Abstract: Oral biopsy is a common surgical practice. To date, few authors have described the healing of mucosal wounds following oral biopsy. The aim of the present single-center, prospective, randomized, controlled study is to evaluate the differences between two chlorhexidine (CHX) mouthrinses and a treatment-free approach in the immediate postoperative period following oral mucosal biopsy. 354 patients were included in this study and were randomly allocated to three groups: A) CHX 0.12% mouthrinse (twice daily, 10 mL rinse for 30 s), B) CHX 0.20% mouthrinse (twice daily, 10 mL rinse for 30 s), and C) no topical treatment. 118 patients were treated in group A and 115 in group B; 121 received no therapy in group C, reporting the greatest improvement in related symptoms. Outcome variables included the age, gender, site and size of lesions, visual analog score (VAS), Oral Health Impact Profile questionnaire (OHIP-14), and number of painkillers taken during the first week post-surgery. Use of a CHX 0.12% mouthrinse exhibited the poorest outcome. On the other hand, best healing was found for patients treated with CHX 0.20%. These findings suggest that regardless of its concentration, CHX was found to be ineffective in reducing related symptoms, whereas CHX 0.20% can be recommended to facilitate enhanced healing.

Keywords: chlorhexidine, mouthrinse, oral biopsy, outcome, trial

Introduction

Oral mucosal biopsies are considered to be minor surgeries, typically performed under local anesthesia [1,2]. Chlorhexidine (CHX) is a commonly used topical anti-septic with bactericidal, fungicidal, and virucidal activities [3]. To date, very few studies have considered possible treatment modalities for patients following oral mucosal biopsy [1,4].

In this study, the differences between two CHX mouthrinses formulations (0.12% vs 0.20%) in the immediate postoperative period for oral mucosal biopsies were evaluated, and the acquired findings were compared with the results from patients undergoing no topical treatment. The null hypothesis is that there is no difference between the three arms in the immediate postoperative period.

Materials and Methods

Ethical approval of the study protocol

The present trial has been registered with ISRCTN (#14440167) and was conducted in line with the principles of the Helsinki Declaration of 1975, as revised in 2000. This study was also accepted by the Research Board of the CIR-Dental School, University of Turin (CIR-2017-22, 2017). All subjects were informed about the surgical procedures, postoperative complications, and provided written informed consent.

Study design

This randomized, controlled, single-blind, parallel design clinical trial was designed according to the CONSORT statement for improving the quality of reports of randomized controlled trials (http://www.consort-statement.org/). In total, 354 consecutive Caucasian patients referred for histological determination of oral lesions participated in this study from May to August 2017.

The exclusion criteria focused on patient’s past medical history such as development of systemic infective disease, cardiovascular, hepatic and hematological disease, coagulation deficiency, uncontrolled diabetes (and previous/current, neoplastic disease), immunosuppression either drug-related or caused by an underlying disease, head and neck radiotherapy, and pregnancy or/and lactation.

Clinical assessment

All surgical sessions were performed by the same oral physician (R.B.). Mepivacaine hydrochloride (30 mg/mL) with epinephrine (1:100,000) was used for inducing local anesthesia. Resection was performed with the use of a scalpel (number 15 blade) mounted on a number 3 handle. Wounds were sutured with interrupted sutures using silk 4.0 (Perma-Hand, Ethicon, NJ, USA). Patients were given standard postoperative instructions and standard pain-relievers therapy (acetaminophen, 500 mg tablet), if necessary, every 6 h.

A computer-generated randomization list was generated, and only one external investigator who did not participate in the study was aware of its sequence and could have access to the file. The randomized codes were enclosed in sequentially sealed envelopes, opened at the end of the surgical session.

