Research Article

Efficacy of oral exfoliative cytology in diagnosis of oral cancer

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Received: December 15, 2016; **Accepted:** December 18, 2016

ABSTRACT

Background: Oral cancer is the major health hazard prevailing in the South Asia particularly in Indian subcontinent. Unlike other cancer, due to its accessibility the oral cancer can be detected at the early stage. Oral exfoliative cytology (OEC) is the simple, sensitive and noninvasive method that is well accepted by the patient and is, therefore, suitable for screening at risk population for early diagnosis of oral cancer and valuable adjuvant for gold standard biopsy. **Objective:** The purpose of this study is to evaluate the efficacy of OEC in the detection of oral premalignant and malignant lesions in comparison to the histopathology. **Materials and Methods:** In this retrospective study, a total of 1510 lesions from 1481 patients underwent oral scrape cytology and were followed up with punch biopsy. Sensitivity, specificity, positive, and negative predictive values, and diagnostic accuracy were calculated. **Results:** Out of 1510 lesions, 1121 were histopathologically confirmed cases of squamous cell carcinoma, out of which 1101 (98.2%) lesions tested positive on cytological examination, among which 987 (88.0%) lesion conclusive, 44 (3.9%) strongly suggestive of malignancy, and 59 (5.2%) suggestive but not conclusive of malignancy. Sensitivity for cytology was 95.91%, specificity was 99.17%, and accuracy was 96.68%. **Conclusion:** Oral exfoliative cytopathology showed high sensitivity, specificity, positive predictive value, and accuracy with good diagnostic concordance with the histopathological method and that makes it a potentially practical tool in resource challenged settings. However, the histopathological method should always be performed when the cytopathological diagnosis is not conclusive.

KEY WORDS: Oral Scrape Cytology; Squamous Cell Carcinoma; Punch Biopsy; Histopathology; Early Detection

INTRODUCTION

Oral cancer is the most common cancer and constitutes a major health problem in developing countries, representing the leading cause of death. Although representing 2-4% of the malignancies in the West, oral squamous cell carcinoma (OSSC) accounts for almost 40% of all cancers in the Indian subcontinent.[1] They are of utmost concern as the mortality rate of the oral cancer for the past three and a half decades has remained high (over 50%) in spite of new treatment modalities. Despite numerous advances in treatment, the 5-year survival has remained approximately 50% for the last 50 years.[2] Early diagnosis of OSCC and premalignant lesions are the best interventions for improving survival and quality of life.[3] Although histological examination of tissue remains the gold standard for diagnosis of malignant oral lesions, a biopsy is an invasive technique with surgical implications, technique limitations, and psychological implications for most patients.[4] Oral exfoliative cytology is a nonaggressive technique that is well accepted by the patient and is, therefore, an attractive option for the early diagnosis of oral cancer, including epithelial atypia and SCC.[5]

Access this article online	
Website: http://www.ijmsph.com	**Quick** Response code
DOI: 10.5455/ijmsph.2017.1268218122016	

This study has been done to find out the efficacy of oral scrape smear as a screening tool for detecting oral carcinoma and to find its utility in detecting premalignant and malignant lesions in comparison to oral punch biopsy.

MATERIALS AND METHODS

A retrospective analysis was conducted on 1481 patients who reported to the Department of Head and Neck Oncology with suspicious clinical presentation of oral cancer during the period of 2 years spanning from August 2014 to July 2016. The cases details were retrieved from the hospital records. Oral scrape was taken with a metal spatula and two slides were prepared from it and were wet fixed immediately in 95% alcohol and stained with Papanicolaou technique.

Parameters that were analyzed in the smear (both cytosmear and modified brush biopsy samples) included enlarged nuclei, nuclear pleomorphism, nuclear borders, nucleocytoplasmic (N/C) ratio, number of nuclei, binucleation, keratinization, tadpole forms, hyperchromatism, chromatin pattern, and distribution and discrepancy in N/C maturation. The most important diagnostic feature of cancer cells are anisonucleosis, anisocytosis, abnormal nuclear texture and nuclear hyperchromasia and abnormal N/C ratio.

Cytological Analysis

Based on above-specified parameters cytologic specimens were classified as follows:[6-8]

- Class 0: Inadequate specimen
- Class 1: No/abnormal or atypical cells
- Class 2: Atypical cytology but no evidence of malignancy
- Class 3: Cytology suggestive of but not conclusive for malignancy
- Class 4: Cytology strongly suggestive of malignancy
- Class 5: Cytology conclusive for malignancy.

