



Original Research Article

Tobacco related oral cavity lesions, scrape cytology with histopathological correlation: A 5 year study at tertiary level hospital in Mumbai

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ABSTRACT

Introduction: Aims and objectives of our study was to detect the most common tobacco related lesions which occurs in oral cavity with respect to age, sex, duration of tobacco use, distribution of premalignant and malignant lesions and to correlate the scrape cytology findings with histopathology in Mumbai region of Maharashtra.

Materials and Methods: Total of 85 cases having tobacco associated intra oral lesions were analyzed retrospectively 5 years. Cases were studied in detail and included complete clinical history and examination, additions, scrape cytology and histopathological findings.

Result: In our study, males were commonly involved (62 cases, M:F=2.7:1), commonest addiction was smoking tobacco (33%) and tobacco with lime (31%) and producing lesions at right and left buccal mucosa (36.2%) and lateral borders of tongue (20.5%) most frequently, in a age group of 41 to 50 years (31.7%) followed by 51 to 60 years (28.2%). In a 42 premalignant lesions, oral submucosal fibrosis were seen in 40.47% and leukoplakia in 38.09% cases. Remaining 60 malignant cases, well differentiated squamous cell carcinoma were seen in 46.66% and moderately differentiated squamous cell carcinoma in 43.33 % cases. The sensitivity and specificity of scrape cytology in detecting oral squamous cell carcinoma were 88.33% and 100% respectively. Positive predictive value was 100%, negative predictive value was 85.7% with diagnostic accuracy of 93.14%.

Conclusion: Strong association of prolong use of tobacco products and smoking to have premalignant and malignant intraoral lesions at the site of placement of tobacco was established. Intra oral scrape cytology can be a main screening tool for early diagnosis along with histopathology correlation.

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1. Introduction

‘Chewing tobacco is tobacco’s body, smoke is its ghost and snuff is tobacco’s soul.’¹ Tobacco has been smoked, chewed and inhaled in various forms for more than 500 years.^{1,2} Tobacco contain substances that can act by both initiators, promoters of carcinogenesis, irritation and toxicity of mucosa along with reducing oxygen transport

and also enhances the local absorption of carcinogens. Tobacco related deaths are due to carcinoma oral cavity and lung, myocardial infarction and stroke. Oropharyngeal carcinoma is the sixth most common malignancy in western world and account for more than 40% cancers in Indian subcontinent.^{3–5} Continuing ha bit of pan, tobacco chewing and smoking causes annually about 7% of all cancer deaths in male and 4% in female in India and is an important public health problem.^{6,7} The high incidence of oral cancer, associated with widespread use of smoking and smokeless tobacco in the Indian subcontinent,

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mandates the implementation of simple and cost-effective methods to screen the population at risk.⁸ Tobacco induced mucosal changes have been identified in exfoliative cells.⁹ Exfoliative cytology, being a minimally invasive procedure, has a better patient compliance as compared to biopsy.¹⁰ Scrape cytology means scraping the surface of a lesion with the edge of glass slide, scalpel blade, wooden spatula etc.¹¹ Bennett, in 1845 for the first time used scrape cytology technique for rapid diagnosis.² The Scrape cytology method is easy, rapid, reliable, inexpensive and does not require special instruments or set up. The diagnostic accuracy is better than imprint cytology or frozen section alone.¹¹ Therefore scrape cytology examination can be carried out as screening as well as diagnostic procedure, followed by biopsy of tobacco related intraoral lesions in case of suspected atypia or malignancy. Selective cytology of extensive oral lesions could be helpful in assisting the clinicians to choose those areas that should be biopsied for confirmation of diagnosis.