Patients were randomly allocated to three groups as follows:

- Protocol A) CHX 0.12% Chlorhexidine digluconate 0.12% alcohol-free (Paroex, Sunstar Butler, Mölndal, Sweden) twice daily for 6 days, a 10 mL rinse for 30 s 30 min following oral hygiene;
- Protocol B) CHX 0.20% Chlorhexidine digluconate 0.20% alcohol-free (Paroex, Sunstar Butler) with the same posology of protocol A;
- Protocol C) no treatment (control).

Patients were reassessed on the day of suture removal (after 1 week) and the reported pain levels, quality of life, and healing of tissue were evaluated and documented. Products were freely provided to subjects in their original packages.

A single oral health care provider (PGA) evaluated the clinical aspects of the lesions by recording their size and site of appearing. Concerning the latter, oral cavity was split into three macro-zones: zone 1) gingiva, palate and retromolar area; zone 2) buccal mucosa and lips; and zone 3) tongue and floor of the mouth.

Outcomes

The primary outcomes of the study focus on assessing quality of life and postoperative pain. The Italian version of the Oral Health Impact Profile-14 (OHIP-14) [5] and a Visual Analog Scale (VAS) were used to assess patients that were subsequently completed in the evening of the surgical day, as well as on day 1, day 3, and day 6 following surgery.

Furthermore, subjects had to record the total amount of painkillers taken during the first 6 days after surgery. The forms were collected on the 7th day after surgery by the same independent examiner (Y.N.) who had...
knowledge of the respective allocation groups.

Clinical healing of the biopsied site, mainly in terms of cicatrization, was assessed via a semi-quantitative scale (good/satisfactory = POSITIVE, insufficient/very bad = NEGATIVE) [6].

**Statistical analysis and sample size**

Sample size was a challenging issue to estimate due to the lack of any earlier reported changes in patients treated under these protocols. However, with a minimum of 115 subjects for each group, thereby fixing a number of painkillers received by patients. Similar findings were obtained for OHIP and for the number of painkillers used). Because of the failure to randomize the generation of three comparable groups, the models were adjusted by means of CHX at “day zero” (the day of the surgical procedure), age, lesion diameter, intervention duration, gender, lesion location, and smoking status.

Finally, multivariable logistic regression models were constructed to evaluate the effect of these protocols on healing. Models were adjusted by age, lesion diameter, intervention duration, gender, lesion location, and smoking status.

Hypothesis tests were two-sided, and statistical significance was set at $\alpha = 0.05$. The statistical software SAS, v9.3 (SAS Institute, Cary, NC, USA) was used to perform the statistical analysis.

**Results**

Of the 422 patients initially screened, 354 patients participated in this study, of which 221 were women (62.4%). The mean age for females was 56.7 years (± 13.8) and for males was 58.2 years (± 12.1).

118 patients (33.3%) were treated with protocol A, 115 (32.5%) with protocol B, and 121 (34.2%) received no therapy (Fig. 1).

Despite the randomization, significant differences were identified between these three groups regarding gender, smoking status, lesions' size, and sites of involvement (Table 1). More specifically, the size of lesions in patients treated with CHX 0.20% ($P = 0.0008$) was greater, although such difference did not influence significantly the duration of the underlying surgical treatment.

Patients treated with CHX 0.12% had more lesions in the keratinized areas and in the tongue when compared with subjects in the other two protocols ($P = 0.0003$).

With respect to the primary outcomes, patients treated with CHX 0.12% had a higher VAS baseline, regardless of the duration of surgery. Symptoms were improved the most in patients belonging to the group that had a higher VAS baseline, regardless of the duration of surgery.

The differences between continuous variables were evaluated with Kruskal-Wallis tests because of the non-normality of the distribution. Furthermore, differences between qualitative variables were evaluated using the Chi-square test. Multivariable linear regression models were constructed in order to test the effect of the respective protocols on several outcomes (VAS at day one, VAS at day 3, VAS at day 6, OHIP-14, and number of painkillers used). Because of the failure to randomize the generation of three comparable groups, the models were adjusted by means of CHX at “day zero” (the day of the surgical procedure), age, lesion diameter, intervention duration, gender, lesion location, and smoking status.

