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# Prophylactic Effects of Chlorine Compounds on Recurrent Aphthous Ulceration

SUMMARY

***Objective**: Purpose of the study was to evaluate the effects of 2 different chlorine compounds on recurrent aphthous ulcerations (RAU).*

***Method**: The study was performed on 30 RAU patients divided into 2 groups. None of these patients had aphthous ulcer at presentation. In the first group 0.2% chlorhexidine gluconate and in the latter 0.1% Chloramin-T mouthwashes were applied. The follow-up period was 3 months and cytological smear examination of the buccal mucosa was done before and at the end of the study.*

***Results**: Aphthous ulcer formation did not occur clinically during this study in both groups. In cytological examination no statistically significant difference could be demonstrated in maturation index to examine the keratinisation and oral flora between 2 groups (p>0.05).*

***Conclusion**: We have observed the same effect of both compounds concerning prophylaxis of RAU; however, by cytological examination, we could not prove keratinisation effect of chlorine compounds. Further studies must be carried out to evaluate mechanisms of chlorine compounds activity in the prophylaxis of RAU.*

**Keywords:** Recurrent Aphthous Ulceration, prophylaxis; Chlorhexidine Gluconate Mouthwash

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## Introduction

Recurrent aphthous ulceration (RAU) or recurrent aphthous stomatitis is the most common oral mucosal disease known to human beings. RAU is divided into 3 varieties: minor recurrent aphthous stomatitis, major recurrent aphthous stomatitis and herpetiform ulcers[10,18,21]. The most common presentation is minor recurrent aphthous stomatitis: recurrent, round, clearly defined, small, painful ulcers that heal in 10 to 14 days without scarring[10,18,21]. Lesions are larger in major recurrent aphthous stomatitis (greater than 5mm), and can last for 6 weeks or more, and frequently scarring[20]. The third variety of recurrent aphthous stomatitis is herpetiform ulcer, which presents as multiple small clusters of pinpoint ulcers that can fuse together to form large irregular ulcers and last 7 to 10 days[10,18,20,21].

There have been numerous proposed etiologic mechanisms for RAU, including trauma[20]; autoimmune disease such as cyclic neutropenia[17], Behçet's disease[20]; deficiencies in iron, folic acid and vitamins B1, B2, B6, B12[14]; genetic basis[10]; microbial factors; gastrointestinal dysfunction[10, 18, 21]. Some studies have found a correlation between stress and RAU[10,16]; however, a more recent investigation revealed no association between stress in psychological life and RAU[10,13, 20]. RAU may be more common in HIV-infected patients because it has been suggested that RAU represents a local breakdown in immunoregulation, a condition that could be amplified by HIV disease[12]. Despite much clinical and research attention, as mentioned above the causes remain poorly understood.

Immunologic studies clearly demonstrated that ulcers of RAU represent a cell-mediated immunologic

dysfunction in which infiltrating T-lymphocytes play a primary role[15].

There is no specific treatment for RAU, and management strategies depend on the symptoms, duration, severities and associated systemic conditions. Management of RAU includes the use of analgesics, antimicrobials, and immunomodulatory drugs[1,4-9,11,20]. However, ulcers of RAU heal in 10 to 14 days spontaneously.

The purpose of this study was to evaluate and compare the cytological and clinical effects of 2 different chlorine compounds, which are not harmful to the tissues on patients with recurrent aphthous ulceration whatever the etiologic factor could be.

## Material and Method

### *Clinical Method*

A total of 30 RAU patients between 20 and 60 years of age, with a history of minor aphthous ulceration, but with an ulcer-free period not exceeding 3 weeks and non-smoker, entered the study. Each individual was asked to stop any other ulcer therapy, if applicable, at least 3 weeks before entering the study. None of these patients had aphthous ulcer at presentation. Patients were divided into 2 groups of 15 patients.

In the first group 5 ml mouthwashes with 0.1% Chloramin-T granules were applied twice a day for 1 minute during 3 months. Patients were advised not to drink and eat anything at least 1 hour after this application.

In the second group 0.2% Chlorhexidine gluconate (Orohex®, Bilim) mouthwash was applied in the same protocol with the first group.

### *Cytological Method*

Cytological smear examination of the buccal mucosa was done before and at the end of the study. The PAP smear was performed.

Examination of keratinisation was evaluated according to the maturation, which is a method of examination in the vaginal cytological smear. It was modified to the maturation index for mouth according to the following criteria[3]:

0. Non-keratinised surface cells: Intermediate maturity may be slightly flattened, with somewhat irregular cytoplasmic morphology and some degree of anuclear contraction. Para-basal and immature prickle cells are spherical or cuboidal and have a centrally placed nucleus with even distribution of chromatin;

1. Keratinised surface cells: Mature or cornified cells are more flattened and irregular in appearance and have small, pyknotic nuclei or are anucleated.

