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Short Communication

Cryotherapy on exfoliative cytological changes for oral mucositis in cancer patients undergoing chemotherapy: A randomized control trial

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Abstract

Oral mucositis is a common complication of chemotherapy that significantly impacts quality of life and may reduce treatment efficacy. While oral cryotherapy has been widely studied as a preventive intervention due to its cost-effectiveness, safety, and ease of use, most research focused on clinical outcomes without incorporating objective cytological assessments of mucosal changes. The aim of this study was to evaluate the effectiveness of oral cryotherapy in managing chemotherapy-induced mucositis using exfoliative cytology to monitor oral mucosal changes. A single-blinded, randomized controlled trial was conducted involving 50 cancer patients undergoing chemotherapy, who were randomly assigned to either the intervention or control group. The control group (n=25) received standard oral hygiene care, while the intervention group (n=25) received oral cryotherapy in addition to routine oral hygiene. A 20-minute oral cryotherapy was administered twice daily after breakfast (09:00 A.M.) and lunch (01:00 P.M.) for 14 days. This study found a significant reduction in mucositis scores was observed in both groups ($p<0.05$). However, post-hoc analysis indicated that mucositis severity declined earlier in the cryotherapy group, whereas improvement in the control group was noted only after 14 days. Serial oral mucosal smears analyzed via exfoliative cytology revealed a reduction in inflammatory cells and the absence of coccus microorganisms by days 7 and 14 in the intervention group. In conclusion, this study demonstrated that oral cryotherapy effectively reduces the severity and duration of mucositis and accelerates recovery in cancer patients undergoing chemotherapy. Oral cryotherapy can be applied as a viable alternative to mitigate the severity of oral mucositis in this patient population.

Keywords: Cancer, chemotherapy, exfoliative cytology, oral cryotherapy, oral mucositis

Introduction

Chemotherapy is a common cancer treatment modality that may induce adverse effects, including oral mucositis [1]. Currently, oral mucositis is considered severe complication of anticancer therapy [2,3], with a prevalence of up to 51.7% in patients undergoing chemotherapy and 90% in those receiving head and neck radiotherapy [4,5]. Oral mucositis is characterized by oral pain, reduced salivary production, dysphagia, decreased nutritional intake, and an increased risk of secondary infection [6-8]. Furthermore, it may compromise the effectiveness of cancer

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therapy [9]. Therefore, effective strategies for the prevention and management of oral mucositis are essential to mitigate its impact on patient outcomes and optimize cancer treatment efficacy.

Current cancer treatment strategies emphasize minimizing therapy-related adverse effects, with oral mucositis being a primary focus of ongoing research [10,11]. Effective prophylactic and symptomatic management may facilitate the administration of more intensive therapeutic regimens, thereby improving treatment efficacy and patient survival [9]. Various strategies have been investigated to mitigate oral mucositis in patients receiving chemotherapy [11], including pre-treatment interventions designed to prevent its onset [12]. The International Society of Oncology has systematically reviewed the literature and developed evidence-based guidelines for the prevention, assessment, and management of oral mucositis [11,13]. Among the recommended approaches, topical cooling of the oral mucosa, known as oral cryotherapy, has demonstrated the potential to reduce both the incidence and severity of this condition [14-17].

Oral cryotherapy mitigates chemotherapy-induced mucosal injury by inducing vasoconstriction and reducing blood flow to the oral cavity, thereby limiting chemotherapeutic exposure to the buccal mucosa [18,19]. This intervention offers several advantages over alternative strategies, including material availability, cost-effectiveness, ease of administration, safety, and high patient tolerance [20,21]. Previous studies have demonstrated its efficacy as a prophylactic measure, significantly reducing the incidence of mucositis in patients undergoing chemotherapy and radiotherapy [22,23]. A systematic review conducted by the Multinational Association of Supportive Care in Cancer/International Society of Oral Oncology (MASCC/ISOO) confirmed that oral cryotherapy effectively decreases both the incidence and severity of oral mucositis [24]. Additionally, another systematic review identified oral cryotherapy as a moderately to highly effective nursing intervention for managing all grades of chemotherapy-induced mucositis [25]. As an evidence-based approach, oral cryotherapy has been widely recognized for its role in minimizing mucositis in patients receiving chemotherapy and radiotherapy [25,26].

A previous study evaluated the effectiveness of cryotherapy by assessing cytological changes in oral mucositis among cancer patients, particularly those receiving 5-fluorouracil therapy [25]. However, the impact of a 2-week cryotherapy intervention on exfoliative cytopathological changes in oral mucositis among chemotherapy patients remains unexplored. Cryotherapy is a non-pharmacological intervention with no reported adverse effects, as it does not involve chemical agents [27]. This approach allows for the assessment of oral mucositis severity by identifying normal desquamated cells or those with pathological abnormalities, thereby facilitating the evaluation of cryotherapy effectiveness [28]. Additionally, exfoliative cytology provides rapid results and serves as a less invasive alternative to surgical biopsy [28]. As a non-invasive diagnostic technique, exfoliative cytology analyzes the cellular structure of samples obtained through mucosal scraping [29]. Therefore, the aim of this study was to evaluate the effectiveness of oral cryotherapy in mitigating exfoliative cytological changes associated with oral mucositis in cancer patients undergoing chemotherapy. A 2-week intervention is anticipated to enhance patient comfort and improve quality of life.