### Table 1 Descriptive statistics. P values computed using Kruskal-Wallis test for continuous variables and chi-squared test for qualitative ones.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall n = 354</th>
<th>CHX 0.12% n = 118</th>
<th>CHX 0.20% n = 115</th>
<th>No therapy n = 121</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.4 (16.3)</td>
<td>57.5 (16.7)</td>
<td>58.1 (16.1)</td>
<td>59.3 (15.0)</td>
<td>0.056</td>
</tr>
<tr>
<td>Lesion diameter (mm)</td>
<td>7.2 (2.5)</td>
<td>7.2 (2.7)</td>
<td>7.8 (2.0)</td>
<td>8.7 (2.6)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>3.5 (1.6)</td>
<td>3.3 (1.2)</td>
<td>3.8 (2.0)</td>
<td>3.3 (1.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>VAS 0</td>
<td>1.9 (2.1)</td>
<td>2.3 (2.4)</td>
<td>1.6 (2.1)</td>
<td>1.7 (1.8)</td>
<td>0.06</td>
</tr>
<tr>
<td>VAS 1</td>
<td>1.3 (2.0)</td>
<td>1.6 (2.2)</td>
<td>1.2 (2.3)</td>
<td>1.0 (1.6)</td>
<td>0.008</td>
</tr>
<tr>
<td>VAS 3</td>
<td>1.0 (1.9)</td>
<td>1.5 (2.2)</td>
<td>0.8 (1.8)</td>
<td>0.7 (1.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>VAS 6</td>
<td>0.5 (1.1)</td>
<td>0.8 (1.3)</td>
<td>0.4 (1.1)</td>
<td>0.2 (0.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OHIP 14</td>
<td>3.1 (5.4)</td>
<td>4.6 (5.7)</td>
<td>2.5 (4.9)</td>
<td>2.4 (5.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Analgesics (acetaminophen 500 mg tablet)</td>
<td>1.3 (3.0)</td>
<td>1.8 (3.5)</td>
<td>1.4 (3.4)</td>
<td>0.8 (1.8)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

VAS* = Visual Analogue Scale; Analgesics* = acetaminophen 500 mg tablet; Oral site§ = zone 1: gingiva, palate and retromolar area; zone 2: buccal mucosa and lips; zone 3: tongue and floor of the mouth.

**Fig. 1** CONSORT explanatory flow chart.
Table 2: Multivariable linear regression models for the effect of protocol on: VAS at day 1, 3, and 6, OHIP-14, and number of painkillers