For analysis, Classes 3-5 were to be considered positive and Classes 1 and 2 as negative (Figure 1).

Punch biopsy was performed in all those patients who showed positive results in exfoliative cytology for histopathological confirmation, and in those cases where in spite of negative results in cytology, the clinical judgment warranted the need for biopsy. Biopsies were fixed in 10% formalin and routinely processed and stained with hematoxylin-eosin stain. On histopathological examination, the dysplasia was graded as mild, moderate, severe and carcinoma *in situ*, while SCC was graded as well differentiated, moderately differentiated, and poorly differentiated. The histopathological diagnosis was considered as gold standard and the cytopathological findings obtained from all the cases were compared with the respective histopathological findings.

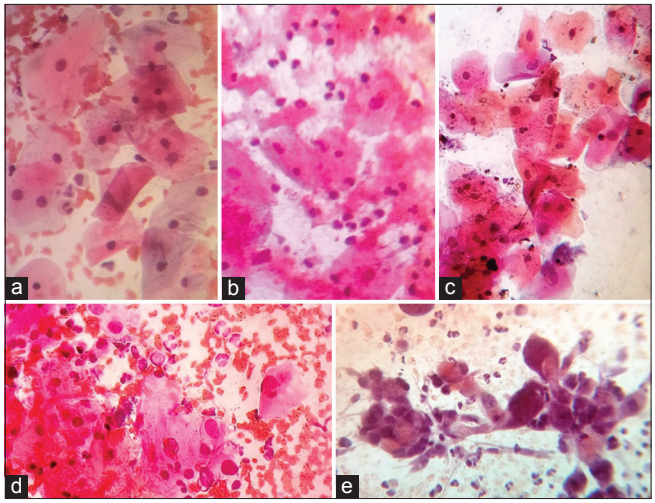

Figure 1: (a) Class 1 – No atypical/abnormal cells (Papanicolaou, ×400), (b) Class 2 - Atypical cytology due to inflammation but no evidence of malignancy (Papanicolaou, ×400), (c) Class 3 - Cytology suggestive of but not conclusive for malignancy (Papanicolaou, ×400), (d) Class 4 - Cytology strongly suggestive of malignancy (Papanicolaou, ×400), (e) Class 5 - Cytology conclusive for malignancy (Papanicolaou, ×400).

Statistical Analysis

Following formulas were used to analyze the efficacy of cytology and brush biopsy with biopsy as a gold standard:

- True positive (TP): Samples that were positive on both histology and cytology
- True negative (TN): Samples that were negative on both histology and cytology
- False positive (FP): Samples those were negative on histology and positive on cytology
- False negative (FN): Samples that was positive on histology and negative on cytology
- Sensitivity = TP/TP + FN = (Number of TP assessment)/(Number of all positive assessment)
- Specificity = TN/TN + FP = (Number of TN assessment)/(Number of all negative assessment)
- Positive predictive value (PPV) = Probability that the disease is present when the test is positive = TP/(TP + FP)
- Negative predictive value (NPV) = Probability that the disease is not present when the test is negative = TN/(TN + FN)
- Accuracy = (Number of correct assessments)/(Number of all assessments) = (TN + TP)/(TN + TP + FN + FP).

RESULTS

A total of 1510 lesions, in 1481 patients, who underwent cytology and then followed by histopathology, were included in the study. Table 1 shows the age and sex distribution of all the histopathologically diagnosed carcinomas. The male to female ratio was found to be 1.9: 1. The age ranged from 19 to 93 years. The most common age groups affected were 31-40 years, 41-50 years, and 51-60 years.

Among 1510 lesions, histopathologically – 1121 (74.43%) cases were diagnosed as SSC, 4 (0.2%) were carcinoma *in situ,* 19 (1.2%) were verrucous carcinoma, 2 (0.1%) were SSC-spindle cell type, 2 (0.1%) were adenoid cystic carcinoma, 1 (0.06%) was mucoepidermoid carcinoma, 159 (10.5%) were epithelial dysplasia, 55 (3.6%) were papilloma, and 147 (9.7%) were nonneoplastic lesions.

Among the 1121 SSCs - 1082 (96.5%) were well differentiated, 31 (2.7%) were moderately differentiated, and 8 (0.7%) were poorly differentiated.