Methods

Study design and setting

A single-blinded, double-arm, randomized controlled trial was conducted at the Oncology Ward of Wahidin Sudirohusodo Hospital and Universitas Hasanuddin Hospital, in Makassar, Indonesia, between July 2020 and September 2021. Pre- and post-intervention assessments were performed to evaluate the effects of a 20-minute oral cryotherapy treatment. Patients meeting the inclusion criteria were recruited, and outcomes were assessed using exfoliative cytology and an oral mucositis severity scale. The primary outcomes included the severity of oral mucositis and cellular alterations detected through exfoliative cytology analysis.

Patients and criteria

The inclusion criteria included hospitalized cancer patients undergoing chemotherapy who presented with grade 1 or 2 mucositis during either the first or subsequent chemotherapy cycles.

Eligible patients were required to be over 18 years of age and have no tooth sensitivity to ice. Patients diagnosed with oral cancer, those with oral complications, or individuals with confirmed COVID-19 were excluded. The dropout criteria included patient death, withdrawal of consent during the study, discontinuation of participation, or a confirmed COVID-19 diagnosis.

Sample size, sampling method, allocation, and randomization

The required sample size was determined using a formula referenced from established elsewhere [30]. Based on this calculation, a total of 50 participants were needed, with 25 allocated to each group. Randomization was conducted using sealed envelopes containing numbered labels, with odd numbers assigned to the intervention group and even numbers to the control group. The allocation process was concealed and exfoliative cytological examinations were performed by a laboratory assistant who was blinded to group assignments. Participants were randomly assigned to either the intervention group, which received oral cryotherapy in addition to usual care, or the control group, which received usual care without cryotherapy. Usual care in this study referred to the standard oral care practices routinely performed by patients.

Data collection

During data collection, patient characteristics that can influence oral mucositis development, including age, body mass index (BMI), chemotherapy cycle, sex, dental caries, cancer stage, and cancer type were recorded. Cancer staging was classified into early (Stage I–II) and advanced (Stage III–IV) categories according to the TNM (Tumor, Node, Metastasis) staging system. Early-stage cancer was defined by localized tumor growth, whereas advanced-stage cancer encompassed regional or distant metastases. Trained enumerators assessed oral inflammation and collected oral swabs before chemotherapy (pre-test) on day one at the hospital. Follow-up assessments were conducted on days 7 and 14 post-chemotherapy through home visit.

Intervention

In the intervention group, 21 patients underwent oral cryotherapy, which involved gargling unflavored ice crystals shaped into cubes with non-sharp edges (3.2 × 3.3 × 1 cm) for 20 minutes [31,32]. The intervention was administered twice daily, after breakfast (09:00 A.M.) and lunch (01:00 P.M.), for 14 days. On day one, oral cryotherapy was performed under supervision at the hospital, after which patients self-administered the intervention and documented adherence in an observation sheet. Patients were trained in the correct application of oral cryotherapy and provided with a container for ice cube preparation. Daily adherence was monitored through telephone follow-ups and verified during home visits on days 7 and 14, during which enumerators collected oral swabs in the morning at 07:00–09:00 A.M. In the control group, consisting of 21 patients, routine oral care was provided, including standard toothbrushing practices.

End points

End points of the study was mucositis severity and exfoliative cytology findings. The severity was assessed using a staging instrument developed from the integration of three scoring systems (**Table 1**): the World Health Organization (WHO) Mucositis Scale was used to evaluate pain levels and the ability to eat [33]; the Radiation Therapy Oncology Group (RTOG) mucositis grading system assessed the extent of ulceration [34]; and WCCNR Organization evaluating the severity of oral mucositis based on the extent of ulceration and its impact on oral function [35]. Integrating the WHO Mucositis Scale, WCNNR, and RTOG provides a comprehensive mucositis assessment, enhancing the evaluation of cryotherapy's impact. The mucositis severity scores were interpreted as follows, a score of 0 indicates normal mucosa, a score of 4 represents mild mucositis, scores ranging from 5 to 10 indicate moderate mucositis, and scores between 11 and 16 reflect severe mucositis. Mild mucositis (score 4) was characterized by erythema or a single small ulcer, causing discomfort during speech or eating, though solid food intake remains possible. Moderate mucositis (score 5–10) presented with 2–4 ulcerations (<1.5 cm) and mild pain even at rest, restricting the diet to semi-solid foods. Severe mucositis (score 11–16) was marked by extensive ulcerations, defined as more than four lesions or large, deep wounds, resulting in severe pain and an inability to consume solid or semi-solid foods, necessitating liquid nutrition or enteral/parenteral support.