<table>
<thead>
<tr>
<th>Variables</th>
<th>VAS 1 β (P value)</th>
<th>VAS 3 β (P value)</th>
<th>VAS 6 β (P value)</th>
<th>OHIP-14 β (P value)</th>
<th>Painkillers used β (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>model r²: 0.65</td>
<td>model r²: 0.37</td>
<td>model r²: 0.29</td>
<td>model r²: 0.27</td>
<td>model r²: 0.23</td>
</tr>
<tr>
<td>CHX 0.12%</td>
<td>0.03 (0.87)</td>
<td>0.40 (0.05)</td>
<td>0.35 (0.005)</td>
<td>1.58 (0.004)</td>
<td>0.99 (0.01)</td>
</tr>
<tr>
<td>CHX 0.20%</td>
<td>0.20 (0.23)</td>
<td>0.27 (0.19)</td>
<td>0.26 (0.04)</td>
<td>1.34 (0.04)</td>
<td>0.47 (0.28)</td>
</tr>
<tr>
<td>VAS* 0</td>
<td>0.75 (&lt;0.0001)</td>
<td>0.49 (&lt;0.0001)</td>
<td>0.24 (&lt;0.0001)</td>
<td>1.13 (&lt;0.0001)</td>
<td>0.50 (&lt;0.0001)</td>
</tr>
<tr>
<td>Age</td>
<td>0.0005 (0.90)</td>
<td>−0.004 (0.36)</td>
<td>−0.001 (0.75)</td>
<td>0.008 (0.60)</td>
<td>−0.01 (0.24)</td>
</tr>
<tr>
<td>Lesion diameter (mm)</td>
<td>−0.01 (0.70)</td>
<td>0.02 (0.56)</td>
<td>0.06 (0.01)</td>
<td>−0.41 (0.02)</td>
<td>0.05 (0.48)</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>0.02 (0.68)</td>
<td>−0.06 (0.35)</td>
<td>−0.20 (&lt;0.0001)</td>
<td>0.25 (0.21)</td>
<td>0.30 (0.008)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Female</td>
<td>0.24 (0.10)</td>
<td>−0.26 (0.14)</td>
<td>−0.07 (0.49)</td>
<td>−2.57 (&lt;0.0001)</td>
<td>−0.40 (0.21)</td>
</tr>
<tr>
<td>Oral site</td>
<td>Zone 1</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Zone 2</td>
<td>−0.004 (0.98)</td>
<td>0.33 (0.16)</td>
<td>0.13 (0.35)</td>
<td>−1.09 (0.14)</td>
<td>1.09 (0.02)</td>
</tr>
<tr>
<td>Zone 3</td>
<td>0.53 (0.14)</td>
<td>0.93 (0.03)</td>
<td>0.09 (0.74)</td>
<td>1.90 (0.17)</td>
<td>0.31 (0.69)</td>
</tr>
<tr>
<td>Yes</td>
<td>0.24 (0.15)</td>
<td>0.68 (0.001)</td>
<td>0.28 (0.03)</td>
<td>1.11 (0.09)</td>
<td>−0.53 (0.16)</td>
</tr>
</tbody>
</table>

Models adjusted by: VAS at day 0, age, lesion diameter, intervention duration, gender, lesion location, smoking status. VAS*: Visual Analogue Scale; Painkillers = acetaminophen 500 mg tablet; Oral site = zone 1: gingiva, palate and retromolar area; zone 2: buccal mucosa and lips; zone 3: tongue and floor of the mouth.

0.004). Early postoperative complications revealed no statistical differences (P > 0.05).

Healing was more efficient in patients treated with CHX 0.20% (P = 0.0001).

Table 2 shows five multivariate linear regression models that evaluate the effect of the three protocols on the following outcomes: VAS at day 1, day 3 and day 6 following surgery, OHIP-14, and number of painkillers. When equalizing baseline VAS, age, diameter of the lesion, duration of intervention, gender, site and smoking habits, treatment with CHX 0.12% lead to an average increase in 6th-day-VAS of 0.35 units (P = 0.005), and in OHIP-14 of 1.86 (P = 0.004) units.

On the other hand, treatment with CHX 0.20% lead to an average increase in 6th-day-VAS of 0.26 units (P = 0.04), and in OHIP-14 of 1.34 units (P = 0.04).

The CHX 0.12% treatment increased the number of painkillers used, with an average of 0.90 units (P ≤ 0.01), when compared to those without therapy, whereas CHX 0.20% treatment increased that number by 0.47 units, being not statistically significant (P ≥ 0.20).

In the model for VAS at day 1, only the score of VAS at baseline counted (β = 0.75, P < 0.0001), while in the model for VAS at day 3 only the effect of smoking (β = 0.68, P = 0.001) and of the site of the tongue (β = 0.93, P = 0.03) was taken into consideration.

In the model for VAS at day 6, VAS at baseline was significantly weighed (β = 0.24, P < 0.0001). Moreover, the effect of the intervention’s duration demonstrated strong association (β = −0.20, P < 0.0001), as well as the effects of smoking (β = 0.28, P = 0.03) and of the lesions’ diameter (β = −0.06, P = 0.01).

