

## Original Research Article

# A study on scrape smear cytology in oral leukoplakia in a tertiary health care centre of southern Assam

Sania S. Ullah\*, Monoj K. Deka, Nitumani Khakhlari

Department of Pathology, Silchar Medical College and Hospital, Assam, India

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### \*Correspondence:

Dr. Sania S. Ullah,

E-mail: [dr.saniaullah@gmail.com](mailto:dr.saniaullah@gmail.com)

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### ABSTRACT

**Background:** Oral cavity is susceptible to countless changes with advancing, environmental, and lifestyle related habits and factors. Oral mucosal lesions especially related to chewing and smoking of tobacco have led to the increased incidence and prevalence of potentially malignant and malignant disorders worldwide. Oral leukoplakia has been a very frequent finding in patients coming to our department and with an increasing rise of oral squamous cell carcinoma in the region, the study was conducted.

**Methods:** Patients referred to our section from various department with white oral lesion. Scrapping smears were prepared. Stained with MGG and PAP stain. Study period: January 2019 to May 2020.

**Results:** Total number of patients with white oral lesion were 83. Out of these 65 were male and 18 were female. Most of the patients had addiction to betel nut and leaf with tobacco and few had history of intake of bidi for several years.

**Conclusions:** The study reveals that most of the oral leukoplakia cases is attributed to smoking, betel nut and lime intake. Exfoliative cytology of oral mucosa serves to be an easy, convenient and reproducible technique for early diagnosis of premalignant condition. Biopsy can be used as an adjunct in cases showing atypia and mixed type of keratosis.

**Keywords:** Cytology, Leukoplakia, Oral cavity

### INTRODUCTION

Oral white lesions that cannot be clinically or pathologically characterized by any specific disease are referred to as leukoplakia. Such lesions are well known for their propensity for malignant transformation to the extent of 10-20%. In 1978 a World Health Organization (WHO) group defined oral leukoplakia as: "A white patch or plaque that cannot be characterized clinically or pathologically as any other disease". It is therefore a diagnosis of exclusion from other oral white lesions such as leukokeratosis, infective lesions (candidiasis, syphilitic oral lesion, oral hairy leukoplakia caused by Epstein Barr virus), lichen planus, lupus erythematosus, dyskeratosis

congenita, white sponge nevus, submucosal fibrosis and frank carcinomas. It is common in adults beyond 40 years of age, and affects 1% of the total population. Oral cancer is the most frequent neoplasm of the head and neck region. Among this the most common is oral squamous cell carcinoma (OSCC).<sup>1</sup> Cancerous lesions are usually benign in appearance and asymptomatic in nature in their early stages.<sup>2</sup> Oral leukoplakia (OL) is a potentially malignant disorder, it is defined as white plaque of questionable risk having excluded (other) known disease or disorders that carry no increased risk for cancer.<sup>3</sup> It may occur either as a single, localized change of the oral mucosa or as diffuse, often multiple lesions.<sup>4</sup> As a clinical entity, it may have varied histological presentations,

ranging from mildly hyperkeratotic lesions to the lesions that exhibit severe dysplastic features.<sup>5</sup> Oral squamous cell carcinoma(OSCC) is a very frequent finding in our institution and comprises of around 45% of biopsy proven malignancy. There has been found very strong association between smoking, chewing tobacco, betel nut consumption of alcohol or combination with both leukoplakia and OSCC. Leukoplakia, the most frequent precancerous lesion of mouth, was first described in the second half of the last century by Hungarian dermatologist, Schwimmer. The use of cervical smear has proved to be of great significance in detection of cervical cancer in females in mass screening. With the rapid rise of OSCC in the region, an attempt was made by us to study the significance of scrape cytology in diagnosing premalignant lesions of oral cavity as an adjunct to biopsy. Since biopsy requires significant time and expenses, scrape cytology is rapid and convenient method for mass screening. Results are obtained quickly and can be repeated for follow up cases. The objective of the current study was to study the various morphological patterns of oral leukoplakia, the cytological findings associated with various patterns and the histopathological correlation of suspicious lesions in order to detect high risk cases at an early stage of disease.

**METHODS**

It was a cross sectional study for a period of 1 year and 5 months done in The Department of Pathology of Silchar Medical College and Hospital. The study was approved by the ethical committee of the institution.

The study consisted of 83 patients, 65 male and 18 female.

**Inclusion criteria**

All patients above 30 years. All must have oral leukoplakia. History of betel nut chewing, tobacco intake and smoking for atleast 5 consecutive years.

**Exclusion criteria**

Patients less than 30 years. Patients having infrequent intake of betel nut, tobacco. Patients not giving consent for biopsy as further investigation.

The cytological smears were made as per following procedure:

The procedure was explained to the patient and consent was taken. The patient was asked to rinse the mouth with normal saline to remove any necrotic slough. Any dry lesion was moistened with normal saline. The lesions were scrapped with wooden spatula and material obtained were smeared on glass slide. The slides were fixed in methanol for 10 minutes to be stained with MGG. The slides for PAP stain were fixed immediately with ultra fixative.

**Staining of smear**

**MGG stain:** routine MGG stain was done to study basic cellular morphology.

**PAP stain:** PAP staining lies in the fact that the dehydration and better clearing solutions help in causing cellular transparency. This detects the overlapped cells and their individual morphology, which otherwise would be confused for bi or multinucleated cells. It also shows a stability of stain over long periods, stability of color and of course, the better reproducibility of results.<sup>6,7</sup>

The MGG stained smears were screened for various pathological lesions and classified as 1) Orthokeratotic, 2) Parakeratotic, 3) Combined, as per criteria laid down by Sahiar et al.

