was established at $p<0.05$.

RESULTS

A total of 32 participants diagnosed with minor recurrent aphthous stomatitis (RAS) who underwent treatment at the Universitas Padjadjaran Dental Hospital (RSGM Unpad) were included in this study. All participants completed the entire intervention according to the study protocol, with no dropouts or exclusions after randomization. The distribution of participant characteristics is presented in Table 1.

Table 1. Distribution of demographic characteristics of patients with minor RAS according to sex and age

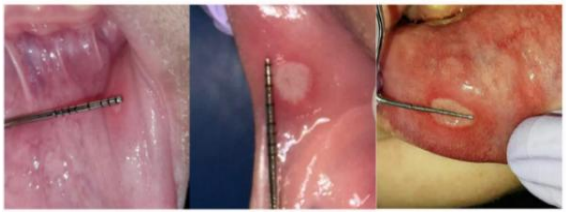
Patient Characteristics	Chlorhexidine + CPC Group (n=16)	Chlorhexidine Group (n=16)	Total	(%)
Sex				
Male	4	4	8	25
Female	12	12	24	75
Age (mean ± SD)	22.19 ± 2.69	21.31 ± 2.39		

Analysis according to sex showed that minor RAS was more frequently observed in female participants, accounting for 24 participants (75%). The participants were between 18 and 28 years of age, with a mean age of 22.19 years in the chlorhexidine + CPC group and 21.31 years in the chlorhexidine group.

Table 2. Ulcer characteristics of patients with minor RAS

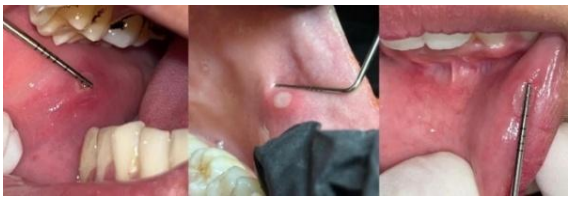
Ulcer Characteristics	Chlorhexidine + CPC Group (n=16)	Chlorhexidine Group (n=16)	Total	(%)
Ulcer Number				
Single	**16**	**16**	**32**	**100**
Ulcer Location				
Floor of the mouth	2	0	2	6.2
Buccal mucosal	1	7	8	25
Labial mucosa	12	9	21	65.6
Ventral surface of the tongue	1	0	1	3.1
Ulcer size				
1-3 mm	8	7	15	46.9
4-5 mm	7	7	14	43.8
>5 mm	1	2	3	9.4
Ulcer shape				
Round	8	5	13	40.6
Oval	8	11	19	59.4

Based on Table 2, all participants had a single ulcer (32 participants; 100%). The most common ulcer location was the labial mucosa, affecting 21 participants (65.6%). The most frequently observed ulcer size ranged from 1 to 3 mm, occurring in 15 participants (46.9%). Oval ulcers were the most common shape, observed in 19 participants (59.4%).



A **B** **C**

Figures A, B, C. Representative clinical characteristics of ulcers in the chlorhexidine + CPC group



D E F

Figures D, E, F. Representative clinical characteristics of ulcers in the chlorhexidine group

Table 3. Distribution of predisposing factors among patients with minor RAS

Predisposing Factors	Chlorhexidine + CPC Group (n=16)	Chlorhexidine Group (n=16)	Total	(%)
One factor				
Genetic factor	1	0	1	3.1
Hematologic deficiency	0	1	1	3.1
Traumatic factor	0	0	0	0
Psychological stress	5	4	9	28.1
Hormonal factor	0	0	0	0
Two factors				
Genetic factor and psychological stress	1	2	3	9.4
Traumatic factor and psychological stress	0	1	1	3.1
Psychological stress and hormonal factor	4	4	8	25
Three factors				
Genetic factor, traumatic factor, and hormonal factor	0	2	2	6.3
Genetic factor, traumatic factor, and psychological stress	1	2	3	9.4
Genetic factor, psychological stress, and hormonal factor	3	0	3	9.4
Psychological stress, hematologic deficiency, and hormonal factor	1	0	1	3.1

Based on Table 3, psychological stress was the most frequently identified single predisposing factor, occurring in 9 participants (28.1%). The combination of psychological stress and hormonal factors was the second most frequently reported predisposing factor, occurring in 8 participants (25.0%).