Table 1. Mucositis grading scale for evaluating the severity of oral mucositis in patients undergoing chemotherapy or radiotherapy

Grade	Assessment			
	Number of ulcerations	Ulceration area	Pain in the mouth	Eating ability
0	-	-	-	Normal
1	Erythema, ulceration 1 piece	Small	Pain when talking or eating	Solid food
2	Ulceration 2–4 pieces	<1.5 cm	Mild pain when not eating	Semi-solid food
3	Ulceration >4 pieces	≥1.5 cm	Moderate pain	Liquid food
4	Ulceration very much	wide and deep	Severe pain	Enteral/parenteral nutrition

This study also assessed the exfoliative cytology of patients oral to evaluate cellular morphology, detect potential cytopathological changes, and identify any inflammatory or degenerative alterations. The analysis was conducted using the Papanicolaou staining technique, which provided detailed visualization of nuclear and cytoplasmic characteristics.

Exfoliative cytology: specimen collection and procedures

The patient's oral cavity was cleansed by swabbing the area with sterile cotton, followed by repeated unidirectional scraping using a wooden spatula. Exfoliative cytology was performed by gently rubbing the buccal mucosa with a wooden spatula to obtain tissue samples. To ensure consistency and reliability, trained enumerators conducted the procedure. The collected sample was transferred onto a clean, pre-labeled glass slide marked with the patient number or designated sample area. Following collection, the samples were preserved in 95% ethanol and stained using the Papanicolaou method for cytological examination at the Laboratory of Anatomical Pathology, Universitas Hasanuddin, Makassar, Indonesia. To minimize contamination and maintain consistency in cytological analysis, samples were collected in the morning (07:00–09:00 A.M.) before the patients consumed any food or beverages.

The slides then stained with Papanicolaou staining technique to facilitate the evaluation of epithelial abnormalities, infections, and inflammatory conditions. The technique improved cellular resolution, enabling the identification of morphological changes. Briefly, the slides were immersed in 96% alcohol for 5 minutes in two separate containers, followed by a 1-minute rinse with water or distilled water. Staining was performed using Harris hematoxylin (3 minutes) and rinsed for 10 minutes. The slides were then sequentially immersed in 96% alcohol and Orange-G (20 dips, 2 minutes), 96% alcohol and eosin Azure-50 (30 dips, 2 minutes), and 96% alcohol and xylol (30 dips, 2 minutes) in three containers. After drying for 5–10 minutes at room temperature, the slides were mounted with Entellan liquid and covered with a glass slip. The final dried slides were examined under a light microscope (Olympus CX23, Evident Corporation, Tokyo, Japan).

Inflammatory cells, including neutrophils, lymphocytes, and macrophages, were identified based on distinct morphological features, and the quantity and distribution were analyzed to assess the degree of inflammation. The presence of inflammatory cells was categorized as follows: 0 (no inflammatory cells observed), + 1 (mild inflammatory cells present), +2 (moderately dense inflammatory cells present), and + 3 (dense inflammatory cells present).

Cell shrinkage was evaluated based on cytoplasmic condensation, nuclear pyknosis, and irregular cell margins, indicative of apoptotic or degenerative changes, and was classified as either normal or reduced cell size. Microorganisms were identified based on morphological characteristics and staining properties, with Papanicolaou staining enabling the visualization of coccus formations. The presence of microorganisms was classified as either (–) (no microorganisms observed) or (+) (microorganisms observed).

Statistical analysis

For continuous variables, normally distributed data were expressed as mean±SD, whereas non-normally distributed data were reported as median (interquartile range, IQR), as determined by the Shapiro-Wilk test (p<0.05). Categorical data were presented as frequency (n) and percentage (%). The homogeneity between the intervention and control groups was assessed using the independent t-test for normally distributed data and the Mann-Whitney U test for non-normally

distributed data. Categorical variables were analyzed using the Chi-squared test or Fisher's exact test, with $p>0.05$ indicating homogeneity between groups. For repeated measures within groups, the Friedman test and Cochran's Q test were applied, with statistical significance set at $p<0.05$. Post hoc analysis using the Wilcoxon signed-rank test was conducted for significant outcome variables to assess within-group differences. Data were analyzed using SPSS 22.0 (IBM, New York, USA).

Results

Patients' selection

Among 1,174 cancer patients screened, 1,116 were excluded for not meeting the inclusion criteria, and eight declined participation (**Figure 1**). Consequently, 50 eligible patients were randomly assigned to the intervention or control group. In the intervention group, four patients withdrew, including one who missed the second session due to suboptimal chemotherapy conditions and three who discontinued participation. In the control group, four patients withdrew, including one who missed the third session due to suboptimal chemotherapy conditions, two who discontinued participation, and one with confirmed COVID-19. Therefore, the final analysis included 42 patients who completed the study per protocol.