Out of 147 cases diagnosed as nonneoplastic lesion: 7 were fibroepithelial polyp, 69 were leukoplakia, 17 were erythroleukoplakia, 18 were verrucous leukoplakia, 3 were oral tuberculosis, 1 was pemphigus, and 32 were inflammatory lesions, however, 3 (2.0%) cases were cytologically class 3 or given as suspicious smear (FP).

Table 2 shows the comparison of cytopathological and histopathological diagnosis. Among the 1121 histopathologically confirmed cases of SCC, 1101 (98.2%) lesions tested positive on cytological examination, out of which 987 (88.0%) lesion were conclusive (Class 5), 44 (3.9%) were strongly suggestive of malignancy (Class 4), and 59 (5.2%) were suggestive but not conclusive of malignancy (Class 3). However, 22 (1.9%) cases of SCC were negative in initial cytological examination and 25 (2.2%) cases were inadequate smears.

Table 1: Age and sex distribution of patients with histologically diagnosed oral cancer

Age group (in years)	Male	Female	Total
11-20	2	0	2
21-30	40	6	46
31-40	168	48	216
41-50	193	105	298
51-60	182	123	305
61-70	96	64	160
71-80	54	40	94
81-90	20	4	24
Above 90	4	0	4
Total	759	390	1149

Table 3 shows TN, TP, FN, and FP for cytological examination as compared with the gold standard histopathologic evaluation. Sensitivity based on the above values for cytology was 95.91% and specificity recorded as 99.17%. Accuracy was 96.68%.

DISCUSSION

In this study, most of the patients with histologically diagnosed carcinoma belonged to 4th, 5th, and 6th decade of age group with strong male predominance. SSC was the predominant malignancy seen. Among all histopathologically confirmed cases of carcinomas, 95.2% lesions tested positive on cytological examination with 4.8% FN cases. Out of 306 nonneoplastic and benign lesions 2.9% cases were FP. The sensitivity exfoliative cytology in detecting OSSC was 95.91% and specificity was 99.17%. The PPV was 99.73%, NPV was 88.4%, and the diagnostic accuracy was 96.68%.

The age and sex incidence observed in this study were in concordance with other studies by Gupta et al. and Mehrotra et al., who have also observed oral malignancies occurring in about two times more frequently in men, and 95% found in persons older than 40 years of age.[1,9,10] Out of all the histologically confirmed OSCC, 1090 (97.2%) lesions were cytologically positive and showed a good diagnostic concordance between histopathological (gold standard) and

Table 2: Comparison of cytopathological and histopathological diagnosis

Histopathology diagnosis	Cytological diagnosis						
	Class 5 Conclusive for malignancy	Class 4 Strongly suggestive of malignancy	Class 3 Suggestive but not conclusive	Class 2 Atypical but no evidence of malignancy	Class 1 No abnormal cells	Class 0 Inadequate smear	Total
SCC	987	44	59	8	-	23	1121
Carcinoma *in situ*	1	3	-	-	-	-	4
Verrucous carcinoma	-	-	5	12	-	2	19
SCC-spindle cell type	1	1	-	-	-	-	2
Adenoid cystic carcinoma	-	-	-	-	2	-	2
Mucoepidermoid carcinoma	-	-	1	-	-	-	1
Epithelial dysplasia	-	-	9	113	35	2	159
Papilloma	-	-	-	-	33	22	55
Nonneoplastic lesions	-	-	-	28	102	17	147
Total	989	48	74	161	172	66	1510

SCC: Squamous cell carcinoma

Table 3: Evaluation of cytopathological diagnosis of all the cases

Statistic	Value
TP	1102
FP	9
TN	352
FN	47
Sensitivity	95.90%
Specificity	97.51%
PPV	99.19%
NPV	88.22%

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

cytopathological methods. This was slightly higher than the studies by Fontes et al. (71.5%) and Sousa et al. (86.7%).[11,12] This may be due to the higher number of cases included in our study. Furthermore, the cytopathological method resulted in at least a suspicion of a malignant lesion in 98.2% of cases 88.0% lesion were conclusive of SCC, 3.9% were strongly suggestive of malignancy, and 5.2% were suggestive but not conclusive of malignancy.[1,9,13] The sensitivity was slightly higher in our study in comparison to Goel et al., who observed a sensitivity of 83.1%, however, specificity reported by Gupta et al. was 100% which was because they did not encounter any FP cases in their study.[1] Several studies had observed wide range of results in similar studies with PPV ranging between 10.6% and 100.0%, NPV between 60.0% and 100%, and accuracy between 13.2% and 96%.[10,15] The discrepancy among the values in these studies, including ours, can be explained by differences in the study design, nonstandardization of the technique, different methods of statistical analysis, nonrepresentative samples, different sample collection sites, differences in the professional who performed the smear, inadequate cellularity of the smear, and mainly, the experience of the pathologist.[11]