In the model for OHIP-14, there was a strong effect of VAS at baseline (β = 1.13, P < 0.0001) and diameter of lesion (β = −0.41, P = 0.002). Females presented an average reduction of 2.57 units (P < 0.0001) when compared to male patients.

Finally, in the model regarding the number of painkillers received by patients, the effect of VAS at baseline (β = 0.50, P < 0.0001), and the surgery’s duration (β = 0.30, P = 0.008) were both significantly weighed.

Multivariable logistic regression models for the effect of the three protocols on POSITIVE healing, adjusted by age, lesion diameter, intervention duration, gender, site and smoking status revealed that CHX 0.20% increased the probability of a proper healing by 96% (95% CI 1.02-3.94), when compared to patients who did not follow any therapy (Protocol C). When it comes to assessing the healing site, buccal mucosa and lips exhibited improved healing processes when compared to the other macro-zones (14.45; 95% CI 6.47-32.26). Finally, and with respect to the side effects experienced, two patients treated with CHX 0.12%, and three with CHX 0.20% complained about a bitter taste at the time of the suture removal.

Discussion

This is the first randomized controlled study to assess the effect of CHX mouthrinses in the immediate postoperative period following oral mucosal biopsies.

Microbial infection of oral postsurgical area can inhibit normal tissue healing process. Therefore, the aim of this study was to investigate and evaluate the efficacy of CHX, a potent antibacterial agent belonging to the biguanide family, characterized by a broad-spectrum antimicrobial activity [3].

The findings of the present trial demonstrate no significant differences between the evaluated mouthrinses in terms of reported pain or related quality of life on the 7th postsurgical day. Hence, CHX mouthrinses were found to be ineffective in terms of reducing related symptoms, regardless of the concentration used.

However, it is important to acknowledge that patients treated with CHX 0.12% exhibited the most intense peak of pain, with the highest level of perceived pain occurring in the first evening after surgery, followed by gradual decrease over the next days. The weekly pain score never surpassed a mean of 2.3, thus confirming previous findings regarding the mild pain experienced during oral mucosal biopsy [4,7]. Additionally, in these groups, smokers were included and a great number of biopsies were performed in the palate, gingiva and tongue, which are usually the most painful sites in the oral cavity after surgery.

On the other hand, Lopez-Jornet et al. showed that the use of CHX 0.2% digluconate gel following oral biopsy could reduce pain significantly, perhaps because its direct application to the wounded tissue and its prolonged release could form a barrier against infection [2].

Wound healing seemed to be better in patients treated with CHX 0.20%, especially when compared with CHX 0.12%. However, the differences between the three groups with respect to the gender, lesions’ size, site of involvement and smoking status may have introduced a bias on this critical outcome. Interestingly, and concerning the oral site of biopsy, buccal mucosa and lips were found to heal more easily when compared to other macro-zones, and this finding is reported for the first time. Similarly, no evidence is available regarding a differential perception of immediate postoperative pain after minor oral surgery between males and females. Concerning smoking status, only 21.7% of patients in Group 2 were smokers, whereas Group 1 included 35.6% smokers, whereas Group 1 included 35.6% smokers, whereas Group 1 included 35.6% smokers. Although smoking could influence the healing rate of oral surgical wounds as a result of catecholamines release, vasoconstriction and decreased tissue perfusion [8], the present trial identified that smoking primarily affects the reported pain levels, with no objective wound deterioration.

This report suffers from several limitations. The main limitation is that this study lacks a placebo arm—as opposed to a control group—which
could have increased the validity and effectiveness of the results of this study. Moreover, a double-blind design using identical bottles administered by clinicians to unaware patients, could have been a more appropriate methodological design for the purposes of this study.

Further investigations should focus on large-scale randomized double-blind controlled trials to identify the optimal selection of concentration, timing of administration, and ideal dosage of CHX rinses in the immediate postoperative time after oral biopsy.

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Conflict of interest
None declared.

References