An oral lesion is defined as ‘a visible alteration of the oral mucosa that persist for at least 7 days after discontinuation of tobacco use; an alteration with little probability of resolving within 7 days’.¹²

In 1978, WHO collaborating center for oral precancerous lesion defined leukoplakia as “A white patch or plaque that cannot be removed by rubbing and cannot be characterized clinically or pathologically as any other disease”.^{13,14} This definition does not carry any histologic connotation. Oral submucosal fibrosis is a high risk precancerous condition predominantly occurs in tobacco addicted Indian patients defined by investigators as “An insidious chronic mucosal disease affecting any part of the oral cavity and sometimes the pharynx, characterized by mucosal rigidity of varying intensity due to fibro elastic transformation of the juxtraepithelial connective tissue layer.”¹⁵

2. Materials and Methods

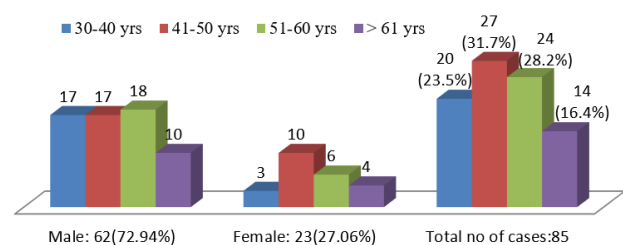
This retrospective study was carried out on patients admitted in wards having tobacco related addictions and oral cavity lesions, for a period of 5 years in a tertiary care hospital, Mumbai. Patients relevant information about detailed history including addiction, a thorough clinical examination of oral cavity was noted along with oral hygiene. Scrape cytology was done from the visible intra oral lesions. The patients asked to rinse the mouth with water before the procedure to prevent the mixing of food particles with the scrape material and for proper examination of oral cavity. Surface of the lesion was blotted with wet cotton or gauge piece to remove any excess blood or purulent exudate. Scraping is done with a metallic ‘Vectis’ having long handle and flattened fenestrated end was used for scraping. The scrape material was spread uniformly over a glass slides and immediately wet fixed with 95% ethyl alcohol for two minutes to avoid drying artefact. The slides were stained

with Hematoxylin & Eosin, Papanicolaou stain. Air dried smears were stained with Giemsa stain. Biopsies were taken from most suspicious, non- necrotic area of the lesion s. Biopsy tissue was processed by routine paraffin techniques and the sections were stained with Hematoxylin & Eosin for microscopic examination for confirmation of diagnosis.

The scrape cytology smears were reported based on the following parameters: increased nucleocytoplasmic ratio, variation in nuclear size and shape (pleomorphism), enlarged nuclei, nuclear membrane irregularity, number of nuclei, binucleation, keratinisation, tadpole forms, hyperchromasia, chromatin pattern and distribution as well as discrepancy in nucleocytoplasmic maturation. Cytology of homogenous leukoplakia demonstrated keratinized hyper mature, polygonal cells or anucleated squamous cells with or without nuclear atypia. Squamous-cell carcinoma was graded as well differentiated, moderately differentiated and poorly differentiated. Thus findings of scrape cytology were correlated with histopathological findings in paraffin embedded sections. Data were presented as frequencies and percentages.

3. Result

Our tertiary level medical college in Mumbai receives the patients from local medical practitioners, rural hospital, district hospital etc. and referred in advanced stage of disease. The number of cases appears to be less in our study as in Mumbai there are many specialized cancer institutes and hospital, also mostly we received referred patients in advanced stages of disease in ENT and Surgical wards.



Graph 1: Age and sex wise distribution of tobacco related oral cavity lesions

Our study included a total numbers of 85 cases having tobacco associated oral cavity lesions. In which we had found 62 male (72.94%) and 23 female (27.06%) patients so that M: F ratio was 2.7:1, suggested male preponderance, [Graph 1]. The age group for male ranges between 30 to 70 years with average age of 50 years. The age group for female ranges between 35 to 75 years with average age of 49 years. The most common age group including male and female with tobacco related precancerous and cancerous lesions was in between 41 to 50 years (31.7%) followed by 51 to 60 years (28.2%), [Graph 1]. Thus