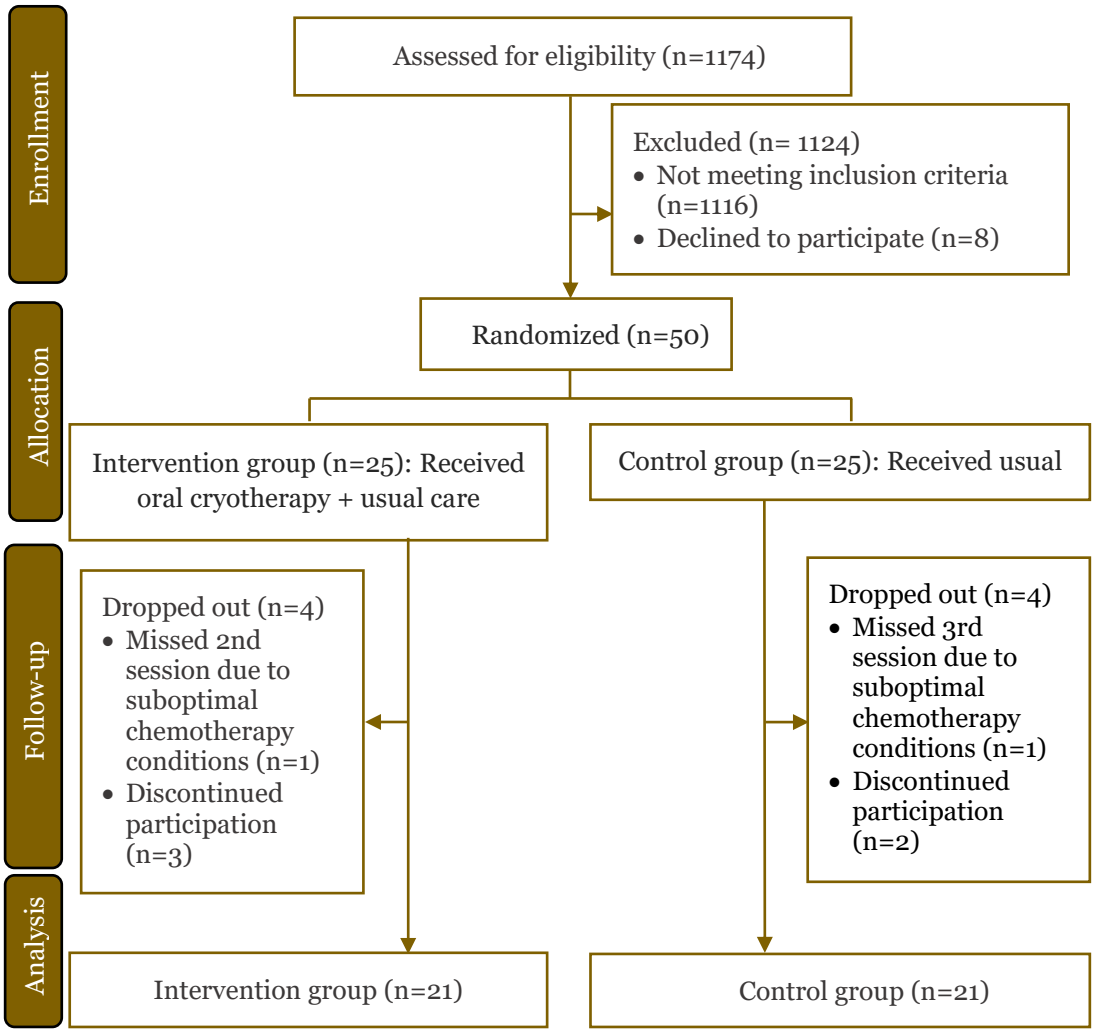

Figure 1. Flowchart of patient selection, allocation, and dropout reasons.

Characteristics of the patients

The mean age of patients was similar between the intervention (44.9±3.0 years) and control groups (47.2±2.9 years) with no significant difference (p=0.585) (**Table 2**). Similarly, BMI showed no significant difference between groups (p=0.508). The median number of chemotherapy cycles was slightly higher in the intervention group (3, IQR: 2–5) compared to the control group (2, IQR: 2–4), but this difference was not statistically significant (p=0.165). For sex distribution, the intervention group had a higher proportion of female patients (42.9%) compared to the control group (33.3%). In contrast, male patients were more prevalent in the control group (16.7%) than in the intervention group (7.1%), with no significant difference (p=0.277). The prevalence of dental caries was comparable between the intervention (19.0%) and control groups (23.8%) (p=0.755). Cancer stage distribution was also similar, with early-stage cases accounting for 16.7% in the intervention group and 14.3% in the control group (p=1.000). In cancer type, breast cancer was the most common diagnosis in both groups (35.7% in the intervention group vs. 26.2% in the control group), with statistical comparison did not reveal significant differences between groups (p=0.34). Other cancer types were infrequent; lymphoma (7.1%) and ovarian, vulvar, and sarcoma cancers (each 2.4%) were only present in the intervention group, while lung (4.8%), neck (4.8%), thyroid (2.4%), thymus (2.4%), and skin cancer (2.4%) were observed exclusively in the control group.

