

Original Research Article


Cytodiagnosis of oral lesions in chronic tobacco users

Shweta P. Bijwe^{1*}, Arun P. Bakshi²

¹Department of Pathology, IGGMC, Nagpur, Maharashtra, India

²Associate Professor, IGGMC, Nagpur, Maharashtra, India

*Corresponding author email: dr.shwetabijwe@gmail.com

	International Archives of Integrated Medicine, Vol. 5, Issue 1, January, 2018. Copy right © 2018, IAIM, All Rights Reserved. Available online at http://iaimjournal.com/	
	ISSN: 2394-0026 (P)	ISSN: 2394-0034 (O)
	Received on: 10-12-2017	Accepted on: 18-12-2017
	Source of support: Nil	Conflict of interest: None declared.
How to cite this article: Shweta P. Bijwe, Arun P. Bakshi. Cytodiagnosis of oral lesions in chronic tobacco users. IAIM, 2018; 5(1): 17-27.		

Abstract

Background: In the country like India where oral cancer with its associated tobacco chewing habit exists in epidemic proportions, constitutes a major public health hazard. The high percentage of oral cancer if not preventable is curable only if detected in its early stage which require large scale screening of the population at risk carried out repeatedly from time to time. Exfoliative cytology plays important role in this.

Aim: To study age and sex wise incidence of oral lesion, to study the clinical presentations, to study the role of tobacco chewing, gutkha, pan with areca nut and smoking in benign, premalignant and malignant lesions of oral cavity, to study the cytomorphological changes in oral cavity of different clinico- pathological lesions of chronic tobacco users, to correlate oral cytology with histopathology where ever possible.

Materials and methods: A prospective study was carried out in total 135 chronic tobacco users. Patients above 15 years age who presented with oral lesion and with history of tobacco use in any form for more than 1 year were included in the study. Samples were obtained by scrape method using wooden spatula. All the smears were stained by Papanicolaou stain.

Results: In the present study of 135 chronic tobacco users 73.33% were male and 26.67% were females. Male to female ratio was 2.75:1. Maximum 34.07% chronic tobacco users were in the age group of 31-40 year. 30 cases of oral submucous fibrosis, 5 cases of leukoplakia and 59 cases of clinically suspected malignancy seen. Most common presenting symptom were ulceration in oral cavity in 51.11%. Commonest site of lesion was buccal mucosa in 56.30% cases. Cytological examination of smear showed dysplasia in 58.52% cases, smears positive / suspicious for malignancy in 37.04% cases and inflammatory smears in 3.70% cases. Clinically suspected malignant lesions were seen maximum (40 cases) in age group of 41-60 years which were using tobacco+lime, kharra, gutkha and tobacco smoking as habits. Incidence of malignancy was seen increasing with increased

duration and frequency of tobacco use. Maximum 24 cases of malignancy (40.68%) were seen with use of tobacco for more than 25 years, affecting males commonly. Squamous cell carcinoma was the only histological type seen in all the cases. Percentage accuracy of cytologic diagnosis in oral cancer when compared with histopathological diagnosis was 84.75%.

Conclusion: Cytology is a reliable method for diagnosis of oral cancer but histological examination remains the gold standard.

Key words

Chronic tobacco users, Oral cytology, Histological correlation, Type of tobacco preparation, Changes related with duration of tobacco use.

Introduction

Tobacco chewing is a widespread habit in India and is practiced in variety of different ways. In the country like India where oral cancer with its associated tobacco chewing habit exists in epidemic proportions, thus constituting a major public health hazard Tobacco use was the leading cause of disease burden measured in disability adjusted life years (DALYs) lost in developed countries, and one of the top 10 risk factors even in the poorest developing regions [1]. The detection of cancer in an individual person is not sufficient. It will necessarily require large scale screening of the population at risk and this will have to be carried out repeatedly from time to time.

The only diagnostic technique that fits in admirably with this scheme is the use of exfoliative cytology. The credit for the revival of the interest in cytology goes to Papanicolaou (1917-1954) who's devoted, meticulous and painstaking work over a number of years but back on a firm foundation.

Today not only cytology is accepted as part of the routine training of pathologists, but it is fast approaching the status of super speciality.

The use of cytological techniques for an early detection of cancer of various organs is now practically well established. However, its acceptance as routine diagnostic procedure in oral disease is still in its infancy. Different opinions have been expressed regarding the usefulness of oral exfoliative cytology by

different investigators. It differs according to the users qualifications, experience and view point, to the country of origin, the methods employed and the expectations towards cytology [2].