The PAP smears were 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

Biopsy was done, tissue was formalin fixed, processed and stained with hematoxylin and eosin.

The results were calculated in Microsoft Office Excel 2007.

**RESULTS**

**Table 1: Age wise distribution of cases.**

Age range in years	No. of cases with oral leukoplakia
31-35	06
36-40	08
41-45	25
46-50	16
51-55	12
56-60	11
≥60	05

**Table 2: Type of clinical lesion and no of cases.**

Type of lesion	No. of cases
Homogeneous,thin and flat	50
Homogeneous, thick and verrucous	18
Non homogeneous, nodular	12
Non homogeneous,speckled	03

The study was carried out in the Department of Pathology, Silchar Medical College and Hospital, Assam and consisted of 83 individuals with a definite history of betel nut, tobacco and bidi smoking for duration of more than 5 years. Cytological study was inconclusive for opinion in 6 cases (7.2%) because of scanty material or degenerative changes in the epithelial cells. Opinion was possible on 77 (92.7%) cases only. The maximum number of cases was in the age group of 44-45 years with

a mean of 44 years (17.6%) as shown in Table 1. A detailed clinical examination of the oral cavity was carried out in each case and lesions were classified as in Table 2.

The comparison between the clinical lesion and the various cytological features of oral cavity has been depicted in Table 3 and Table 4.

**Table 3: Type of clinical lesion and their cytology finding (MGG stained smear).**

Type of lesion	Parakeratosis	Orthokeratosis	Combination	Unsatisfactory
<b>Homogeneous, thin and flat</b>	30	12	6	2
<b>Homogeneous, thick and verrucous</b>	11	04	1	2
<b>Non homogeneous, nodular</b>	07	03	1	1
<b>Non homogeneous, speckled</b>	01	00	1	1
	n=49	n=19	n=9	n=6

**Table 4: Type of clinical lesion and their cytology finding (PAP smear).**

Type of lesion	I (normal)	II (atypical)	III (intermediate)	IV (suggestive of malignancy)	V (malignant)
<b>Homogeneous, thin and flat</b>	10	38	-	-	-
<b>Homogeneous, thick and verrucous</b>	4	12	-	-	-
<b>Non homogeneous, nodular</b>	2	8	-	-	-
<b>Non homogeneous, speckled</b>	-	-	2	1	-
	n=16	n=58	n=2	n=1	0

Maximum number of cases with parakeratosis (49) followed by orthokeratosis (19) and combination of both types (09) were observed.

Biopsy was advised in cases showing combination of ortho and parakeratosis and having class III and class IV atypia. The results are in Table 5.

**Table 5: Biopsy finding of cytologically suspicious lesion.**

HPE finding	No. of cases (n=12)
<b>Inflammatory</b>	8
<b>Moderate dysplasia</b>	3
<b>Carcinoma in situ</b>	1

**DISCUSSION**

Oral exfoliative cytology is the microscopic examination of shed cells from an epithelial surface.<sup>8-10</sup> Papanicolaou and Traut’s staining technique for cytology smears were first used in oral leukoplakia by Montgomery and von Hamm.<sup>8,11</sup> Jolar Bancozy studied 201 oral leukoplakia and found 76.6% of cases correlated with histological findings.<sup>12</sup> The superficial epithelial cells do contain nuclei and alterations in these cells can serve as reliable

indicators of dysplastic or neoplastic changes.<sup>13</sup> Our study revealed that oral leukoplakia is a frequent finding in the population with a male preponderance. Smoking of bidi and chewing of betel nut with lime was found to be positive in almost all the cases. Hence these habits can be considered to have a positive correlation with oral leukoplakia. Though highest case was present in age group 41-45 years group, an interesting trend was observed with gradual increase of cases from 31 to 45 years and slight decrease in cases towards higher age. This can be attributed to frank ulceration and transformation to premalignant or malignant forms. The most common morphological finding of OL was homogenous, thin and flat (60.97%), followed by homogenous, thick and verrucous forms (21.68%). The non homogenous OL comprised of 18.07%. Maximum number of parakeratosis, orthokeratosis and combination were found in homogenous flat type of OL with 61.22%, 63.15%, 66.66%. The presence of hyperkeratosis could have been the reason for not obtaining cells with dysplastic features on cytology. This was also the experience of Jolar Bancozy in his study of 201 cases of oral leukoplakia.<sup>12</sup> Most of the OL cases showed grade II atypia in PAP stain with predominant morphology of lesion being homogenous flat type. Grade III and IV atypia was seen only in the non homogenous speckled type OL. Histopathological examination was advised in

cases showing dual keratosis and grade III and IV atypia. Punch biopsies were done in those case. Tissue was formalin fixed and stained with HnE. 8 out of 12 such cases showed chronic inflammatory features along with varying degree of fibrosis. 3 cases showed moderate dysplasia and 1 insitu carcinoma was found. Several authors have listed the criteria for malignancy in the interpretation of oral smears.<sup>11,14,15</sup> Oral exfoliative cytology is a reliable adjuvant to biopsy with the 69% sensitivity and 100% specificity in oral leukoplakia and 75 % sensitivity and 100% specificity in squamous cell carcinoma.<sup>16</sup>

## CONCLUSION

From the above study it can be concluded that the gross morphology of oral leukoplakia, like non homogenous, speckled pattern, the dual type of keratinisation, and also the higher degree of atypia is associated with early progression of benign lesion to a premalignant or malignant one with time. Punch biopsy can be used as an adjunct in selective cases to reduce the expenses. Exfoliative cytology is quick and reproducible hence can also be used up in long term follow up of cases of oral leukoplakia.

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