Conventional Versus Papanicolaou-Stained Cytobrush Biopsy in the Diagnosis of Oral Squamous Cell Carcinoma

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Abstract

Aims: Squamous Cell Carcinoma (SCC) is the most frequent malignancy of the oral cavity which is known to have a high mortality rate. Early diagnosis of this cancer has a major role in the prevention of its progression and can help increase patient survival. Conventional biopsy is considered the gold standard for diagnosis of oral SCC. However oral brush biopsy is less invasive, quicker and easier than surgical method. The aim of this study was to assess the sensitivity of oral brush biopsy in patients with oral SCC.

Methods: Thirty-five oral SCC subjects underwent. Cyrtobrush sampling followed by conventional biopsy and the latter was used as gold standard to determine the sensitivity of the brush biopsy technique. Papanicolaou and hematoxylin/eosin staining were used for cytolopathologic and histopathologic assessments, respectively.

Results: Only one patient was excluded due to inadequate sample material. A total of six of cytobrush specimens had the same diagnosis as their conventional biopsies (20.6% true positive), while 27 samples (79.4%) showed false negativity.

Conclusions: According to the results obtained in the present study, sampling with cytobrush followed by Papanicolaou staining may not be a good alternative for conventional biopsy in oral SCC cases.

Key words: Brush biopsy, Conventional biopsy, Squamous cell carcinoma.

Intrduction

Cancer is a global health issue and is one of the leading causes of death in many parts of the world. In general, individuals residing in developing countries demonstrate a higher frequency of oropharyngeal cancer as compared to those living in developed nations [1]. Cancer of the oral cavity ranks 8 among worldwide malignancies and is more common in men, with a global incidence of 2% in women and 3% in men [2]. Squamous Cell Carcinoma (SCC) is the most common malignancy of the oral cavity and is known to have a high mortality rate. In this tumor, malignant epithelial cells invade the underlying connective tissue and at some stage penetrate the lymphatic system and spread to distant organs. One of the most critical aspects in treatment of neoplastic diseases is their prognosis. OSCC has a poor prognosis with a five years survival rate of 30-40%.3 which in many cases, may be related to its late delayed diagnosis of this carcinoma [1]. Metastasis decreases this rate from 80% in primary lesions less than 20% for metastatic tumors [4]. SCC occurs in the 6th and 7th decades of life and is uncommon in people under the age of 45 [5] This carcinoma is a multifactorial disease that is influenced by two categories of factors: internal including general or systemic conditions like iron deficiency anemia and malnutrition and external such as tobacco, alcohol, syphilis and sun light (the latter only for lip vermilion cancer) [5]. The most frequent sites of OSCC include the floor of the mouth, tongue, soft palate, anterior tonsilar pillars and retro-molar regions [5].

Despite easy access to the oral cavity, diagnosis of oral cancers is not without its difficulties and can be time-consuming in many cases [6], thus 21% of these lesions are diagnosed when they have already metastasized to cervical areas. Considering the relatively poor prognosis of this cancer, early detection is critical to reduce its mortality rate. The gold standard for diagnosis of OSCC is conventional biopsy [6] during which a sample is taken either partially (incisional biopsy) or in its entirety (excisional biopsy).) Both these methods require application of anesthesia (usually local anesthesia but in some cases general anesthesia is inevitable), infection control and sterilization which are time consuming [7]. Therefore both patients and clinicians would benefit from simpler diagnostic methods which simultaneously cause less morbidity. Oral brush biopsy involves the use of a specific brush to collect epithelial cells and is considered a quick, easy and painless method that despite not being an alternative for conventional biopsy lacks the complications inherent to surgical biopsy and at the same time has been suggested to be preferential to exfoliative cytology [7]. The sensitivity of this method in diagnosis of carcinomatous lesions is reported to be up to 96% in some studies [7].

In the present investigation we aimed to assess the sensitivity of brush biopsy in the diagnosis of oral SCC with conventional biopsy serving as a gold standard for the exact identification of these lesions.

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Materials and Methods

The protocol of the present project was approved by the Ethics committee of our University. Thirty five patients with intraoral lesions suspicious to be SCC and in need of routine diagnostic procedures were recruited from those attending Meraj Cancer Institute; Tehran, University of Medical Sciences. After explaining the procedures used in our investigation, informed consents were obtained from all patients before entering the study. Clinically all lesions were ulcerated (at least in one area), mostly surrounded by erythematous areas and a few demonstrated scattered white patches but no veruciform growths.

A standard micro-brush OTM (Omid-Teb-Mehrdad, Iran) conventionally used to conduct poap smear was employed to collect the samples *(Figure 1)*. Patients were first asked to wash their mouth with water [8] to remove possible debris and then the sterile brush was pivoted with moderate pressure 5 times on the surface of the lesion in order to take a complete sample of the epithelium layer as described previously [8]. A moderate amount of pressure was applied during brush biopsy in order to avoid inadequate material removal and excessive bleeding pinpoint bleedings was acceptable. All samples were obtained by the same investigator and no local anesthesia was necessary throughout the process. Caution was exercised not to include areas clinically suspicious of necrosis to preclude the possibility of obscured cytologic results.

