Comparison between exfoliative cytology and histopathology in detecting oral squamous cell carcinoma

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Abstract

Objectives: To compare the cytological findings of preoperative buccal smears with their histopathological types in oral cavity tumors.

Materials and Methods: This study investigated 45 patients with oral tumors who had undergone preoperative buccal smears of oral lesions and were diagnosed by postoperative histopathological examination. Two samples were taken from patients that were clinically diagnosed with oral squamous cell carcinoma (OSCC): smears for cytological examination and biopsies for histopathological diagnosis.

Results: Of the 45 cases studied, histopathology showed the presence of OSCC (n = 39 [86.7%]), verrucous carcinoma (n = 2 [4.4%]), and hyperkeratosis (n = 4 [8.9%]). OSCC cases predominantly occurred in males, with a ratio of 3.8:1 (P = 0.000); the majority of OSCC cases, in both males (27; 87%) and females (6; 75%), were older than 50 years. Most OSCC cases, 32 (82.1%), were toombak dippers, and 28 out of the 32 were males (87.5%). In addition, the labiogingival, a common site of toombak dipping, was a prevalent lesion site of OSCC (13 patients, 33.3%) (P = 0.004). The cytological findings showed good diagnostic concordance with the histopathological findings; in 39 cases of OSCC, 31 patients had positive smears and biopsies and eight patients had negative smears but positive biopsies, for an overall sensitivity of 79.5% and specificity of 100%.

Conclusion: Despite the small number of cases in this study, we realized that the diagnostic accuracy of preoperative buccal smear cytology for oral lesions was high and that it is a safe, easy-to-perform, and clinically useful diagnostic procedure.

Key words: Exfoliative cytology, histopathology, oral cancer, toombak

Introduction

Oral carcinoma (OC) is increasing in developing countries, overall and substantially among younger patients.[1] Worldwide, OC is estimated to be the sixth most common carcinoma, and its prevalence is highest in Sudan.[2] Furthermore, OC in Sudan is the second most common cancer type, with an incidence rate of 920/year.[2] This is strongly attributed to the use of a local type of snuff known as toombak, a very popular material in the Sudanese community. The association between toombak dipping and OC has been investigated...
thoroughly in many studies. Approximately 94% of all oral malignancies are squamous cell carcinoma, which can be preceded by precursor lesions in the form of leukoplakia and erythroplakia. Oral squamous cell carcinoma (OSCC) afflicts an estimated 500,000 patients annually worldwide and has high mortality and increasing incidence rates. Cancerous lesions are usually benign in appearance and asymptomatic in nature in their early stages. In Sudan, many cases of OSCC are not diagnosed or treated until they reach an advanced stage; the prognosis for patients with OSCC that is treated early is much better. Since oral cancer is a major health problem, early detection is desirable so that successful therapy can be carried out. The diagnosis of precancers is primarily based on cell morphology. Exfoliative cytology is advantageous because it is a painless, noninvasive, quick, and simple procedure. The definitive diagnosis of cancer is made by biopsy. Exfoliative cytology is based on fact that normal epithelium is exposed to regular exfoliation. Benign disease or the occurrence of malignant epithelial formation causes the cells to lose their cohesive force and results in exfoliation. Loss of cohesion between the cells enables the collection of the exfoliated cells for microscopic examination. This noninvasive method, which requires small numbers of cells and has a relatively low cost, could be useful for the early diagnosis of oral cancer. Recent application of quantitative and cytomorphometric techniques has refined the potential role of oral exfoliative cytology, however, in the present study, conventional methods of smear collection, staining, and interpretation were followed because the study aimed to evaluate the efficiency of oral exfoliative cytology in detecting OSCC and to demonstrate the association between oral cancer, gender, and oral habits. In addition, this study sought to shed light on the urgent need for necessary measures for the prevention and early detection of oral cancer.

Materials and Methods

Study design
This is a prospective hospital-based study that investigated patients with oral tumors who had undergone preoperative buccal smears of oral lesions and were diagnosed by postoperative histopathological examination.

Study area
This study was conducted at Khartoum Dental Teaching Hospital from January 2012 to May 2015, together with the Histopathology and Cytology Department of the University of Khartoum, Faculty of Medical Laboratory Sciences. After clinical examination and obtaining written consent, patients who had oral lesions, independent of the size, color, consistency, presence of an ulcerated surface, or other clinical characteristics in the oral cavity, were prepared for buccal smear collection and subsequently for incisional biopsy.