Examination of polymorph nuclear leukocyte (PNL) accumulation was evaluated according to the following criteria:

0. Nil
1. Minimal
2. Moderate
3. Dense

Statistical Analysis

Statistical analysis was done using Graph Pad Prism V.3 program. Qualitative data were analyzed by employing $\chi$-square parametric test. The results were accepted as significant at the $p<0.05$ level.

## Results

A total of 30 patients (age range 22-60, mean age 33.2 years) entered the study, of whom 12 were male and 18 were female. Aphthous ulcer formation did not occur clinically during this study in both groups.

In cytological examination no statistically significant differences ($\chi^2$: 2.226; p=0.329) could be demonstrated in maturation index when keratinisation was examined (Tabs. 1 and 2; Figs. 1 and 2).

*Table 1. Keratinisation during the treatment in both groups*

| | | Chlorhexidine n=15 | Chloramin-T n=15 | p | $\chi^2$ test |
|---|---|---|---|---|---|
| Before | Non-keratinised cells | 3<br>20% | 2<br>13.3% | > 0.05<br>ns* | 0.24 |
| | Keratinised cells | 12<br>80% | 13<br>86.7% | | |
| After | Non-keratinised cells | 11<br>73.3% | 7<br>46.7% | > 0.05<br>ns* | 2.22 |
| | Keratinised cells | 4<br>26.7% | 8<br>53.3% | | |
| | | | | p=0.329 | 2.226 |

* - not significant

*Table 2. Cross-tabulation of keratinisation variation during the treatment*

| Keratinisation | Patient groups | | Total |
|---|---|---|---|
| | Chlorhexidine n=15 | Chloramin-T n=15 | |
| No changes in the keratinised cells | 4<br>13.3% | 8<br>26.7% | 12<br>40.0% |
| Keratinised cells differentiated to non-keratinised cells | 8<br>26.7% | 5<br>16.7% | 13<br>43.3% |
| No change in the non-keratinised cells | 3<br>10.0% | 2<br>6.7% | 5<br>16.7% |
| TOTAL | 15<br>50% | 15<br>50% | 30<br>100% |

