



PREDICTING PREMALIGNANT POTENTIAL OF OSMF USING ORAL EXFOLIATIVE CYTOLOGY: ORIGINAL RESEARCH

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ABSTRACT

Aim: To determine and compare the cytological features in mucosal smears of oral submucous fibrosis patients with normal cells.

Materials & methods: Total 60 patients were included in the study in which 30 were clinically diagnosed cases of oral submucous fibrosis and 30 were healthy controls between the age group of 17 to 78 years of either gender. The study was conducted within the duration of 3 months after the ethical clearance. Oral exfoliative cytology (OEC) was performed on both buccal mucosae of each group and stained under Periodic Acid Schiff (PAS) stain with further evaluation under microscope.

Result: In the present study it was observed that 3(10%) out of 30 OSMF cases, showed class I cytology, 21(70%) and 6 (20%) showed class II and class III cytology respectively.

Conclusion: OEC is a useful early diagnostic method for detection of epithelial atypia and is an adjunct to biopsy in premalignant cases and makes screening of cancer easy to manage as more number of cases can be screened in less time.

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INTRODUCTION

Oral SubMucous Fibrosis (OSMF) is a chronic, premalignant condition of the oral mucosa which was first described by Schwartz in 1952.¹ It is characterized by blanching and stiffness of oral mucosa, trismus, and burning sensation in the mouth. It also produces hypo mobility of the soft palate and tongue, and loss of gustatory sensation. Occasionally there can be mild hearing impairment due to blockade of the Eustachian tube.²

The disease is predominant in India, Bangladesh, Sri Lanka, Pakistan, Taiwan, China, and among other Asians, with a reported prevalence ranging up to 0.4% in Indian rural population. The age range of patients with OSMF is wide ranging between 22 and 61 years of age.³

The aetiology of this crippling disease is complex, but it has been suggested that consumption of chilies, nutritional deficiency, genetic susceptibility, altered salivary constituents, autoimmunity and collagen disorders may be involved in the pathogenesis of this condition.⁴ Currently areca nut/betel quid use is the single-most important etiological factor considered in OSMF. Malignant transformation rate of OSMF is found to be in the range of 7-13%, which is highest among all premalignant lesions and conditions.⁵

Oral exfoliative cytology (OEC) is a non-aggressive technique that is well accepted by the patient, and is therefore a suitable option for the early diagnosis of oral cancer.⁶ Literature suggests that oral cytology may be helpful for detecting potentially malignant disorders or early carcinoma in asymptomatic patients with lesions that appear benign. Early detection of such lesions increases the endurance and decreases the morbidity of such patients. The features of

cytological atypia usually observed in such disorders include cellular and nuclear pleomorphism, nuclear budding, hyperchromatism and micronuclei, inflammatory cells, indented cellular outline, and intracytoplasmic vacuoles.⁷

It is not a substitute for biopsy. Rather, it is a useful although not essential adjunct in the diagnosis of oral surface lesions due to cancer, viral disease, vesiculobullous dermatoses or fungal infection. Although this painless, atraumatic and simple technique for collecting a sample of superficial cells is used extensively in the diagnosis of less visible and accessible lesions, such as those in the uterine cervix on the lung, emphasis is here placed on its role in detecting and monitoring premalignant lesions and squamous cell carcinoma of the mouth. Very few studies have been carried out in this regard. The aim of the present study was to evaluate certain specific cytologic features which are indicative of a premalignant condition and compare them with that of normal cytology.



Fig 1 Clinical photograph of OSMF showing reduced mouth opening and Blanching on Buccal mucosa and Facial Area

MATERIALS AND METHODS

The observational & cross sectional study was carried out in the outpatient department of Oral Medicine and Radiology, Institute of Dental sciences, Bareilly. The study was conducted within the duration of 3 months after the ethical clearance. A total of 60 patients were selected according to the study done by Jaitley *et al* and included in the study.¹⁸ Out of which, 30 were clinically diagnosed with oral submucous fibrosis under the criteria according to the workshop held in Kuala Lumpur, Malaysia in 1996⁸ and 30 subjects were healthy controls with no history of habits and no abnormal clinical features on examination were included in the control group. OSMF patients along with any other systemic disease, patients with OSMF with other superimposed oral lesions like oral leukoplakia, oral lichen planus etc. and patients in which OSMF was associated with another pathology resulting in reduced mouth opening were excluded from the study. Patients included were in the age group of between 17 to 78 years of either gender. Informed written consent was obtained from each patient.

The area of interest was cleaned using cotton swab and normal saline. Smears were taken from the buccal mucosae of the subjects using the wooden spatula and were fixed using 95% ethyl alcohol. The slides were then sent to the department of oral pathology for further cytological evaluation. Slides were stained using PAP stain and studied under microscope. They were then classified according to Papanicolaou's classification (1960) as follows:

- Class I (normal): only normal cells observed

- Class II (atypical): presence of minor atypia but no evidence of malignancy;
- Class III (intermediate): an in-between cytology (the cells display wide atypia that may be suggestive of malignancy but are not clear cut cancer and represent precancerous lesions or in situ carcinoma)
- Class IV (suggestive of cancer): a few epithelial cells with malignant characteristics or cells with borderline characteristics;
- Class V: positive cancer cells that are obviously malignant.⁹

RESULTS

All the smears from clinically normal buccal mucosa showed Class I and class II cytology. The exfoliated cells were of normal size and shape with normal staining intensity and normal nuclear characteristics. A total of 60 patients were included in the study out of which 30 patients were clinically diagnosed cases of OSMF including 7 females and 23 males, in the age range of 17 to 78 years, and 30 patients were age and gender matched in control group. When analysed microscopically, 3(10%) out of 30 OSMF cases, showed class I cytology, 21(70%) and 6 (20%) showed class II and class III cytology respectively (Table 1). Cytological findings in control group revealed class I cytology in 27(90%) patients and class II in 3(10%) cases (Table 2).