Table 2. Characteristics of the included patients (n=42)

Variables	Intervention (n=21) n (%)	Control (n=21) n (%)	p-value
Age (years), mean±SD	44.90±3.03	47.24±2.97	0.585[a]
Body mass index (kg/m²), mean±SD	21.99±0.91	22.86±0.95	0.508[a]
Chemotherapy cycle, median (IQR)	3 (2–5)	2 (2–4)	0.165[b]
Sex			
Male	3 (7.1)	7 (16.7)	0.277[c]
Female	18 (42.9)	14 (33.3)	
Dental caries			
Yes	8 (19.0)	10 (23.8)	0.755[c]
No	13 (31.0)	11 (26.2)	
Cancer stage			
Early	7 (16.7)	6 (14.3)	1.000[c]
Advanced	14 (33.3)	15 (35.7)	
Cancer type			
Breast cancer	15 (35.7)	11 (26.2)	0.34[c]
Lymphoma	3 (7.1)	0 (0)	-
Sarcoma	1 (2.4)	3 (7.1)	-
Ovarium cancer	1 (2.4)	0 (0)	-
Vulva cancer	1 (2.4)	0 (0)	-
Lung cancer	0 (0)	2 (4.8)	-
Neck cancer	0 (0)	2 (4.8)	-
Thyroid cancer	0 (0)	1 (2.4)	-
Thymus cancer	0 (0)	1 (2.4)	-
Skin cancer	0 (0)	1 (2.4)	-

[a] Analyzed using an independent t-test
[b] Analyzed using the Mann-Whitney U test
[c] Analyzed using a Chi-squared test

Effect of oral cryotherapy on exfoliative cytological changes for oral mucositis in cancer patients undergoing chemotherapy

Histopathological findings revealed that the intervention group demonstrated a progressive reduction in inflammatory cell infiltration over the 14-day observation period (**Figure 2**). On day 1, a high density of neutrophilic inflammatory cells was observed among oral squamous cells. By day 7, a noticeable decline in the number of inflammatory cells was evident. By day 14, inflammatory cell infiltration had further decreased, indicating a resolution of the inflammatory response (**Figure 2**).

Serial oral mucosal smears in the intervention group demonstrated a progressive increase in oral squamous cell size over the 14-day observation period (**Figure 3**). On day 1, the cells appeared shrunken, indicative of cytoplasmic condensation. By day 7, cell size had gradually returned to a normal morphology, suggesting the resolution of cellular shrinkage. This trend

persisted through day 14, with oral squamous cells maintaining a normal size and structure. The green arrow highlighted the gradual restoration of cell size (**Figure 3**).

Furthermore, the intervention group revealed the presence of coccus microorganisms during the 14-day monitoring period (**Figure 4**). On day 1, coccus microorganisms were observed adhering to oral squamous cells, as indicated by the green arrow. By day 7, no coccus microorganisms were detected, suggesting a reduction in microbial presence. This absence persisted through day 14, indicating sustained microbial clearance (**Figure 4**).

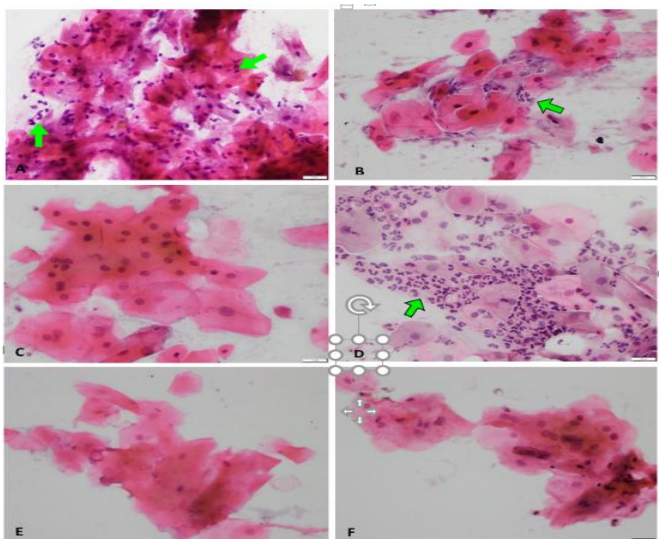

Figure 2. Serial oral mucosal smears in the intervention and control groups demonstrated neutrophilic inflammatory cell presence over 14 days. (A) Abundant neutrophilic inflammatory cells were observed among oral squamous cells in the intervention group on day 1 (green arrow: neutrophilic inflammatory cells). (B) Neutrophilic inflammatory cells were present in the control group on day 1. (C) A reduction in inflammatory cells was observed in the intervention group on day 7. (D) Inflammatory cells remained in the control group on day 7. (E) A further decrease in inflammatory cells was noted in the intervention group on day 14. (F) A reduction in inflammatory cells was also observed in the control group on day 14 (green arrow: neutrophilic inflammatory cells) (400× magnification).