Table 1: Distribution of tobacco related oral cavity lesions to anatomical sites

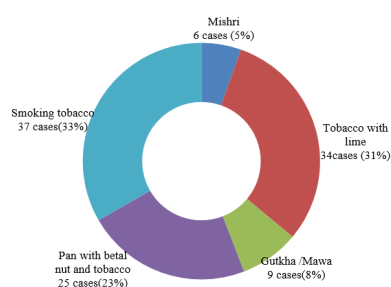
Site	Right side	Left side	Total
Buccal mucosa	16	21	37 (36.2%)
Lateral border of tongue	11	10	21 (20.5%)
Lower gingiva	3	7	10 (9.8%)
Cheek	5	4	9 (8.8%)
Labial mucosa	1	6	7 (7%)
Hard palate	3	3	6 (6%)
Retro molar area	0	2	2 (2%)
Anterior floor of mouth	1	0	1 (1%)
Double lesion	5	4	9 (8.8%)
Total	45 (44.12%)	57 (55.88%)	102

Table 2: Types of premalignant and malignant lesions in male and female. (n=102)

4A) Type of premalignant lesions (n=42: 41.17%)	Male	Female	Total
Oral submucosal fibrosis	15	2	17 (40.47%)
Leukoplakia	13	3	16 (38.09%)
Lichen planus	2	2	4 (9.52%)
Chronic non healing ulcer	1	2	3 (7.14%)
Verruca vulgaris	1	0	1 (2.38%)
Squamous papilloma	1	0	1 (2.38%)
Total	33 (78.57%)	9 (21.43%)	42
4B) Type of malignant lesions (n=60: 58.82%)	Male	Female	Total
Well differentiated SCC	17	11	28 (46.66%)
Moderately differentiated SCC	22	4	26 (43.33%)
Poorly differentiated SCC	3	0	3 (5%)
Verrucous SCC	3	0	3 (5%)
Total	45 (75%)	15 (25%)	60

Table 3: Comparative study of scrape cytology and histopathology.

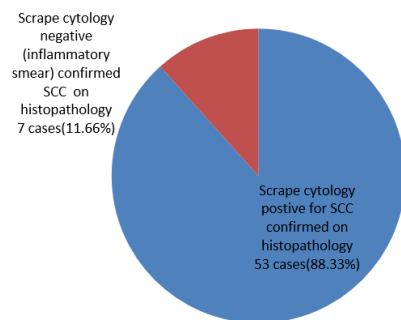
Study	Remmerbach et al. ⁽²⁵⁾	Driemel et al. ⁽²⁶⁾	Mehrotra et al. ⁽¹⁹⁾	M Babshet. et al. ⁽¹⁶⁾	Nazoora Khan et al. ⁽²⁴⁾	Bharati Jha et al. ⁽¹⁰⁾	In our study
Sensitivity	94.6 %	79 %	76.8 %	77 %	93.2 %	91.5 %	88.3 %
Specificity	99.5 %	93 %	93.3 %	100 %	96.8 %	100 %	100 %
Positive predictive value	98.1 %	88 %	—	100 %	—	100 %	100 %
Negative predictive value	98.5 %	88 %	—	38 %	—	66.7 %	85.7 %
Accuracy	—	—	—	—	94.9 %	92.7 %	93.14 %

**Graph 2:** Types of tobacco addiction

there was increased trend of occurrence of lesions towards younger age. In our study smoking (37 cases) and tobacco along with lime (34 cases) were the most common type of addictions followed by pan, betel nut with tobacco (25 cases), gutkha or mawa (9 cases) and mishri (6 cases), [Graph 2]. We found 29 patients were using two or more than two types of addiction. Tobacco products were commonly kept in the left gingivobuccal sulcus (21 cases) and right gingivobuccal sulcus (16 cases). Therefore buccal cavity was the commonest site (37 cases) for tobacco related lesions followed by tongue (21 cases), gingiva (10 cases),



Fig. 1: A) Photograph showing 70 yrs female with ulceroproliferative growth over left buccal mucosa reaching up to left angle of mouth; B): 65 yrs male with large exophytic growth arising from left buccal mucosa) 45 yrs male with large ulceroproliferative mass arising from right buccal mucosa infiltrating through the skin, producing externally fungating cauliflower like growth.