The gathered material was then smeared on a glass labeled slide, fixed with 96% saturated alcohol and stained with Papanicolaou using standard protocols. All samples were evaluated by the same pathologist before observing the histopathology slides. Assessments were based on previously described criteria [9]. Mainly those applied in cervical cancers such as nuclear hyperchromatism, granularity and border plus cytoplasmic characteristics and N/C ratio. Finally the samples were divided into three groups consisting of negative (without malignant changes), positive (with malignant changes) and inadequate specimen (cytobrush failure). The latter included cases in which cellularity was sparse or defective fixation occurred or the specimen was dispersed too thickly [9]. Sparse specimens were those with less than 29 properly maintained intermediate/parabasal cells not overclouded by hemorrhage or necrotic/exudative material [6].

After brush sampling, conventional biopsies were obtained from all patients under local anaesthesia. The specimens were fixed in formalin, routinely processed and stained with hematoxylin and eosin and evaluated by a pathologist according to the WHO criteria [8].

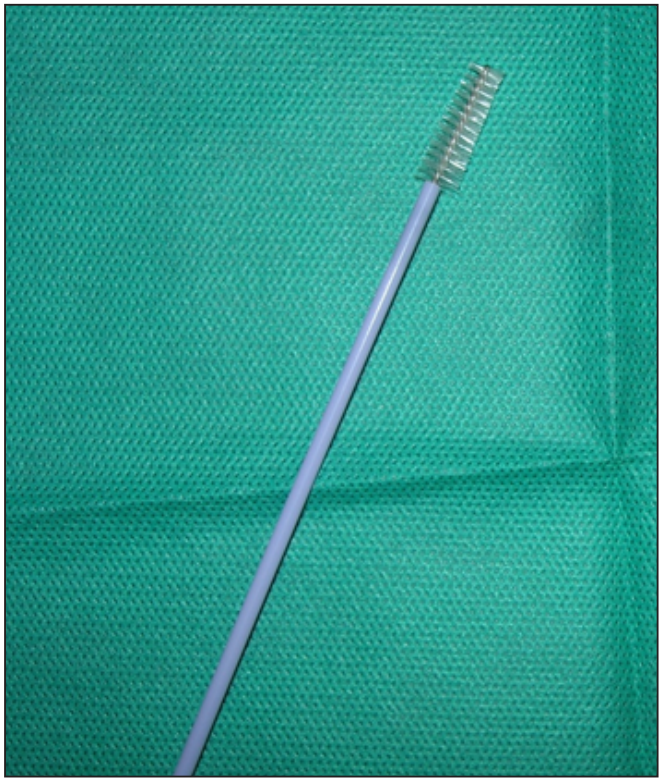

Figure 1. *A standard micro-brush OTM (Omid-Teb-Mehrdad, Iran) conventionally used to conduct poap smear was employed to collect the samples.*

Results

Clinical characteristics of the subjects are shown in *Table 1*. The mean values of patients' age, lesion size, tobacco use duration and alcohol use duration was 61 (SD=13) years, 30 (SD=17) mm, 21 (SD=10) years and 24 (SD=15) years, respectively. Histologic-based diagnosis of conventional biopsies was considered as the gold standard with which clinical diagnosis and brush biopsy results were compared. As demonstrated in *Table 2*. Sensitivity was determined to be 97.14% (95% CI: 85.08-99.93).

For analysis of cytobrush data, one specimen was excluded from the study sample due to cytobrush failure (thickly spread specimen) and of the remaining 34, only 6 were reported as positive (with malignant changes) meaning that they had the same diagnosis as the conventional biopsies. Therefore based on the information presented in *Table 2*, the sensitivity of cytobrush sampling was calculated as 17.6 (95%CI: 6.76-34.53).

Discussion

Oral Squamous Cell Carcinoma is a potentially life threatening

Table 1. *Clinical characteristics of evaluated oral Squamous Cell Carcinoma patients.*

	Number of patients	Percentage
Gender		
Male	23	65.7
Female	12	34.3
Tobacco history		
Yes	15	42.9
No	20	57.1
Alcohol history		
Yes	5	14.3
NO	30	85.7
Family history of OSCC		
Yes	2	5.7
No	33	94.3

Table 2. *Accordance between methods of biopsy and clinical diagnosis.*

Comparison		Number of cases	Percentage
Accordance between biopsy and clinical diagnosis	Yes	34	97.1%
	No	1	2.9%
Accordance between cytobrush and clinical diagnosis	Yes	7	20.6%
	No	27	79.4%
Accordance between cytobrush and biopsy diagnosis	Yes	6	17.6%
	No	28	82.4%

disease that dentists should be able to diagnose and screen. Despite Number of cases technology and surgical methods, the prognosis of OSCC remains poor and its five years survival rate is 30-40% [3]. Conventional biopsy as the gold standard for diagnosis of this disease has its disadvantages, leading to an ongoing search for easier, quicker and more acceptable diagnostic methods [10].