Table 4. Ulcer size and pain intensity in patients with minor RAS on Days 1, 3, and 7

Variable	Chlorhexidine + CPC Group (mean ± SD)	Chlorhexidine Group (mean ± SD)	*p-value*
Ulcer size (mm)			
Day 1	3.47 ± 1.56	3.61 ± 1.24	0.762[b]
Day 3	2.14 ± 1.55	2.72 ± 1.53	0.296[a]
Day 7	0.00 ± 0.00	0.16 ± 0.62	0.317[b]
Pain intensity*			
Day 1	5.62 ± 1.20	5.31 ± 1.25	0.485[b]
Day 3	0.81 ± 1.47	0.75 ± 1.24	0.841[b]
Day 7	0.00 ± 0.00	0.00 ± 0.00	-

Note: *SD = standard deviation; [a]Independent-samples t-test; [b]Mann–Whitney U test; *Pain intensity was measured using the Visual Analog Scale (VAS).*

Based on Table 4, ulcer size decreased in both groups on Days 3 and 7. The mean ulcer size was lower in the chlorhexidine + CPC group than in the chlorhexidine group; however, the difference was not statistically significant ($p > 0.05$). Pain intensity also decreased in both groups, with complete resolution of pain by Day 7. No statistically significant differences in pain intensity were observed between the two groups throughout the study period.

Table 5. Effects of mouthwash use on oral sensations

Question	Chlorhexidine + CPC Group		Chlorhexidine Group	
	(n=16)	(%)	(n=16)	(%)
Experienced a burning sensation after use	9	56.3	7	43.8
Experienced dry mouth after use	3	18.8	4	25
Experienced oral numbness after use	10	62.5	12	75
The mouthwash tasted bitter	11	68.8	8	50
The mouthwash had a refreshing aroma	14	87.5	13	81.3
Felt refreshed after using the mouthwash	15	93.8	13	81.3

Note: n = number of participants who answered "Yes" to each question, out of a total of 16 participants in each group.

Based on the questionnaire results presented in Table 5, the most frequently reported effect was a feeling of freshness after using the mouthwash, particularly in the chlorhexidine + CPC group, where it was reported by 15 participants (93.8%). A refreshing mouthwash aroma was also reported by most participants in the combination group (14 participants; 87.5%). Oral numbness was the next most frequently reported effect, particularly in the chlorhexidine group (12 participants; 75.0%).

Several mild oral discomforts were reported following mouthwash use; however, no harmful or unintended effects or serious complaints leading to discontinuation of the intervention were observed. Both mouthwash formulations were well tolerated by the participants.

Tabel 6. Comparison of ulcer healing outcomes between the Chlorhexidinte + CPC and Chlorhexidine groups

Parameter	Chlorhexidine + CPC Group		Chlorhexidine Group	
	(n=16)	(%)	(n=16)	(%)
Participants whose ulcers healed on Day 3	2	12.5	3	18.8
Participants with 0-mm ulcers on Day 7 (without erythema)	10	62.5	6	37.5
Participants with 0-mm ulcers on Day 7 (with erythema)	6	37.5	9	56.3
Participants with persistent ulcers on Day 7	0	0	1	6.3

Based on Table 6, 10 participants (62.5%) in the chlorhexidine + CPC group had ulcers measuring 0 mm without erythema on Day 7. In the chlorhexidine group, 9 participants (56.3%) had ulcers measuring 0 mm with erythema on Day 7.

DISCUSSION

Based on the data presented in Table 1, female patients with minor recurrent aphthous stomatitis (RAS) were more frequently observed at the Universitas Padjadjaran Dental Hospital (RSGM Unpad) than male patients. This finding is consistent with the 2018 Indonesian Basic Health Research (Riskesdas), which reported that RAS occurs more frequently in females than in males.[5] This finding is further supported by a previous study conducted at RSGM Unpad in 2022, which reported that 74.23% of RAS cases occurred in females, whereas 25.77% occurred in males.[24] Table 1 also shows that the participants were between 18 and 28 years of age, which is consistent with the literature reporting that RAS occurs most frequently during the second decade of life.[2,9]