Graph 3: Correlation of scrape cytology with histology in malignant lesions

cheek (9 cases), lower labial mucosa (7 cases), hard palate (6 cases), retro molar area (2 cases) and a single case at anterior floor of mouth, [Table 1]. This concludes that oral lesions were associated with site of placement of tobacco products. About 9 cases showed more than one lesion associated with mixed tobacco habits. The duration of tobacco with lime addiction in a case of male ranges from 6 to 30 years with a frequency of 1 to 15 times per day and 5 to 40 years with frequency of 1 to 8 times per day in case of female. Thus longer duration and increased frequency of keeping tobacco products inside a mouth were responsible for early development of oral precancerous and cancerous lesions.

Total 102 lesions were found in 85 cases out of which 60 lesions (58.82%) were malignant whereas 42 lesions (41.17%) were premalignant. The most common premalignant lesion encountered in this study was oral submucosal fibrosis (17 cases) and leukoplakia (16 cases) followed by oral lichen planus (4 cases), chronic non healing ulcer (3 cases), squamous papilloma and verruca vulgaris one case each, [Table 2 A]. Well differentiated squamous cell carcinoma seen in more number of cases

(28 cases ; 46.66%) along with moderately differentiated squamous cell carcinoma (26 cases; 43.33 %) while poorly differentiated squamous cell carcinoma constitutes only 3 cases (5%), [Table 2 B]. Scrape cytology smears were positive for squamous cell carcinoma in 53 out of 60 cases, while discrepancy with histopathological diagnosis occurred in 7 cases,[Graph 3].

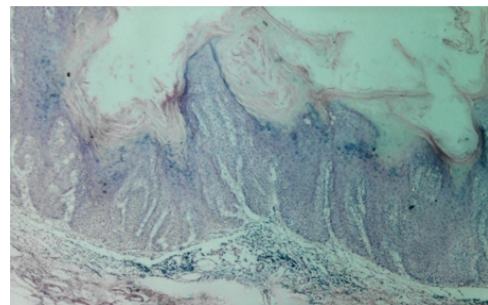


Fig. 2: Photomicrograph showing histopathological features of leukoplakia (H&E stain: 400x) in a 30 yrs male

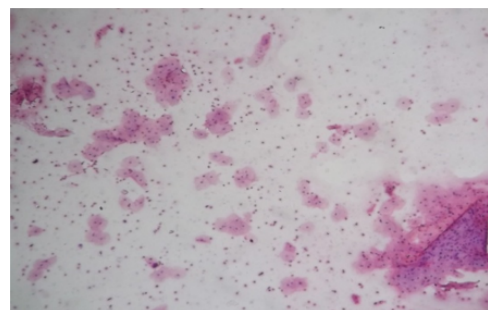


Fig. 3: Photograph showing inflammatory buccal smear (H&E stain: 100 x)