Böcking *et al*. stated that screening for oral cancer and its precursor lesions should be performed by dentists, dental surgeons, and other health care professionals. Exfoliative cytology and obtaining brush biopsies is advocated for evaluation of macroscopically suspicious lesions of the oral mucosa that are detected clinically during screening. This noninvasive approach may lead to a higher number of suspicious oral lesions to be diagnosed and thus to increase the rate of curable cancers, identified in early stages [11].

Although the cytobrush method is used for diagnostic purposes in other areas of the human body, it is not routinely employed in the oral cavity [12,13]. In some cases specimens collected from the mouth do not include sufficient amount of cells, may contain blood or dead cells and/or necrotic tissue all of which make the results hard to interpret and cause problems for its application in this region. [12,14].

A number of studies have evaluated the sensitivity of cytobrush biopsy in the diagnosis of oral lesions. Mehrotra *et al.* assessed the sensitivity of brush biopsy using a similar method to the one employed in the present investigation and reported a sensitivity of 76.8% [15]. Navone *et al.* conducted their research in OSCCs and found a 85% sensitivity rate for brush biopsy [6]. A study by Rich et al. indicated 90% sensitivity for brush biopsy [16]. In 1998 the mean sensitivity rate for the cytobrush method in several studies was reported to be 87.4% [17]. This rate was claimed to be even higher in another study in 1999 which included 945 patients with intraoral lesions. All of the lesions that showed malignancy in conventional biopsies were categorized as positive or atypical cell activity with brush biopsy. Thus, the researcher concluded that brush biopsy could be as sensitive as conventional biopsy [8].

On the other hand, some studies postulated low sensitivity rates for brush biopsy. Fereitas et al. asserted that the high rate of false negative diagnosis of this method due to errors in brushing and collecting insufficient number of cells makes it unreliable for vast usage [18].

Lauren *et al.* in a systematic review, compared evaluated auxiliary diagnostic methods used for detection of oral lesions which included papers on cytobrush application [19]. It was indicated that a number of studies reporting high sensitivities might have suffered from issues like lack of confirmation of the cytopathology results by conventional biopsy. They concluded that since adjunctive cancer diagnostic methods have not been proven to be completely efficient, dental clinicians should base their diagnoses on complete clinical examination along with validation by a specialist and histopathologic analysis [19].

Similarly, Rick (in reference to a study reported with a high sensitivity and specificity) pointed out that interpretation of brush biopsy results in lacking scalpel biopsy confirmation which might be problematic. He also reported his own findings on the sensitivity and specificity of brush biopsy and indicated high sensitivity and low specificity for this technique and suggested gathering of further data and reports on this issue to clarify the effectiveness of cytobrush in detection of oral malignant and premalignant lesions [16].

Huge differences regarding the sensitivity of oral brush biopsy in various reports, including the present study, may be due to discordances in the ability of performers to use brushes [20].

Additionally, according to Koch *et al*., lesion size may affect sensitivity, with smaller lesions demonstrating lower rates compared to larger ones. Furthermore test accuracy can change when different "cytopathologic definitions for malignancy" are employed [21].

Moreover the type, structure, brand and form of the brush might have an impact on the results; however Reboiras-López *et al*. found no differences in either cytological preparations or mean cell numbers and types among three sampling instruments, namely cytobrush, curette and oral CDx [22].

Our study sample did not include precancerous and dysplastic lesions, but considering that previous investigations [8,15] have reported high sensitivity rates of brush biopsy obtained from these lesions, application of this method in high-risk patients and those with premalignancies may prove to be beneficial. Further studies are suggested to help clarify the efficacy of this technique.

In 2012, Bagan *et al*. in a prospective double-blind study, assayed cytological changes in the oral mucosa after using a mouth wash with alcohol. They obtained three cytological samples from the oral mucosa .their results showed no cytological alteration in patients using a mouth wash with alcohol, but these finding should be considered preliminary results, to be confirmed in a greater sample of patients [23].

Conclusion

According to the results obtained in the present study, despite the advantages of cytobrush biopsy including simple and quick performance, no pain, bleeding or need for anesthesia cytobrush sampling followed by Papanicolaou staining good does not seem to be a suitable alternative for conventional biopsy. However, it is noteworthy that other means of cytology assessment including immunohistochemistry and more sophisticated cellular/molecular methods following brush biopsies, may prove to be useful in clinical settings.

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