The ulcer characteristics presented in Table 2 show that all participants had a single ulcer. This finding is consistent with a previous study reporting that 95.2% of cases presented with a single ulcer, whereas only 4.8% presented with multiple ulcers.[25] In the present study, the labial mucosa was the most common ulcer location. This finding is in

agreement with previous studies reporting that minor RAS most frequently occurs on non-keratinized surfaces, particularly the labial mucosa, accounting for 72.6% of cases.[25]

The most frequently observed ulcer size in this study was 1–3 mm. Oval-shaped ulcers were the most common ulcer shape, followed by round ulcers. These findings are consistent with the clinical characteristics of minor RAS, which is typically characterized by small ulcers measuring <10 mm in diameter with a round or oval shape.[2,6] The ulcer characteristics are also illustrated in Figure 1, which demonstrates the variation in ulcer number, location, size, and shape in each treatment group, thereby providing a more comprehensive clinical representation of the ulcer characteristics observed in the study participants.

Based on the data presented in Table 3, five predisposing factors associated with RAS were identified: genetic factors, hematologic deficiency, trauma, psychological stress, and hormonal factors. Previous studies support these findings, reporting that all five factors may contribute to the development of RAS.[10,26,27]

Several participants reported that other family members had experienced similar complaints, although no definite information regarding a history of allergies or other systemic diseases was available. This finding suggests a possible genetic contribution, particularly because the pattern of occurrence appeared to be familial. A previous study supports this assumption by stating that "RAS belongs to the heritage of an atopic background," referring to an inherited predisposition among family members.[28] In addition to genetic factors, hematinic deficiency was also identified as an aggravating factor for the clinical manifestations of RAS. One participant reported systemic symptoms, including dizziness upon standing and fatigue, and infrequent consumption of fruits and vegetables.

This condition may result in deficiencies of essential micronutrients, such as iron, vitamin B_{12}, and folic acid, which are important for epithelial regeneration and tissue healing. Previous studies have reported that hematinic deficiencies, including deficiencies of vitamins B_{12}, D, and E, are associated with increased ulcer size, ulcer number, and pain intensity, as well as delayed healing.[29] Low hematinic levels have also been associated with increased systemic inflammatory activity, reflected by elevated IL-6 and ROS.[30] Furthermore, hematinic deficiency may influence gene expression through epigenetic mechanisms, particularly reduced DNA methylation, leading to increased IL-6 mRNA expression.[31]

Furthermore, trauma was not identified as a single predisposing factor in this study. Participants reported that ulcers developed after accidentally biting the oral mucosa or consuming hot food or beverages. This finding is consistent with the literature indicating that trauma is not the sole cause of RAS, as it is generally associated with RAS only when it occurs in combination with other predisposing factors.[25]

In contrast, psychological stress reported by participants in this study was primarily related to academic pressures, including heavy coursework, demanding class schedules, and examinations, which may trigger RAS recurrence. Psychological stress increases salivary cortisol levels, which stimulate the immune system to recruit leukocytes to sites of inflammation, thereby contributing to the development of RAS.[8]

Hormonal imbalance was also identified as a predisposing factor, particularly during the luteal phase of the menstrual cycle. Decreased progesterone and estrogen levels during the luteal phase are thought to increase the susceptibility of women to RAS.[25] In the present study, medical history taking focused on patients' medical histories and conditions related to the onset of ulcers. Clinical examination was performed by assessing the number, location (keratinized or non-keratinized surfaces), size, shape, margins, and condition of the surrounding tissues without additional diagnostic examinations. This approach is consistent with the literature indicating that medical history taking and clinical examination are useful not only for establishing the diagnosis of RAS but also for identifying its predisposing factors.[3,11,27]

Based on the data presented in Table 4, ulcer size decreased in both groups from Day 1 to Day 3, and almost all ulcers had resolved by Day 7. Statistical analysis showed no significant difference between the two groups ($p > 0.05$). This finding is consistent with the study by Zakaria et al. (2020), which reported that chlorhexidine use in patients with RAS can help reduce ulcer size and pain intensity during the healing period.[12]