Sample collection and preparation
Each patient was asked to wash his mouth thoroughly and then the lesion site was scraped. The sample was smeared on a glass slide and immediately fixed in 95% ethanol for 15 min, then stained with a Papanicolaou stain as described by George Papanicolaou in 1960. Smears were hydrated using 90% alcohol for 2 min, 70% alcohol for 2 min, rinsed in water for 2 min, stained by Harris hematoxylin for 5 min, rinsed in water for 2 min, differentiated in 0.5% hydrochloric acid for 10 s, rinsed in water for 2 min, blued in tap water for 10 min, dehydrated in 70% alcohol for 2 min, treated with two changes of 95% alcohol for 2 min in each, stained by orange G-6 for 2 min, washed in 95% alcohol for 2 min, and finally stained by EA50 for 2 min. Afterward, the smears were washed in 95% alcohol, dehydrated in absolute alcohol, and cleared in xylenes and mounted with DPX. All slides were screened by a cytotechnologist, and the results were confirmed by a cytopathologist. The oral biopsies for histopathological examinations were fixed in 10% buffered formalin, processed with the paraffin wax method, and embedded in paraffin blocks, followed by microtome cutting into 4 μm-thick sections. The deparaffinized sections were stained with Mayer’s hematoxylin for 8 min and subsequently with eosin for 3 min. Staining was performed according to the method described by Luna. The histopathological slides were evaluated by a pathologist.

Ethical consideration
This study was approved by the medical board of the Faculty of Medical Laboratory Sciences and by the administration of Khartoum Dental Teaching Hospital. Participation in this study was voluntary. All samples were collected after informing the patients about the study objectives, methods, and possible benefits, and written consent was obtained from all the participants. The decision to perform both the buccal smear for cytology and oral surgery was based on clinical indications only and was not influenced by this study. Only appropriate physicians reviewed the records, and the individual patient data remained anonymous.

Data analysis
The obtained data were analyzed using the Statistical Package for Social Sciences Program, version 17 (SPSS Inc., Chicago, IL, USA). The significance of the
The difference between the proportions of qualitative characteristics was tested using Chi-square test. \( P < 0.05 \) was considered statistically significant for all the statistical tests in the present study. The numbers of true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) results were calculated. The suspicious/malignant cytological results were considered TPs in cases where the operation revealed a malignancy upon histological examination, and they were considered FPs when no malignancy was found. The benign histopathological finding was considered a TN if the histological finding was benign and a FN in cases of histologically proven malignancy. From these numbers, the following statistical values were calculated:

- **Sensitivity in percentage**: \( \frac{TP}{TP + FN} \times 100 \)
- **Specificity in percentage**: \( \frac{TN}{TN + FP} \times 100 \)
- **Positive predictive value (PPV) in percentage**: \( \frac{TP}{TP + FP} \times 100 \)
- **Negative predictive value (NPV) in percentage**: \( \frac{TN}{TN + FN} \times 100 \).

### Results

Out of the 45 cases studied, histopathology showed the presence of OSCC \( (n = 39 \ [86.7\%]) \), verrucous carcinoma \( (n = 2 \ [4.4\%]) \), and hyperkeratosis \( (n = 4 \ [8.9\%]) \). The OSCC cases were predominantly male, with a ratio of 3.8:1 \( (P = 0.000) \), and most OSCC cases, males (27; 87\%) and females (6; 75\%), were older than 50 years.

Most OSCC cases (32; 82.1\%) were toombak dippers, and the majority was male (28; 87.5\%). In addition, the labiogingival, a common site of toombak dipping, was a prevalent OSCC lesion site \( (13; \ 33.3\% \ (P = 0.004)) \). The cytological findings showed good diagnostic concordance with the histopathological findings; in 39 cases of OSCC, 31 patients were TP results and eight patients had FP results, for an overall sensitivity of 79.5\% and specificity of 100\%. For the OSCC cytology results, a specificity of 100\%, a sensitivity of 79.5\%, a PPV of 100\%, and a NPV of 42\% were obtained when comparing nondysplastic lesions with dysplastic lesions [Figure 1].

