ORIGINAL ARTICLE

Comparison of Oral Brush Cytology and Tissue Biopsy in Diagnosing Oral Lesions

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ABSTRACT

Objective: To assess the diagnostic efficacy of oral brush cytology in the diagnosis of potentially malignant and malignant lesions of the oral cavity.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Outpatient Department of ENT and Maxillofacial Surgery, Pakistan Institute of Medical Sciences and Pathology Department of Pakistan Railway Hospital Rawalpindi from 1st March 2017 to 28th February 2018.

Materials and Methods: A total of 50 patients with oral lesions were enrolled through non-probability convenient sampling. All patients presented with oral potentially malignant lesions were included, while patients with age less than 10 years and with bleeding diathesis were excluded from the study. The oral lesions were first sampled by oral brush biopsy technique using a toothbrush and then later on by scalpel biopsy. Samples were then studied under microscope for diagnosis. The data was analyzed using SPSS software version 21.0. Sensitivity, specificity, positive predictive value and negative predictive values were calculated keeping histopathology as a gold standard. Pearson's Chi-Square Test was used for calculating p-value, where p-value of ≤ 0.05 was considered significant.

Results: In this study, the mean age of patients presented with non-malignant oral lesions was 59 ± 12 years, while those with oral cancers were 60 ± 12 years. Men were affected than women. Among 50 patients 39 were found to have oral cancers. The sensitivity of oral brush biopsy was 88%, specificity was 83.3%, positive predictive value was 97.6% and negative predictive value was 50%. The p-value was calculated as 0.001, which was significant.

Conclusion: Our study found that oral brush cytology is reliable and can be easily performed with less cost and discomfort to the patient. It can be used for screening of suspicious oral lesions. It is useful in those situations where a patient refuses to have a biopsy or where a patient with bleeding diathesis would be exposed to unnecessary surgical risks.

Key Words: *Brush Cytology, Dysplasia, Oral Squamous Cell Carcinoma, Potentially Malignant Disorders.*

Introduction

Oral lesions are a common presentation in our outpatient departments. Oral cancer involves cancer of the lips, tongue, floor of the mouth, cheeks, soft and hard palate and pharynx. It is the 6th most common cancer in the western world and second most common cancer in some parts of the subcontinent.[1] It is a global health problem with increasing incidence and mortality.[2] In 2013 oral cancer resulted in 135,000 deaths, which have increased from 84000 in 1990.[3]

Oral cancer has a multifactorial etiology. It can be caused by genetic reasons as well as environmental influences. Globally tobacco, alcohol and human papillomavirus are associated with oral cancers.[4] Studies have shown that the high incidence of oral cancer in the subcontinent is due to a strong association with tobacco chewing, use of gutka, pan, chaalia, naswar, hukka and cigarette smoking.[5]

In Pakistan, areas of khyber phuktun-khwa have a higher prevalence of oral cancers due to the frequent

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use of naswar and in Karachi due to use of paan and chaalia.[6] Karachi cancer registry shows that oral cancer is the second most common cancer in both men and women.[7] The Shaukat Khanum cancer registry in 2016 also shows oral cancer as the 8th most common cancer in Pakistan.[8] Men have twice the risk of oral cancer as compared to women and the risk increases after the age of 50 years.[9] However, some recent studies have shown an earlier incidence in the younger age group as well.[10]

Early detection of oral lesions has been the most effective approach to reduce morbidity and mortality, especially in the malignant ones.[11] It has been proven that benign oral lesions cannot be distinguished from cancers based on clinical examination alone and so when a suspicious oral lesion is encountered it should always be evaluated.[12]

Oral brush cytology utilizes a brush to obtain a complete trans-epithelial cytology specimen with cellular material from all three layers of the lesion i.e. basal, intermediate and superficial layers.[13] The technique is to make repetitive to and fro movements with the brush until there is punctate bleeding from the lamina propria of the lesion, thus ensuring that cells from all epithelial layers have been taken.[14] The yield of brush cytology can be further increased by using digital aids and other adjunctive techniques such as DNA analysis, Immunohistochemistry, molecular analysis and liquid based preparations. Brush cytology is indicated to aid in the diagnosis of an oral lesion which cannot be identified with clinical certainty or a probable benign lesion when a clinician wants to avoid unnecessary biopsy.[15]

Literature search reveals very limited local studies on efficacy of brush cytology. Our study intended to investigate this simple but useful technique. The objective of our study was to assess the efficacy of oral brush cytology in the diagnosis of potentially malignant and malignant lesions of the oral cavity.