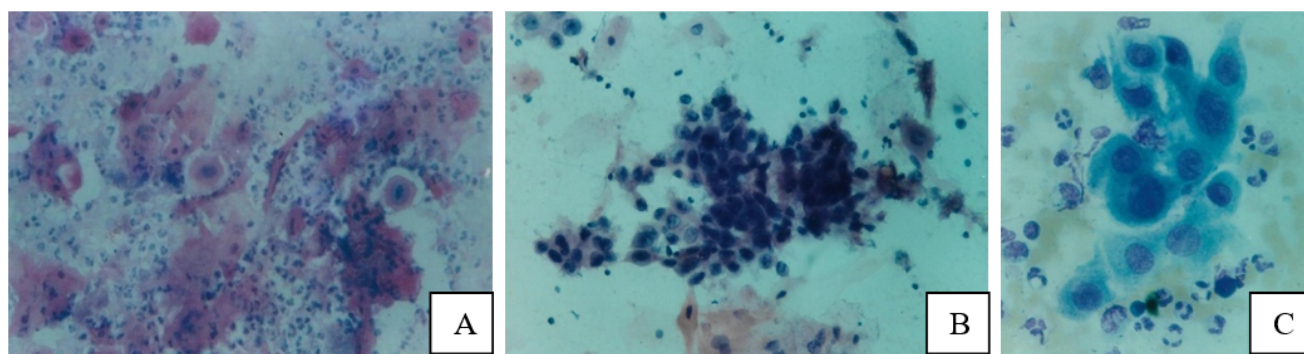


Fig. 4: **A):** Scrape cytology reveals malignant squamousepithelial cells with neutrophils on the background (H&E stain: 400x), **B):** Scrape cytology reveals cluster of malignant squamous epithelial cells(H&E stain: 400x), **C):** Scrape cytology reveals malignant squamous epithelial cells (Giemsa stain: 1000x)

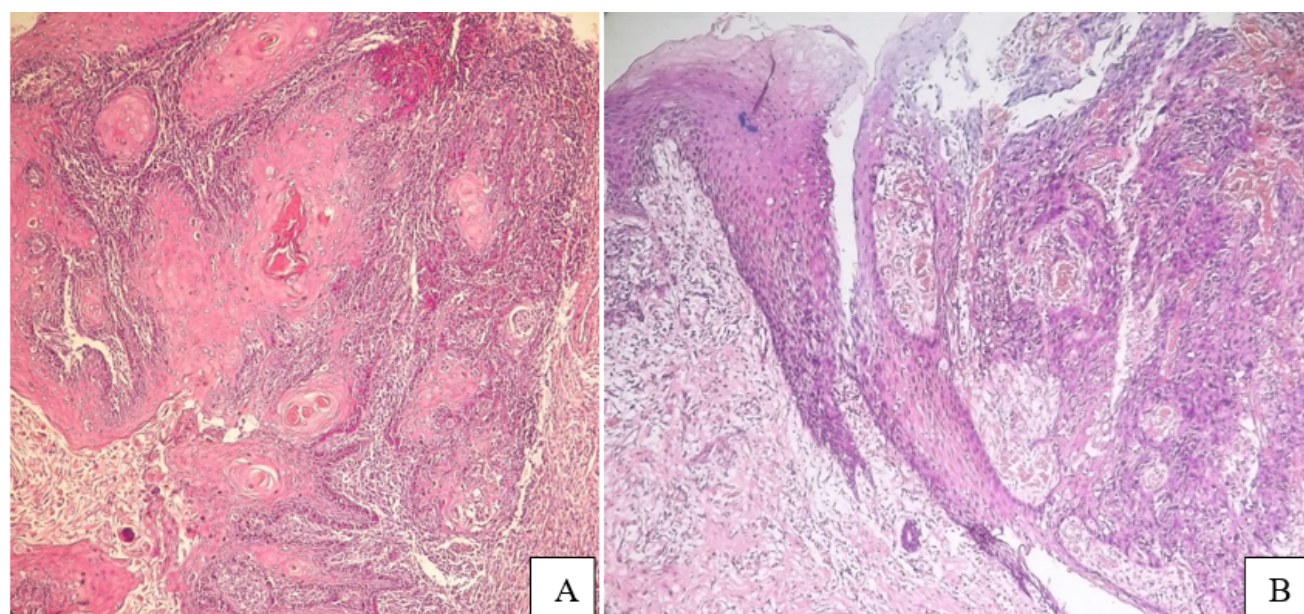


Fig. 5: **A):** Photomicrograph showing histopathological features of well differentiated squamous cell carcinoma (H&E stain: 400x) of ulceroproliferative lesion on left lateral border of tongue in a 57 yrs female, **B):** Photomicrograph showing histopathological features of moderately differentiated squamous cell carcinoma (H&E stain: 400x) of left buccal mucosal ulcer in a 49 yrs male.