### Discussion

In Sudan, few studies have compared histopathology and cytopathology results. Although in recent years, numerous morphometric and molecular factors\[^{14,15}\] have been proposed as prognosticators in OSCCs, these are yet to have an impact on routine clinical care. This study was an attempt to demonstrate the sensitivity and specificity of oral exfoliative cytology compared to histopathology in the diagnosis of oral cancer and to find out the association between OSCC and risk factors that may be linked the etiology of oral cancer. Improvements in the OSCC survival rate, the quality of life after treatment, and costs require reliable early diagnosis of the tumor. In this study, oral cytology had a sensitivity of 100\% and specificity of 79.5\%. Based
on this, oral cytology may be the preferred option in resource-limited settings, given cytology’s comparable performances to histopathology. Surgical biopsy and histopathological examination remain the gold standard for the definitive diagnosis of oral pathology; however, oral biopsy is invasive and involves both psychological implications for the patient and technical difficulties for sample collection. A high variability of histological diagnoses for dysplasia was documented and described by several authors. The most important requirement for a useful diagnostic technique is that it should be easy to use and cause minimal patient discomfort. The diagnostic procedure should be neither time consuming nor complicated but should be highly sensitive. High specificity also avoids FPs and therefore reduces patient anxiety and even unnecessary treatment. The oral cytology technique is one such simple, nonaggressive, relatively painless technique that is tolerated well by patients. Oral cytology can be used for the diagnosis and identification of recurrent, potentially malignant, and malignant lesions. This study showed that males had OSCC at a higher rate than females, in accordance with the literature, which may be attributed to the relatively increased frequency of habits related to toombak dipping, cigarette smoking, or alcohol usage in male patients compared to female patients. The mean age of our study group was 63.9 years; the increased mean age in males also reflects increased exposure to deleterious habits, rendering them vulnerable to the development of precancerous or cancerous lesions. This finding is not consistent with the previous work of Ginawi et al. as their mean age group was 46 years. Bayat reported a mean age of 56.3 years, which is close to our study findings; however, Weir JC et al. reported their patients to be in their fifth and sixth decades of life, which is almost similar to our finding. With regard to the male:female ratio, a similar finding was previously reported from Sudan that oral cancer is more common in males. Moreover, a large number of our patients present with squamous cell carcinoma (37; 82.3%), among whom the males were predominant. toombak dipping is common among the Sudanese population. Our findings support previous studies that reported the major role of toombak dipping in the etiology of oral cancer in Sudan since it is a potent carcinogen and acts as a synergistic factor that enhances the carcinogenesis of other etiological factors, such as alcohol use and cigarette smoking. This study found an association between OSCC, toombak dipping; for other habits, including cigarette smoking and alcohol use, this association needs to be confirmed in future studies.

Table 1: Gender, habit profile of study group and histopathology findings

<table>
<thead>
<tr>
<th>Gender</th>
<th>Negative (%)</th>
<th>Hyperkeratosis (%)</th>
<th>Verrucous (%)</th>
<th>Scc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>37 (82.3)/8 (17.7)</td>
<td>4 (10.8)/0 (0)</td>
<td>2 (5.4)/0 (0)</td>
<td>31 (83.8)/8 (16.2)</td>
</tr>
<tr>
<td>No habit</td>
<td>7 (15.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>Snuff dipping</td>
<td>10 (22.2)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Snuffing and smoking</td>
<td>5 (11.1)</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td>4 (80)</td>
</tr>
<tr>
<td>Snuffing and alcohol drinking</td>
<td>11 (24.5)</td>
<td>1 (9.2)</td>
<td>2 (18.1)</td>
<td>8 (72.7)</td>
</tr>
<tr>
<td>Three habits</td>
<td>12 (26.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>12 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>45 (100)</td>
<td>4 (100)</td>
<td>2 (100)</td>
<td>39 (100)</td>
</tr>
</tbody>
</table>

Conclusion

Despite the small number of cases in this study, oral exfoliative cytology is a useful method for detecting premalignant and malignant oral lesions. Oral exfoliative cytology is a simple, rapid, minimally invasive, and relatively painless method that is well accepted by patients and therefore suitable for population screening programs. Although further work is needed to address the limitations in this study, this method can potentially improve oral screening efforts in low-resource settings, where clinical expertise and resources are often limited. A significant challenge will be the integration of this screening tool into existing primary health-care systems in low-resource settings at a similar scale as existing screening programs for other cancers.

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Conflicts of interest
There are no conflicts of interest.

References

argyrophilic nucleolar organizer region, papanicolaou and crystal violet