Materials and Methods

This cross-sectional, analytical study was carried out at Outpatient Department of ENT and Maxillofacial Surgery, Pakistan Institute of Medical Sciences, and Pathology Department of Pakistan Railway Hospital from 1st March 2017 to 28th February 2018. A total of 50 patients were enrolled through Non-Probability Convenient Sampling. A written informed consent was taken from every patient. Approval for the study was taken from the Ethical Review committee of Riphah International University. Patients with oral lesions suspicious for malignancy, irrespective of the gender were included in the study. These lesions included leukoplakia, erythroplakia, actinic cheilosis and suspected oral carcinoma.

Before sample collection, patient's data were recorded on a pre-designed proforma. For oral brush cytology rinsing of the oral cavity was performed by every patient with ample water. The lesion was viewed with the aid of light. A toothbrush was disinfected in 0.2% of chlorhexidine gluconate mouth wash and was used to obtain a complete trans-epithelial biopsy with minimal discomfort. By using moderate pressure, the brush was repeatedly brushed in one direction over the entire lesion many times until pinpoint bleeding occurred, signaling entry into lamina propria. The material from the brush was smeared on two clean, dry glass slides. The smears were fixed with 95% isopropyl alcohol for staining with hematoxylin and eosin. Cytological smears were graded as follows:[16]

Class 0: Inadequate specimen, Class 1: Benign: No atypical cells identified, Class 2: Dysplastic: Cells exhibiting dysplasia, not sufficient for diagnosis of malignancy, Class 3: Cytology suggestive for malignant.

For biopsy samples a local anaesthetic was injected at the site of oral lesion and a scalpel biopsy was taken. The biopsy specimen was kept in 10% formalin for fixation and sent for histopathology. Gross inspection of tissue was done and submitted for routine processing, slide preparation and then stained with Hematoxylin and Eosin for microscopy.

Based on the degree of dysplasia, architectural loss, invasion deep to the basement membrane and presence of atypical cells, these biopsy specimens were classified as benign, mild to moderate dysplasia, marked dysplasia or Carcinoma in Situ, well-differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma. Olympus CX21 light microscope was used for examination of slides of both brush cytology and biopsy.

The data was entered and analyzed by using SPSS 21.0 (Statistical Package for Social Sciences).

Sensitivity, specificity, positive predictive value and negative predictive value with 95% confidence interval were calculated by 2 x 2 table, keeping histopathology as a gold standard. Pearson's Chi-Square Test was used for calculating p-value, where p-value of ≤ 0.05 was considered significant.

Results

The total numbers of cases were 50. The mean age of patients presented with oral cancer was 60. The age group most commonly affected was in 6th decade of life, with a male preponderance. The most common site of oral cancer was buccal mucosa as shown in Table I.

Table I: Table Showing Age, Gender, Site and Adverse Habits

Groups		Non-Malignant Group	Malignant Group
Age		59±12	60±12
Gender	Female	4 (44.4%)	16 (39%)
	Male	5 (55.5%)	25 (61%)
Site	Buccal	2 (22.2%)	15 (36.5%)
	Tongue	3 (33.3%)	12 (29%)
	Alveolus	1 (11.1%)	10 (24.3%)
	Lip	3 (33.3%)	2 (5%)
	Other	0	2 (5%)
Adverse Habits	Naswar	4 (44.4%)	25 (61%)
	Cigarette smoking	3 (33.3)	9 (22%)
	Paan	1 (11%)	3 (7%)
	Gutka	1 (11%)	3 (7%)

The brush cytology and biopsy results were classified into three classes, i-e Benign, dysplastic and malignant (Table II).

Table II: Table Showing the Classification of Oral Lesions on Brush Cytology and Biopsy

	Brush Cytology		Biopsy	
	Percentage	Frequency (n)/50	Percentage	Frequency (n)/50
Benign	20%	10	10%	5
Dysplastic	4%	2	8%	4
Malignant	76%	38	82%	41
Total	100%	50	100%	50

The analysis of the results of the study was done with the help of the 2x2 Table (Table III).

Table III: 2x2 Table Showing Brush Cytology Results Against the Tissue Biopsy

	Tissue Biopsy Positive	Tissue Biopsy Negative	Total
Brush Cytology Positive	(True Positive) 39	(False Positive) 1	40
Brush Cytology Negative	(False Negative) 5	(True Negative) 5	10
Total	44	6	50

In our study sensitivity and specificity, PPV and NPV were calculated. (Table IV). The true and false positives and negatives were based on the following:

- True positive: Samples that were positive on both biopsy and brush cytology.
- True negative: Samples that were negative on both biopsy and brush cytology.
- False positive: Samples those were negative on biopsy and positive on brush cytology.
- False negative: Samples those were positive on biopsy and negative on brush cytology.