4. Discussion

This study was undertaken with the view of evaluation of the most common tobacco related oral cavity lesions and to correlate the scrape cytology findings of these lesions with histopathology, so that with the help of scrape cytology as a rapid, reliable, easy diagnostic procedure we can identify premalignant and malignant lesions as early as possible. That will help the patients in early, rapid diagnosis and prompt treatment. There are certain premalignant conditions like leukoplakia, erythroplakia; oral submucosal fibrosis if not treated promptly may lead to a squamous cell carcinoma. Although surgical biopsy followed by histopathology is considered the gold

standard for diagnosing the oral lesions, it may not be possible to carry out biopsy in every case as some of the patients may be medically compromised and a few patients with asymptomatic lesion may not give their consent for biopsy.¹⁶

4.1. Age and sex related incidence of lesions

Our study included 62 male (72.94%) with age range of 30 to 70 yrs. (average age 50.35yrs) and 23 female (27.06%) with age range of 35 to 75 yrs. (average age 49.52yrs.) patients, so that male to female ratio was 2.7:1, suggesting male preponderance for tobacco related oral cavity lesions directly reflecting the increased use of tobacco and tobacco

products in male. Exactly similar observation was stated by Bagate A. et al¹⁷ in his study of 100 oral mucosal lesions showing male preponderance and male to female ratio of 2.7:1.

The most common age groups in our study was between 41 to 50 years (27 patients-31.76%) followed by 51-60 years (24 patients-28.23%) thus >60 % lesion found after 40 years of age. In a study of total 67 Indian patients by M Babshet. et al,¹⁶ males were predominantly affected, fourth and fifth decades of life, majority of patients being in the 40 to 70 years age group. Ali Mahmoud M Edris. et al¹⁸ in his hospital based case control study of 100 Sudanese patients ranging from 18 to 78 years with mean age of 45 years and M:F ratio of 1.3:1 found vast majority of the oral squamous cell carcinoma in lip(28.5%) followed by tongue (25%) and buccal mucosa(14.3%).

4.2. Types of addiction

Tobacco addiction in our patients was in the following form, commonly chewing tobacco with lime (34 patients: 31%), pan with betel nut and tobacco(25 patients :23%), gutkha or mawa (9 patients :8%), and mishri application over gum and teeth.(6 patients: 5%). The 37 patients (33%) had mixed addiction means they were chronic smokers and alcohol addiction.

Bagate A et al¹⁷ observed chewing gutkha, pudi or mixed habit in 48.93% followed by tobacco with lime users in 36.17% cases. M Babshet et al,¹⁶ found 38.3% had the habit of tobacco chewing, 28.3% had smoking habit, and 25% of the patients had a combination of quid, tobacco or gutkha chewing habits along with alcohol consumption. In the study performed by Mehrotra et al.¹⁹ tobacco consumption was the most common with 54% patients having used it in one form or another, 35.4% gave a history of exposure to more than one carcinogen. Castellsagu et al. reported that simultaneous exposure to both alcohol and tobacco increases the risk of oral cancer by 13-fold.²⁰ heavy alcohol consumption and smoking have been identified as primary risk factors for nearly 80% of oral malignancies.²¹ Mohammed S. Abdelaziz et al. In their study suggested that alcohol consumption and cigarette smoking are risk factors for oral atypical cellular changes.²² Mixed addiction is associated with increase incidences of malignant lesions than individually due to synergistic effects.