It would appear that oral exfoliative cytology is most suitably used when there is clinically apparent lesion of unknown origin. If a lesion is clinically suggestive of malignancy it should be biopsied immediately under most circumstances [3].

With respect to the oral cytology for detection of oral lesion there is general agreement on several points-

- Oral cytology is simple, reliable, accurate, easy, reproducible, screening method for detection of early intra oral malignant lesions without producing trauma to patient.
- Oral cytology is useful as an adjuvant for a more precise categorization of visible lesions not warranting biopsies.
- The value of oral cytology is limited if cancer is clinically suspected but a combined cytology biopsy approach may be of value.
- Oral cytology is useful in cases where biopsy is contraindicated.
- Oral cytology is not a substitute for biopsy [2-6].

Now-a days the use of tobacco, betel nut products, instant preparation of betel nut products i.e. pan masala and gutkha is very popular in young generation and even in school

teenagers, which leads to the premalignant condition known as submucous fibrosis [7-10].

Many studies have proved efficacy of cytologic diagnosis in oral pathology especially cancer. The present study is under taken to study the cytological changes occurring in the oral mucous membrane of chronic tobacco chewers with oral lesions, attending outpatient and inpatient department of hospital.

Materials and methods

The present study was prospective study carried out in Department of Pathology, Indira Gandhi Government College, Nagpur, Maharashtra, during a period of December 2011 to October 2013. The study included clinical and cytological examination of the oral cavity lesions in chronic tobacco users. The study included 135 cases.

Inclusion criteria

Patients above 15 years age who presented with oral lesion and with history of tobacco use in any form for more than 1 year.

Exclusion criteria

Patients with oral lesions without any history of tobacco addiction in any form.

Most of the lesions required no preparation. If the lesion was covered with debris or slough,

then surface was cleaned by wiping with gauze moistened in normal saline solution. Dry lesions were moistened with saline solution before the specimens were obtained, at least for 15 minutes.

We have obtained the samples by scrape method using wooden spatula.

Scrape method

In chronic tobacco users having particular quid bed or oral lesion the entire surface of lesion was scraped with blunt wooden spatula, several times in one direction.

As soon as the specimen was obtained on the scrapping instrument, it was transferred to the glass slides which were kept ready labeled and numbered. The smears were prepared by spreading the material thinly and uniformly with a circular motion on middle third of the slide. Then the glass slide was placed immediately in the fixative that was 95 % ethanol mixture.

Results

Table - 1 shows age and sex wise distribution of 135 chronic tobacco users. The total number of males was 73.33% and females were 26.67%. The maximum number of tobacco users was in the age group of 31-40 years i.e. 34.07%, followed by 41-50 years i.e. 23.70%.

Table – 1: Age and sex wise distribution of chronic tobacco users (n= 135).

Age group (Years)	Male No of cases	Female No of cases	Total No of cases (%)
11 – 20	7	-	7 (5.18 %)
21 – 30	14	6	20 (14.81%)
31 – 40	30	16	46 (34.07%)
41 – 50	27	5	32 (23.70%)
51 – 60	13	7	20 (14.81%)
61 -70	7	1	8 (5.92%)
71 – 80	1	1	2 (1.48%)
Total	99 (73.33%)	36 (26.67%)	135 (100%)

Table - 2 shows distribution of lesions to anatomical sites. The maximum number of cases were of buccal mucosa i.e. in 76 cases (56.30%)

followed by tongue in 23 cases (17.04%) and gingivoalveolar sulcus in 22 cases (16.30%).

Table - 2: Age and sex wise incidence of various sites of lesions in chronic tobacco users.

Site	Male	Female	Total
Buccal mucosa	59	17	76 (56.30%)
Tongue	16	7	23 (17.04%)
Lip	3	1	4 (2.96%)
Gingivoalveolar sulcus	13	9	22 (16.30%)
Palate	3	1	4 (2.96%)
Retromolar region	2	0	2 (1.48%)
Floor of mouth	1	0	1 (0.74%)
Alveolus	1	0	1 (0.74%)
Corner of mouth	1	1	2 (1.48%)
Total	99	36	135 (100%)

Table – 3: Various clinical presenting features in chronic tobacco users.