One factor that may have contributed to the absence of a significant difference between the two groups is the relatively short intervention period of only 7 days. This duration falls within the natural healing period of minor RAS ulcers, which are generally self-limiting and typically resolve spontaneously within 4–14 days without scarring.[2,6,7] The healing process of RAS consists of three stages: the pre-ulcerative, ulcerative, and healing phases. The ulcerative phase is characterized by epithelial disruption and superficial tissue loss, whereas the healing phase is characterized by epithelial regeneration covering the ulcer surface.[32]

The reduction in ulcer size observed during the intervention suggests that most ulcers had likely entered the healing phase, during which epithelial regeneration becomes the predominant process. The use of chlorhexidine mouthwash may have accelerated this process by suppressing microbial colonization that could otherwise delay epithelial regeneration.[13,33] A similar trend was observed in the chlorhexidine + CPC group, which demonstrated a greater reduction in ulcer size, with a smaller mean ulcer size on Day 7.

This additional effect may be attributed to the antiseptic properties of CPC, which act synergistically with chlorhexidine to inhibit microbial growth. Consequently, the combination of chlorhexidine + CPC may not only help maintain a clean ulcer surface by reducing microbial colonization but also support epithelial regeneration, as reflected by the observed reduction in ulcer size.[21,34,35]

Pain assessment data presented in Table 4 also showed a reduction in pain intensity in both groups by Day 3, with complete resolution of pain by Day 7. Statistically, no significant difference in pain intensity was observed between the two groups throughout the study period ($p > 0.05$). The reduction in pain may be associated with the natural inflammatory response to ulceration. During this phase, epithelial cell damage is accompanied by infiltration of immune cells, including neutrophils, macrophages, and lymphocytes, as well as the release of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukins. Activation of these inflammatory mediators stimulates nociceptors, resulting in the perception of pain.[32,36,37]

The use of chlorhexidine may facilitate the transition from the inflammatory phase to the healing phase.[16] Chlorhexidine helps regulate the inflammatory response to prevent prolonged inflammation. As inflammation subsides, epithelial cell migration and regeneration can proceed more effectively, resulting in gradual reductions in both inflammation and pain.[18,32,38,39] As an antiseptic, chlorhexidine exerts its antimicrobial activity by forming electrostatic interactions with the negatively charged bacterial cell wall, leading to cytoplasmic leakage, coagulation, and bacterial cell death through interactions with adenosine triphosphate (ATP) and nucleic acids[16]

This mechanism effectively suppresses secondary infection and maintains a favorable environment for ulcer healing, thereby promoting epithelial regeneration and accelerating pain reduction. Owing to its substantivity, chlorhexidine remains active in the oral cavity for approximately 8–12 hours after application, helping to maintain a stable oral environment.[40] This prolonged activity may also contribute to pain relief during the healing process.

A reduction in pain intensity was also observed in the chlorhexidine + CPC group. This combination may enhance the antiseptic effect through two distinct but synergistic mechanisms of action. As a quaternary ammonium compound, CPC acts by displacing divalent cations such as Mg^{2+} and Ca^{2+} from the bacterial cell membrane and inserting its hydrophobic tail into the membrane lipid bilayer, thereby disrupting membrane integrity. This process results in leakage of intracellular potassium (K^{+}) ions and other cellular

components, disrupting osmotic balance and causing damage to proteins and nucleic acids, ultimately leading to bacterial cell lysis and death.[23,41]

Becker et al. (2021) reported that the combination of chlorhexidine and CPC is effective in reducing dental plaque and bacterial load.[21] Similar findings were reported by Bollain et al. (2021), who demonstrated that this combination reduced the number of microorganisms in the oral cavity.[20] The findings of the present study support previous evidence that the combination of chlorhexidine and CPC effectively controls the oral bacterial load. This plays an important role in preventing secondary inflammation that may exacerbate ulcer severity and delay the healing process. Therefore, the combination may also contribute to pain reduction.[2,7,17]

Although the combination of chlorhexidine and CPC is theoretically expected to produce a stronger antimicrobial effect, the present study showed that pain intensity in the combination group was slightly higher than that in the chlorhexidine-only group on Day 3. This finding may be related to lower patient tolerance and acceptance of the combined antiseptic formulation, which may cause local irritation of the oral mucosa and consequently influence the level of pain perceived by the participants.[42]