4.3. Duration and frequency of addiction:

The duration of tobacco addiction ranges from 6 to 30 years in male with frequency of 1 to 10 times per day and duration of 5 to 40 years in female with frequency of 1 to 8 times per day. Thus the average period was 19.5 years for oral tissue alteration associated with tobacco addiction. Bagate A et al¹⁷ reported malignant cases with history of tobacco chewing for >20 years.

4.4. Site of placement of tobacco products

The habit of placing tobacco mixed with lime, usually in the canine premolar region of the mandibular sulcus is widespread in rural population of central Maharashtra.⁹ Common site for keeping tobacco products was left gingivobuccal sulcus (31 patients), followed by right gingivobuccal sulcus (22 patients), anterior gingivobuccal sulcus (11 patients) and 3 patients an anterior floor of mouth. Bharati Jha et al¹⁰ concluded that frequent involvement of tongue and floor of mouth, compared to lip in European's³ may be due to the more use of chewable tobacco in Indian population.

4.5. Number and site of intra oral lesions

Right and left buccal mucosa (37 patients:36.2%) was the most common site of tobacco related oral cavity lesions followed by lateral border of tongue (21 patients: 20.5%), lower gingiva (10 patients:9.8%), involvement of cheek (9 patients), lower labial mucosa (7 patients), hard palate(6 patients), retro molar area(2 patients) and a single patient had lesion in the anterior floor of mouth. It is well correlated with intra oral lesions and the most common sites of placement of tobacco products i.e. gingivobuccal sulcus in buccal cavity. Two oral lesions in single patients were found in 9 cases (8.8%) mostly associated with mixed tobacco habit.

M Babshet. et al,¹⁶ found buccal mucosa was the most frequently involved site (67%), followed by alveolus and gingiva (21%), lip (7%) and tongue (5%). Mehrotra et al,^{4,19} in their study, found that buccal mucosa and tongue were the most frequently involved sites (35.7% and 21.4%, respectively) correlating with our study. Cancer Registry, Indian Cancer Society, Mumbai showed in case of males, 7% of all cancers in buccal mucosa & 5% of all cancers in tongue.²³

5. Types of oral lesions

Total 102 lesions were found in 85 patients out of which 42 lesions (41.17%) were premalignant condition and 60 lesions (58.82%) showed malignancy. The malignant lesions are more because most of the patients with early oral cancer lesions are usually painless and come to hospital after a progression of lesions hence got unnoticed. Also they are treated at private or other smaller hospitals at primary level. Most of the patients were referred from rural parts of Maharashtra.

In 42 patients (41.17%) premalignant lesions were seen, oral submucosal fibrosis (17 patients) and leukoplakia (16 patients) were most common lesions followed by oral lichen planus (4 patients), chronic no healing ulcer (3 patients), squamous papilloma and verruca vulgaris one patient each. However, our study didn't found erythroplakic lesion. Bagate A et al¹⁷ also seen maximum number

of oral submucosal fibrosis cases. In the study sample comprised of total 67 patients by M Babshet. et al,¹⁶ 32 being premalignant lesions and other 35 being frank oral carcinomas. Nazoora Khan et al.,²⁴ showed Leukoplakia was the most common premalignant lesion (26 in 28 cases) and squamous cell carcinoma was the most common tumor in malignant category (91 in 104 cases).

In 58.82% malignant lesions, in our study of 60 patients, 45 were male and 15 female patients thus tobacco related oral malignancy male were affected more commonly (75%) due to addicted to tobacco, smoking and alcohol than female. The age range for male was 30-70 years with mean age 50.82 years and age range of female 35-75 years with mean age 53.80 yrs. Thus there is shift of oral malignancy towards early age group might be due increased tobacco habits towards younger age. The most common site of oral carcinoma in our study was buccal mucosa (26 patients: 43.33%) which is also the most common site of placement of intraoral tobacco products followed by tongue (13 patients: 21.66%), cheek with skin involvement (9 patients: 15%), gingiva (8 patients), lower lip (7 patients), hard palate (2 patients), retro molar area (2 patients) and a single case at anterior floor of mouth. Similar findings were noted by Bagate A et al¹⁷ with maximum number of malignant lesions in right & left cheek buccal mucosa (50%), followed by tongue (31.25%) in an age group of 41 to 70 years.