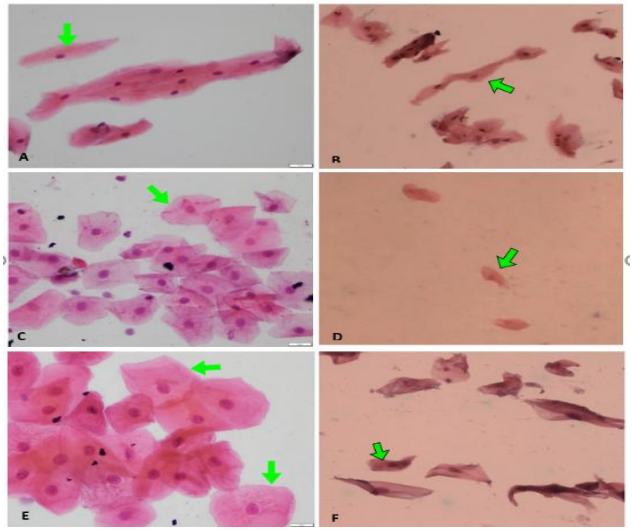

Figure 3. Serial oral mucosal smears in the intervention and control groups demonstrated changes in oral squamous cell size over 14 days. (A) Shrunken cells were observed in the intervention group on day 1. (B) Shrunken cells were also noted in the control group on day 1. (C) Cell size returned to normal in the intervention group by day 7. (D) Some cells remained shrunken in the control group on day 7. (E) Normal cell size was maintained in the intervention group on day 14. (F) Cell shrinkage persisted in the control group on day 14 (green arrow: gradual restoration and changes in cell size) (400× magnification).

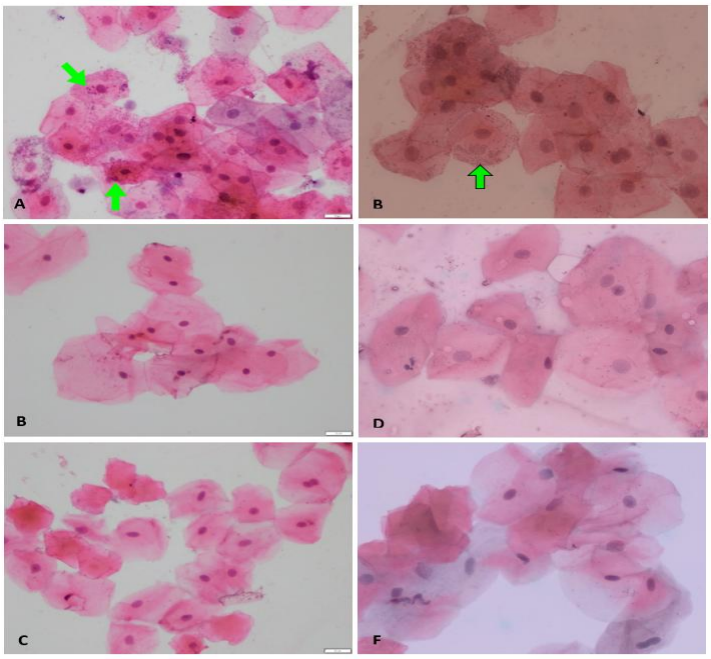

Figure 4. Serial oral mucosal smears in the intervention and control groups demonstrated the presence of coccus microorganisms over 14 days. (A) Coccus microorganisms were observed within oral squamous cells in the intervention group on day 1. (B) Similar findings were noted in the control group on day 1. (C) No coccus microorganisms were detected in the intervention group by day 7. (D) The control group also showed no coccus microorganisms on day 7. (E) The absence of coccus microorganisms persisted in the intervention group on day 14. (F) The control group remained free of coccus microorganisms on day 14 (green arrow: coccus microorganisms) (400× magnification).

The intervention group showed a significant reduction in mucositis severity over 14 days, with the median mucositis score decreasing from 5 (IQR: 4–7) on day 1 to 0 (IQR: 0–0.5) on day 14 (p=0.0001) (**Table 3**). In the control group, scores declined from 4 (IQR: 3–5) to 2 (IQR: 0–5) (p=0.009). The proportion of patients with normal mucosa increased significantly in the intervention group, reaching 76.2% by day 14 (p=0.001), while in the control group, only 38.1% had normal mucosa by day 14 (p=0.020) (**Table 3**).

Inflammation grades remained stable over time, with no significant differences between groups (**Table 3**). Most patients in both groups had minimal to no inflammation. Cell shrinkage patterns showed no significant changes between time points or groups. Microorganism presence decreased in the intervention group from 23.8% on day 1 to 14.3% on day 14 (p=0.607), while in the control group, it increased from 0% on day 1 to 19.0% on day 14 (p=0.074) (**Table 3**). These findings indicated that the intervention effectively reduced mucositis severity and promoted oral mucosal recovery compared to the control group.