In this study, histologically diagnosed 8 (0.7%) cases of SCC, 12 (63.1%) cases of verrucous carcinoma and 2 cases of adenoid cystic carcinoma were initially given as cytologically negative (FN) for malignancy whereas 66 (4.3%) lesions were unsatisfactory or inadequate on evaluation by cytology, which was comparable to other studies by Gupta et al. and Jha et al.[1,16] These findings, which demonstrate the lack of efficiency of exfoliative cytology, may be explained by nonrepresentative sampling and/or individual subjectivity,[12,15] since this was a retrospective study in which the cytological tests were not performed by the same examiner. Various studies have given different reasons for FNs: [18] (i) Smear from a nonrepresentative site and painful lesions may not allow proper scrapping; (ii) intramucosal malignant change with an intact mucosa cannot be detected by oral scrapping; (iii) hyperkeratotic lesions will not allow underlying malignant cells to be scrapped,[16] so lesions should be scrapped till pinpoint bleeding is present; (iv) cancers with ulceration, fungation will not yield malignant cells in the smears because of presence of necrotic debris;[14] (v) improper fixation by air drying or using a wrong fixative may produce artefacts and alterations in the cellular morphology; (vi) staining and processing errors; (vii) subjective errors: It is essential to screen the slide completely and mark the more characteristic cells; (viii) lack of clinical information may also lead to improper interpretation of the cytological smear.[18] We encountered 2.4% FP cases cytologically. Gupta et al. found 5 (4.6%) out of 107 cases to be FP, whereas Jha et al. and Fontes et al. did not encounter any FP cases.[16,11] The FP results of the oral cytology are possible in inflammatory oral lesions with a certain grade of epithelial atypia. Other reason for such FP result could be improper fixation or air drying. Hence, proper technique is a requisite to obtain good results.[1,19]

The oral cytopathology method is a simple, noninvasive, relatively painless, and rapid diagnostic technique. Exfoliative cytology of oral mucosa is also very effective in detecting certain infectious lesion such as oral tuberculosis.[20] Therefore, it is suitable for routine application in screening programs, early analysis of suspicious lesions, and posttreatment monitoring of malignant lesions. Exfoliative cytology is useful in those situations when a patient refuses to have a biopsy performed or when medically compromised patients would be exposed to unnecessary surgical risks. In addition, anxious patients can be reassured quickly about the nature of oral mucosal changes, especially when a fear of cancer or a family history of cancer accounts for their apprehension.[14] However, FN results in cytologic evaluation present a real hazard in cancer detection and management, since biopsy will often not be done until such time as clinical features of a proliferating and spreading malignancy render the biopsy necessary, giving a false sense of security to the patient and doctor. Hyperkeratotic lesions such as leukoplakia can pose diagnostic challenge, as they yield scanty cellularity on scrape smear, which may erroneously appear benign on cytology, which compromises the accuracy of the technique.[16,12] This limitation was observed in the present study. We believe that if the sample is obtained by a professional who performs a high quality oral examination, carefully selects the best site and type of procedure to collect the sample, and if the sample is analyzed by an experienced pathologist, the rate of inadequate sample can be reduced.

CONCLUSION

Although exfoliative cytology should not be used as a substitute for histopathological examination, this study has demonstrated that exfoliative cytopathology had good diagnostic concordance with the histopathological method and also showed high specificity, sensitivity, PPV, and accuracy, that makes it a potentially practical tool in resource challenged settings. However, the relatively less sensitivity

indicates that it does not rule out malignancy in all cases. Thus, we believe that the histopathological method should always be performed when the cytopathological diagnosis is not conclusive, i.e., suspicious for OSCC or positive for epithelial dysplasia; and when the cytopathological diagnosis is conclusive for OSCC, this result should be used to refer the patient to the oncology center for therapy, reducing the time between diagnosis and treatment. Although there is a very small risk of FN and FP results, it should be kept in mind and these cases should be followed by biopsy where there is a strong clinical suspicion.

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How to cite this article: Besra K, Samantaray S, Pathy PC, Das PK, Panda S, Rout N. Efficacy of oral exfoliative cytology in diagnosis of oral cancer. Int J Med Sci Public Health 2017;6(5):896-900.

Source of Support: Nil, **Conflict of Interest:** None declared.