The data presented in Table 5 were obtained from a questionnaire completed by the participants after the intervention. The questionnaire results showed that oral numbness and dry mouth were the most frequently reported effects among participants in the chlorhexidine group. These effects are commonly associated with the use of chlorhexidine in the oral cavity and have been reported in previous studies.[15,19,43]

In contrast, participants in the chlorhexidine + CPC group more frequently reported burning and bitter taste sensations. These effects have also been reported with chlorhexidine used alone and may therefore be considered clinically acceptable responses.[19,20,43] One possible explanation for these effects is the presence of ethanol in chlorhexidine-based mouthwash formulations. Ethanol functions as a solvent for the active ingredients, an additional antiseptic agent, and a preservative; however, it is also known to cause local irritation, which may contribute to burning sensations or discomfort in the oral mucosa.[44] These findings may also be explained by the presence of 0.1% peppermint in the chlorhexidine + CPC formulation. Peppermint is commonly incorporated into mouthwash formulations because of its antiseptic properties, which help create an environment that is less favorable for microbial growth.[45,46]

However, these antiseptic properties may also cause oral discomfort, such as the burning sensation that appeared to be more frequently reported in the combination group.[47] On the other hand, peppermint may provide a stronger refreshing aroma and sensation than chlorhexidine alone. This refreshing sensation may enhance patient comfort during mouthwash use.[46]

Based on the data presented in Table 6, five participants across both groups experienced ulcer healing by Day 3 after the intervention, with initial ulcer sizes ranging from 1 to 3 mm. This healing was likely attributable to the small ulcer size, as ulcers of this size generally heal spontaneously.[2,6,7] The use of antiseptic mouthwash in this study may also have accelerated the healing process. By Day 7, six participants in the chlorhexidine + CPC group still exhibited residual erythema despite complete ulcer closure, whereas nine participants in the chlorhexidine group demonstrated the same finding. The persistence of these residual lesions is related to the tissue repair process, which requires varying durations depending on the size of the ulcer. Larger ulcers generally require a longer period for epithelial regeneration and tissue reconstruction.[48]

Ulcer healing occurs gradually through tissue regeneration until complete wound healing is achieved.[38] Another factor that may have influenced healing is oral hygiene. All participants in this study had moderate OHI-S scores, indicating the presence of plaque and calculus accumulation. These local factors may create an environment favorable for microbial growth, potentially exacerbating RAS.[49] Accordingly, the use of antiseptic mouthwash in this study was relevant for suppressing microbial colonization that could

delay epithelial regeneration while simultaneously contributing to pain reduction, as reflected by the VAS scores in both groups.

In addition to pharmacological therapy, participants also received non-pharmacological management through communication, information, and education tailored to their identified predisposing factors. The information provided included stress management and the avoidance of local trauma, such as injury from toothbrushing or accidental cheek biting.[25,26,33,50] Participants were also advised to avoid irritative foods and beverages, particularly those that were hot or spicy. A soft diet rich in calories and protein, together with adequate consumption of vegetables, fruits, and water, was recommended to support ulcer healing.[33,51]

This study has several limitations. First, the sample size was relatively small and may not fully represent the target population. In addition, the management of RAS requires not only pharmacological therapy but also appropriate control of factors that influence the healing process, as well as participants' adherence to the prescribed treatment. Future studies with longer follow-up periods are warranted to evaluate long-term outcomes, including tooth discoloration associated with the use of both mouthwash formulations.

CONCLUSION

The use of chlorhexidine mouthwash, with or without cetylpyridinium chloride (CPC), demonstrated comparable effectiveness in reducing ulcer size and pain in patients with minor recurrent aphthous stomatitis (RAS). Both mouthwash formulations represent effective topical treatment options, with the chlorhexidine + CPC combination offering an alternative that may be selected based on patient tolerance and preference, particularly given their different tolerability profiles. These findings also emphasize the importance of managing predisposing factors to support optimal ulcer healing. Further studies with longer intervention periods are warranted to evaluate the long-term effects and potential adverse effects associated with these mouthwash formulations. The findings of this study may serve as a reference for clinicians in selecting the most appropriate topical therapy according to individual patient conditions and treatment needs without compromising therapeutic effectiveness.

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