Table IV: Statistical Values

Statistics	Value
Sensitivity TP/ (TP + FN)	88.6%
Specificity TN/ (TN + FP)	83%
PPV* TP/ (TP+FP)	97.5%
NPV TN/ (FN+TN)**	50%
Accuracy (TN + TP)/ (TN+TP+FN+FP)	88%

True Positive: TP, True negative: TN, False positive: FP, False Negative: FN. *PPV: Positive predictive value **NPV: Negative Predictive Value.

Pearson's Chi-Square Test was applied through SPSS version 21 and p-value was calculated as 0.001, which was significant.

Discussion

Our results showed that the mean age of patients presenting with malignant oral lesions was 60 years. The age group most commonly affected (30.7%) was 60-69 years. Majority of patients with oral malignancies were males accounting for 61% of the total patients while female patients were 39%. Previous studies also support this finding. Mehrotra et al[17] have documented that 58.9% of malignant oral lesions were males as compared to 41% in females. Naseem et al[18] have documented that 73.4% of cases with malignant oral lesions were males and 26.6% in females. The higher male incidence is attributed to the fact that males are more predisposed to the risk factors such as smoking, alcohol and smokeless tobacco like paan, gutka, naswar causing oral cancers.[19] The most common site of oral cancer in our study was buccal mucosa (37%) followed by the tongue (30%) and then alveolus (23%). This finding was consistent with other studies conducted in the South Asia region. Sharma et al[20] reported buccal mucosa as the most common site with involvement of 63.5%. The likely reason for buccal mucosa being

the most common site for oral cancers can be smokeless tobacco; naswar which is the most common addiction in our patients which is kept against the cheek. Secondly, cheek mucosa is also very thin and non-keratinized and hence more prone to irritants and carcinogens.

There was 88 % agreement among brush cytology and scalpel biopsy results, with a p-value of 0.001 which showed statistically significant agreement between two the tests. This show that the diagnostic accuracy of brush cytology in comparison with the scalpel biopsy was fair and hence brush cytology can be used as an adjunctive test for diagnosis of oral cancers.

In our study, 38 out of 50 patients were diagnosed as malignant, two cases were dysplasia and 10 cases were benign on cytology. When we compared the same cases on histopathology, we found that among 10 patients classified as benign on cytology, only 5 were benign while 5 were malignant (Table II). We found on biopsy that 41 cases were malignant as compared to 38 malignant cases on brush cytology. Hence true positive in our study were 39, true negative were 5, false negative results were 5 and false positive was 1 (Table III). This was consistent with other studies which showed that brush cytology had higher false negative cases than false positive cases.[21] The reason for the higher value of true positives in our study is firstly, being the inclusion of the cases which look malignant on visual examination and secondly the late presentation of oral malignancies in our setup. Reasons for the false negatives on brush cytology can be small sample size, wrong sampling technique, loss of malignant cells in toothbrush bristles and topographic error between the site of brush and scalpel biopsy. This false negative rate suggests that the suspicious oral lesions should undergo scalpel biopsy before they are labelled as benign on cytology.

In our study, the sensitivity was 88.6% and specificity was 83%, positive predictive value was 97.5% and the negative predictive value was 50 % (Table IV). These values are consistent with other studies. Trakroo et al[22] have found that the sensitivity and specificity of brush cytology in detecting dysplasia and oral squamous cell carcinoma were 84.37 % and 88.09 % respectively, and positive and negative predictive values were 93.10% and 76%, respectively. Moreover, when histopathology and brush cytology were compared, they showed good correlation with insignificant P values.[23] Mehrotra et al.[24] found in their study that when compared to scalpel biopsy, the statistical sensitivity of the brush cytology was greater than 76.8% ($P < .05$) while the statistical specificity was greater than 93.3% ($P < .05$). The limitations of this study were that this study was conducted at only one hospital. Strength of the study can be improved by conducting a multicentre study with a larger sample size. Secondly, most of the patients in this study were malignant and hence sensitivity and specificity of the brush cytology for benign lesions could not be ascertained beyond doubt.

Conclusion

Our study finds that oral brush cytology is reliable and can be easily performed with less cost and discomfort to the patient. It can be used for screening of suspicious oral lesions and may have applications in resource-constrained areas. It is useful in those situations where a patient refuses to have a biopsy or where a patient with bleeding diathesis exposes to unnecessary surgical risks.

Recommendation

Brush cytology can be used as a useful adjunct to scalpel biopsy in diagnosing oral lesions especially when the index of suspicion for malignancy is high.

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