Table 3. Comparison of mucositis score, inflammation grade, cell shrinkage, and microorganism count in the intervention and control groups (n=42)

Variables	Intervention (n=21), n (%)				Control (n=21), n (%)			
	Day 1 (n=21)	Day 7 (n=21)	Day 14 (n=21)	p-value	Day 1 (n=21)	Day 7 (n=21)	Day 14 (n=21)	p-value
Mucositis score, median (IQR)	5 (4–7)[a]	3 (2–4)[b]	0 (0–0.5)[c]	0.0001[d*]	4 (3–5)[a]	3 (2–5)[b]	2 (0–5)[c]	0.009[d*]
Mucositis level								
Normal	0 (0)	4 (19.0)	16 (76.2)	0.001[d*]	0 (0)	2 (9.5)	8 (38.1)	0.020[d*]
Mild	6 (28.6)	13 (61.9)	5 (23.8)		15 (71.4)	13 (61.9)	8 (38.1)	
Moderate	15 (71.4)	4 (19.0)	0 (0)		6 (28.6)	6 (28.6)	5 (23.8)	
Inflammation grade								
0	15 (71.4)	17 (81.0)	15 (71.4)	0.380[d]	16 (76.2)	13 (65.0)	16 (76.2)	0.378[d]
+1	3 (14.3)	4 (19.0)	5 (23.8)		3 (14.3)	4 (20.0)	3 (14.3)	
+2	2 (9.5)	0 (0)	1 (4.8)		1 (4.8)	3 (15.0)	2 (9.5)	

Variables	Intervention (n=21), n (%)				Control (n=21), n (%)			
	Day 1 (n=21)	Day 7 (n=21)	Day 14 (n=21)	*p*-value	Day 1 (n=21)	Day 7 (n=21)	Day 14 (n=21)	*p*-value
+3	1 (4.8)	0 (0)	0 (0)		1 (4.8)	0 (0)	0 (0)	
Cell shrinkage								
Normal	8 (38.1)	6 (28.6)	9 (42.9)	0.505[d]	9 (42.9)	7 (35.0)	7 (33.3)	0.758[d]
Small number	3 (14.3)	8 (38.1)	6 (28.6)		3 (14.3)	8 (40.0)	7 (33.3)	
Majority of the cell	10 (47.6)	7 (33.3)	6 (28.6)		9 (42.9)	5 (25.0)	7 (33.3)	
Microorganism								
Absent	16 (76.2)	17 (81.0)	18 (85.7)	0.607[e]	21 (100)	17 (85.0)	17 (81.0)	0.074[e]
Present	5 (23.8)	4 (19.0)	3 (14.3)		0 (0)	3 (15.0)	4 (19.0)	

[a-c] Analyzed using Wilcoxon-signed ranks post hoc test; median with different superscripts are significantly different, while those with the same superscript are not, significant at $p<0.05$.
[d] Analyzed using a Friedman test
[e] Analyzed using a Cochran's Q test
*Statistically significant at $p<0.05$

Discussion

Oral cryotherapy significantly reduced mucositis severity, with earlier improvement compared to the control group. In the cryotherapy group, a significant reduction was observed from the first day of intervention, whereas the control group showed improvement only after 14 days. These findings are consistent with previous studies demonstrating the efficacy of oral cryotherapy in mitigating mucositis severity in cancer patients undergoing chemotherapy [36]. However, a study reported significant effects only on days 7 and 14, suggesting variability in response timing across different patient populations and treatment protocols [37]. In this study, the observed improvement in the control group, despite the absence of cryotherapy, highlights the role of routine oral hygiene in mucositis management. Prior study has indicated that standard oral care can mitigate mucositis severity, although its effects typically become evident after a longer duration [38]. This suggested that while oral hygiene alone may contribute to mucosal healing, the addition of cryotherapy accelerates the recovery process, providing an early and more pronounced therapeutic benefit. A meta-analysis further supported the efficacy of oral cryotherapy in reducing the incidence and severity of mucositis among chemotherapy patients [39]. Given its effectiveness and minimal side effects, oral cryotherapy is considered a first-line strategy for mucositis prevention. Aligned with these findings, the MASCC recommends oral cryotherapy as an evidence-based approach for mucositis management [40]. The results in this study highlighted the clinical relevance of cryotherapy in supportive cancer care, reinforcing its role as a non-invasive and easily implementable intervention for improving patient outcomes [24,41].

The findings of this study demonstrated that cryotherapy is effective in preventing oral mucositis in cancer patients undergoing chemotherapy, with its protective effects attributable to several molecular mechanisms. The primary mechanism involves the induction of vasoconstriction, which reduces blood flow to the oral mucosa, thereby limiting the exposure of epithelial cells to chemotherapeutic agents [19]. This restricted drug delivery minimizes direct cytotoxic damage to the basal epithelium, preserving mucosal integrity [19]. Beyond its vascular effects, cryotherapy modulates inflammatory pathways by inhibiting the activation of nuclear factor kappa B (NF-κB), a key transcription factor involved in the upregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). Suppression of these inflammatory mediators reduces neutrophil and macrophage infiltration, thereby mitigating excessive tissue damage and ulcer formation. Additionally, cryotherapy attenuates the inflammatory response by decreasing macrophage infiltration and the accumulation of key inflammatory markers, contributing to the preservation of tissue architecture without adversely affecting muscle injury area or extracellular matrix remodeling [42]. These molecular mechanisms collectively highlight the therapeutic potential of cryotherapy in minimizing chemotherapy-induced mucosal injury. By reducing both direct cytotoxic effects and inflammatory responses, cryotherapy serves as a non-invasive and effective strategy for preventing oral mucositis, thereby improving patient outcomes in cancer treatment.