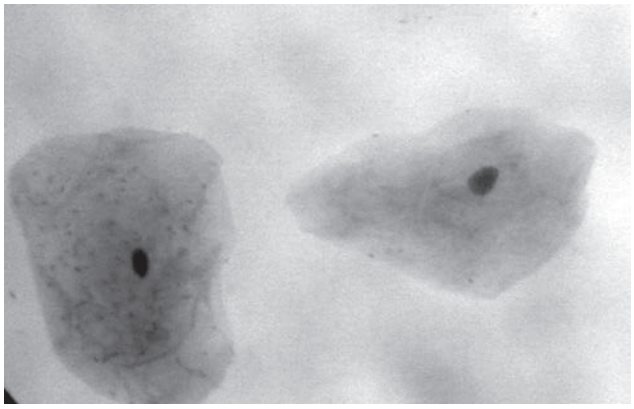

*Figure 1. Mature non-keratinised cells before the treatment (PAP; x400)*

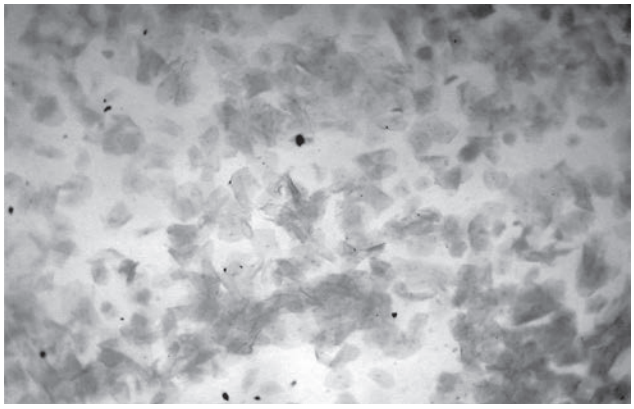

*Figure 2. Anucleated mature keratinised cells before the treatment (PAP, x100)*

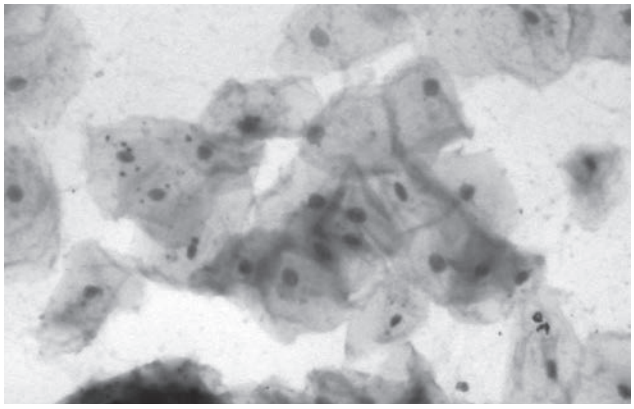

*Figure 3. Immature keratinised cells after the treatment (PAP; x400)*

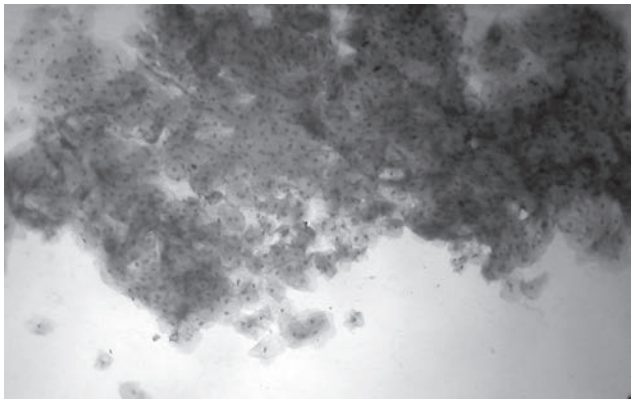

*Figure 4. Intermediate mature non-keratinised cells after the treatment (PAP; x100)*

Variations in keratinisation in both groups are described in table 3. Keratinised cells differentiated to non-keratinised cells after the treatment in 8 patients of the first group and 5 patients of the second group (Fig. 3 and 4). No statistically significant difference ($\chi^2$: 9.714; p=0.286) could be demonstrated in polymorphonuclear leukocyte (PNL) accumulation in both groups (Tab. 3).

Hif/pseudohif was encountered in 3 patients before the therapy. At the end of the therapy all were cured (Fig. 3).

*Table 3. Table of PNL accumulation during the treatment in both groups*

| TREATMENT | PNL Accumulation | Chlorhexidine n=15 | Chloramin-T n=15 | p | $\chi 2$ test |
|---|---|---|---|---|---|
| BEFORE | Nil | 2 | 2 | > 0.05 ns* | 4.261 |
| | Minimal | 6 | 11 | | |
| | Moderate | 3 | 1 | | |
| | Dense | 4 | 1 | | |
| AFTER | Nil | 7 | 7 | > 0.05 ns* | 0.41 |
| | Minimal | 7 | 6 | | |
| | Moderate | 1 | 2 | | |
| | Dense | 0 | 0 | | |
| | | | | p=0.286 | 9.714 |

* - not significant

## Discussion

Recurrent aphthous ulcers are common, painful lesions of the oral mucosa. There is no specific treatment for RAU. Although ulcers of RAU heal in 10 to 14 days spontaneously, antimicrobial rinses have some clinical efficacy for the treatment of RAU[1].

Chlorine containing mouthwashes have been available for a number of years. There have been relatively few studies of the antimicrobial properties of this mouthwash. Several studies have reported that mouthwashes with chlorine compounds reduce the number of ulcer days, increase ulcer-free days and interval between bouts of ulceration[1,4,6,10,19]. The role of chlorine compounds in prophylaxis of RAU may be due to the antibacterial effect of these agents[1,19,20]. In this study cytological examination demonstrated that there are no statistically significant differences in PNL accumulation in both groups. But we have observed that PNL accumulation diminished after application of these chlorine compounds.

Chloramin-T, which is a swimming pool disinfectant, has been used as a mouthwash in the prophylaxis of RAU since many years in our clinic. Chlorhexidine gluconate, which is also a chlorine compound, was evaluated and compared with Chloramin-T in this study. We observed the same effect of both compounds in the prophylaxis of RAU. Aphthous ulcer formation did not occur clinically during this study in both groups.

We assumed that the effect of chlorine compounds on patients with RAU was increased keratinisation of oral mucosa. But by cytological buccal mucosal smear examination, keratinisation was not observed - it was decreased in 43.3% of patients.

On the other hand, several studies reported that cigarette smoking prevents aphthous ulcers by causing increased keratinisation of oral mucosa[1,2]. Grady et al[8] reported that smokeless tobacco also prevents aphthous stomatitis. 1456 professional baseball players were examined, about half of whom were smokeless tobacco users. Usage of smokeless tobacco significantly reduced the risk of aphthous ulcers among healthy young men. They suggested that smokeless tobacco may protect aphthous ulcers by the increasing of keratinisation[8]. As a result, the antibacterial effect of chlorine compounds in the prophylaxis and treatment of RAU is open to discussion.

In this study, we have seen that chlorine compounds are effective in prophylaxis of RAU without increasing keratinisation. On the other hand, several studies have shown that smoking and smokeless tobacco can prevent RAU by increasing the keratinisation. In conclusion the effects of chlorine compounds mouthwashes in prophylaxis of RAU needs further investigation.

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