Table 1 Cytological findings in OSMF group

Total no. of patients.	Class I	Class II	Class III	Class IV	Class V
30	3(10%)	21(70%)	6(20%)	0	0

Table 2 Cytological findings in control group

Total no. of patients.	Class I	Class II	Class III	Class IV	Class V
30	27(90%)	3(10%)	0	0	0

Statistical Analysis

Statistical analysis was done using chi square test with χ^2 value 115.45 and the p value was found out to be 0.0001 which was highly significant.

DISCUSSION

Countless changes in oral cavity are evident with advancing, environmental, and lifestyle related habits and factors. Incidence and prevalence of potentially malignant and malignant disorders has increased due to increase in the habit of chewing and smoking of tobacco worldwide. OEC is the microscopic examination of exfoliated cells from epithelial surface.¹⁰ In India and its subcontinents the incidence of oral potentially malignant disorders is high.¹¹ The prevalence of OSMF being 0.03% to 3.2% which is gradually increasing owing to the excessive usage of areca nut and tobacco products among various groups of population.¹²

OSMF is preceded by symptoms such as burning sensation of the oral mucosa, ulceration and pain. The characteristic features of OSMF are loss of pigmentation of oral mucosa, leathery texture and blanching of oral mucosa, depapillation and reduced movement of tongue, progressive reduction of mouth opening and sunken cheeks.¹³ Blanching may be localized, diffuse or reticular and is caused by impairment of the local vascularity because of increasing fibrosis of the oral mucosa and results in a marble like appearance. Blanching may be associated with small vesicles that rupture to form

erosions. Patients usually complain that these vesicles form after they eat spicy food, suggesting the possibility of an allergic reaction to capsaicin. These features can be observed at all stages of OSMF.¹⁴ In the more advanced stage of the disease, the essential feature is a fibrous band restricting mouth opening and causing difficulty in mastication, speech, swallowing and maintaining oral hygiene.¹⁵

Exfoliative cytology is a rapid and simple method and has gained importance. The diagnostic oral exfoliative cytology, despite being a useful, cost-effective and convenient tool in the diagnosis of oral precancerous and cancerous lesions, is not yet applied as widely as cervical cytology.¹⁶ Early diagnosis is of great importance for oral squamous cell carcinoma, oral exfoliative cytology, a simple, painless and inexpensive method has become a preferred method for both early diagnosis of the lesion and for establishing quantitative techniques. OEC can be the preferred method for screening of oral mucosal lesions. It was found that, 4.5% of clinically benign-appearing lesions have dysplastic or carcinomatous features.¹⁷ Thus, cytological screening of the patients, at high risk of oral neoplastic lesions and without any macroscopically apparent oral lesion, can be of tremendous importance.

In the present study it was observed that 3(10%) out of 30 OSMF cases, showed class I cytology, 21(70%) and 6 (20%) showed class II and class III cytology respectively, which was in accordance to a similar study conducted by Jaitley *et al* & Arpita *et al* in which class II cytology was observed in maximum number of patients.^{18, 19} In a study from Sudan, cytological analysis of buccal scrapings has been proposed as a useful early diagnostic method for epithelial atypia and malignant oral lesions where nearly 5% of clinically benign appearing mucosal lesions were sampled by this technique and later confirmed by typical scalpel biopsy to represent dysplastic epithelial changes or invasive cancer.²⁰ In one more study by Singh *et al* the role of exfoliative cytology was elucidated in determining the cellular atypical features of oral submucous fibrosis.⁹

The features of cytological atypia that were recorded in the present study included cellular pleomorphism, nuclear pleomorphism, nuclear budding, hyperchromatism and micronuclei, bacterial colonies, inflammatory cells and indented cellular outline, and intracytoplasmic vacuoles indicative of cytolysis.⁷ Micronucleus refers to the small nucleus that forms whenever a chromosome or a fragment of a chromosome is not incorporated into one of the daughter nuclei during cell division. These may serve as marker for increased cancer risk, since they have been reported to arise in response to DNA damaging agents. Micronuclei are found at increased frequencies from normal mucosa to potentially malignant disorders to carcinoma, especially in the head and neck region suggesting ever increasing chromosomal instability.¹⁸

OEC is widely advocated as an addition to clinical conclusion and an adjunct to biopsy. The smear technique cannot replace tissue biopsy but can be valuable and useful for detecting early malignant changes or recurrence, where biopsy is contraindicated. It certainly promises to improve the survival rate of patients suffering from such conditions. With OEC being an easy, noninvasive, cost-effective technique, it can be used for mass screening and regular follow-up of potentially malignant disorders. However, further studies should be

conducted on a larger population to establish the role of OEC in potentially malignant disorders.

CONCLUSION

Cytology is reliable as a diagnostic tool so far as diagnosis of presence or absence of malignancy in a lesion is concerned with high accuracy rate in diagnosing oral cancer. The smear technique is not intended to replace tissue biopsy, but it serves as a supplement to biopsy. Indications for its use include oral mucosal lesions, and follow up for patient with a history of either a premalignant or malignant lesion. The oral cytologic technique is easy to do and can help surgeon / physician, in cases where he/she might hesitate to perform an invasive procedure, like a biopsy, or desire more information regarding a lesion before referring the patient.

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