The optimal duration of oral cryotherapy remains a subject of ongoing investigation, with studies yielding mixed results regarding its efficacy across different time frames. While one study reported no significant difference in mucositis prevention between 2-hour and 7-hour applications [43], other research demonstrated that administering ice cubes for 35 minutes—before, during, and after chemotherapy—significantly enhanced its effectiveness [44]. The MASCC/ISOO guidelines currently recommend a 30-minute cryotherapy duration; however, emerging evidence suggests that a shorter 20-minute protocol provides comparable benefits [24,36]. In this study, all patients received a 20-minute oral cryotherapy intervention. These findings highlighted the potential for optimizing oral cryotherapy by reducing treatment duration without compromising clinical efficacy. A shorter protocol could improve patient adherence and comfort while maintaining mucosal protection against chemotherapy-induced cytotoxicity.

Serial assessments of oral mucosal smears in this study demonstrated a progressive reduction in neutrophilic inflammatory cells over 14 days. A significant decline in inflammatory cell infiltration was noted on days 7 and 14 post-intervention, coinciding with the restoration of oral squamous cell size to normal and the absence of cocci. These findings are particularly relevant given that mucositis symptoms typically manifest 7–14 days after chemotherapy initiation [28]. The administration of oral cryotherapy on days 1, 7, and 14 effectively mitigated mucosal injury, as evidenced by the cytological findings. The protective effect of cryotherapy is likely mediated through multiple mechanisms. Local cooling of the oral mucosa inhibits the release of proinflammatory cytokines, thereby attenuating the inflammatory cascade and reducing mucosal damage [45]. Additionally, cryotherapy-induced vasoconstriction limits chemotherapeutic drug distribution to the mucosal epithelium, minimizing direct cytotoxic injury [46,47].

This study highlighted oral cryotherapy as an effective and accessible therapeutic strategy for reducing oral mucositis in patients undergoing chemotherapy. The findings supported its integration into routine clinical practice as both a preventive and therapeutic intervention. Cryotherapy, a non-invasive and cost-effective modality, can be administered by oncologists and nursing professionals as part of a multidisciplinary approach to supportive cancer care. By alleviating mucositis severity, cryotherapy has the potential to enhance treatment adherence, improve patient comfort, and ultimately contribute to better overall quality of life. Despite its clinical benefits, several limitations must be acknowledged. The relatively small sample size may limit the generalizability of the findings to a broader patient population, considering variations in age, sex, genetic predisposition, and comorbid conditions. Additionally, the study primarily focused on short-term outcomes without evaluating the long-term effects of cryotherapy on mucosal healing, recurrence rates, or potential delayed adverse effects. Individual responses to cryotherapy, including variability in patient compliance and inconsistencies in chemotherapy schedules, were not fully explored. Moreover, the COVID-19 pandemic posed significant challenges, including disruptions in chemotherapy visits due to mandatory pre-treatment screening, prolonged waiting times, and patient attrition resulting from loss to follow-up or COVID-19-related complications. These factors may have influenced study adherence and outcome assessments, emphasizing the need for further research under more stable clinical conditions.

To address these limitations, future research should focus on conducting larger, multicenter randomized controlled trials to validate these findings in a more diverse patient population. Expanding the study cohort enhances the generalizability of the results and provides a more comprehensive understanding of the efficacy of oral cryotherapy across different demographic and clinical subgroups. Long-term investigations remain necessary to assess the sustained impact of cryotherapy on mucosal healing, patient-reported outcomes, and potential modifications to the oral microbiome and localized immune response. Evaluating these parameters over an extended period helps determine whether cryotherapy offers lasting benefits beyond the immediate reduction of mucositis severity. Increasing the study duration or sample size allows for better accommodation of patient attrition and strengthens the robustness of efficacy assessments. Implementing strategies to minimize dropout rates, such as enhanced patient follow-up protocols and flexible study designs accommodating variations in chemotherapy schedules, improves the reliability of findings. Future studies should also explore the integration of cryotherapy with other

supportive care strategies to optimize mucositis management in patients undergoing chemotherapy.

Conclusion

Oral cryotherapy serves as an effective intervention for mitigating the severity of oral mucositis in patients receiving chemotherapy. Exfoliative cytology analysis of serial oral mucosal smears revealed a progressive reduction in neutrophilic inflammatory cells, with significant decreases observed by day 7 and sustained through day 14. These findings supported the therapeutic potential of oral cryotherapy as a practical, cost-effective, and well-tolerated intervention. Given its ease of administration and minimal adverse effects, professionals may integrate oral cryotherapy into routine supportive care protocols to alleviate mucositis severity and improve patient outcomes during chemotherapy.

Ethics approval

Protocol of this study was approved by the Health Research Ethics Commission, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia (Approval number: UH20060254).

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Competing interests

The authors declared no conflict of interest

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tool and methodology of which AI-based language model ChatGPT was employed in the language refinement (improving grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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