We observed ulcerative or ulceroproliferative types of cancerous lesions (48 cases) on gross examination followed by exophytic growth (7 cases) and verrucous growth (5 cases). Squamous cell carcinoma constitutes the most common (57 cases :95%) types of oral malignancy with remaining 5% cases showed verrucous carcinoma (3 cases), which is a variant of squamous cell carcinoma. In our study, well differentiated squamous cell carcinoma were seen in more number of cases (28 cases; 46.66%), followed by moderately differentiated squamous cell carcinoma (26 cases; 43.33%) while poorly differentiated squamous cell carcinoma constitutes only 3 cases (5%). The spread of squamous cell carcinoma to regional cervical lymph nodes was seen in 15 patients (25%).

We found one case of 35 years, seropositive male patient with pulmonary Koch's, having gutkha addiction for 9 years showing ulcer on left side of hard palate which was diagnosed as well differentiated squamous cell carcinoma on scrape cytology and confirmed on histology.

5.1. Scrape cytology correlation with histology

According to our study, out of 60 oral carcinoma cases, 53 patients showed true positive for squamous cell carcinoma. Thus diagnostic accuracy of scrape cytology compared with histopathology was 88.33% and false negative cases were 7(11.66%), it means scrape cytology smears revealed features of inflammation ; however biopsy of the lesion

turned out to be squamous cell carcinoma. As not a single false positive case was found in our study, sensitivity and specificity of scrape cytology in detecting oral squamous cell carcinoma were 88.33% and 100% respectively.

In present study, positive predictive value was 100% similar to studies by Bharati Jha et al,¹⁰ M Babshet et al¹⁶ and Remmerbach et al.²⁵ and negative predictive value was 85.7% correlating with Driemel et al.²⁶ and diagnostic accuracy of 93.14% which was similar with other studies by Nazoora Khan et al.,²⁴ Bharati Jha et al.¹⁰ Thus the above results were well correlated with various other studies, where the sensitivity ranges from 77% to 95% and specificity 93% to 100% [Table 3]. Bagate A. et al,¹⁷ also found diagnostic accuracy of 92.3% which were comparable with our results.

The scrape cytology of premalignant lesions showed only inflammation without evidence of malignancy. The deeply located lesions were not easily approachable due to trismus; also most of them were well differentiated squamous cell carcinoma reducing the diagnostic accuracy in our study. False negative results were probably due to cautious approach to the diagnosis of the malignant lesion and mature squamous cells diluting and responsible for paucity of the number of immature cells, lack of clarity to cell structures and indefinite malignant character. Limitation includes its inability to detect invasion and its false negative potential in well differentiated squamous cell carcinoma.^{11,27}

Scrape cytology can be done by Ayre's spatula, cytobrush, wooden tongue depressor /spatula. Scrape cytology is a rapid, simple & inexpensive diagnostic test which can be easily performed on day to day practice. Scrape cytology with PAP staining can be utilized effectively to identify and differentiate between various mucosal diseases as well as early diagnostic method for epithelial atypia.^{18,28}

6. Conclusion

Prolonged use of tobacco products, smoking and alcohol which are causes synergistically carcinogenic effect to have premalignant and malignant intra oral lesions at the site of placement of tobacco. The lower socio economic status, lack of education, awareness and well equipped diagnostic centers at rural levels and fear of cancer itself accounts for larger extent of delay in early and rapid diagnosis and treatment of the premalignant and malignant oral cavity lesions. Therefore scrape cytology can be a useful adjuvant along with histopathological correlation if required for these patients and should be practice in a general laboratory set up.

7. Source of funding

None.

8. Conflict of interest

None.

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