Presenting features	Male	Female	Total
Ulceration	51	18	69
Dysphagia	2	-	2
Swelling with ulceration	22	8	30
Whitish patch	10	2	12
Difficulty in opening mouth	22	8	30

Table – 4: Age and sex wise distribution of various types of oral lesions in chronic tobacco users. -

Age group (Years)	Oral submucous fibrosis			Leukoplakia			Clinically suspected malignancy			Other benign lesions (Benign ulcers, glossitis, cheilitis)		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
11-20	6	-	6	-	-	-	-	-	-	1	-	1
21-30	7	4	11	1	-	1	1	-	1	5	2	7
31-40	7	4	11	1	1	2	6	6	12	15	6	21
41-50	1	-	1	1	-	1	18	4	22	6	1	7
51-60	-	-	-	-	-	-	12	6	18	2	1	3
61-70	-	1	1	-	-	-	5	-	5	2	-	2
71-80	-	-	-	1	-	1	-	1	1	-	-	-
Total	21	9	30	4	1	5	42	17	59	31	10	41

Males showed most common site of involvement as buccal mucosa in 59 cases (59.59%) followed by tongue in 16 cases (16.16%) and gingivoalveolar sulcus in 13 cases (13.13%). In females most common site was buccal mucosa, in 17 cases (47.22%), followed by gingivoalveolar sulcus in 9 cases (25.00%) and tongue in 7 cases (19.44%).

Table - 3 shows most common clinical presentation of cases as ulceration in 69 cases, followed by swelling with ulceration in 30 cases and difficulty in opening mouth in 30 cases.

Table - 4 shows age and sex wise distribution of clinical lesions in chronic tobacco users. The maximum 59 cases were of suspected malignancy (43.70%), followed by other benign lesions like stomatitis, glossitis and benign ulcers in 41 cases (30.37%), followed by oral submucous fibrosis in 30 cases (22.22%). Leukoplakia was reported in 5 cases (3.70%). All above lesions were more commonly seen in males.

Table - 5 shows following findings, oral submucous fibrosis is more common in males than in females. It is more common in persons

chewing gutkha (30.00%) followed by persons using tobacco and kharra (20.00%) and with use of tobacco and gutkha (16.67%).

Leukoplakia was seen in persons using tobacco lime (40.00%) and tobacco gutkha and persons with mixed habits of tobacco chewing and smoking (40.00%). Leukoplakia was more common in males than females.

Malignant lesions were seen higher in persons with mixed habit of tobacco chewing and

smoking (45.76%) followed by habit of tobacco, betel nut (pan) and areca nut use (16.95%). Males outnumbered females.

2 cases of malignant lesion were reported with use of nuswar [snuff] for teeth cleaning purpose. Both cases were females.

Habit of tobacco smoking was exclusively found in males only.

Table – 5: Distribution of clinically premalignant and malignant lesions in relation to tobacco preparations used.

Tobacco preparation	Oral submucous fibrosis (n=30)			Leukoplakia (n=5)			Malignancy (n=59)		
	M	F	Total	M	F	Total	M	F	Total
Tobacco + lime	1	3	4(13.33%)	2	-	2(40.00%)	6	1	7(11.86%)
Gutkha	5	4	9(30.00%)	-	-	-	-	1	1(1.69%)
Tobacco + gutkha	5	-	5(16.67%)	-	1	1(20.00%)	-	-	-
Tobacco + kharra	6	-	6(20.00%)	-	-	-	4	-	4(6.78%)
Tobacco + areca nut + lime+betel nut (pan)	2	2	4(13.33%)	-	-	-	2	8	10(16.95%)
Bidi smoking	-	-	-	-	-	-	2	-	2(3.39%)
Mixed habit (Tobacco chewing and smoking)	2	-	2(6.67%)	2	-	2(40.00%)	27	-	27(45.76%)
Tobacco+ pan+ gutkha + kharra	-	-	-	-	-	-	2	4	6(10.17%)
Nuswar	-	-	-	-	-	-	-	2	2(3.39%)
Total	21	9	30(100%)	4	1	5(100%)	43	16	59(100%)

Table – 6: Cytological findings in chronic tobacco users (n =135).

Cytological findings	No of cases
Negative	1 (0.74%)
Inflammatory	5 (3.70%)
Mild dysplasia	48 (35.55%)
Moderate dysplasia	20 (14.81%)
Severe dysplasia	11 (8.15%)
Suspicious of malignancy	15 (11.11%)
Positive for malignancy	35 (25.92%)
Total	135 (100%)

Table - 6 shows most common cytological finding was mild dysplasia in 35.55%. Moderate dysplasia in 14.81 % and severe dysplasia in

8.15% cases. Total 50 cases were suspicious or positive for malignancy (37.03%).

Table - 7 shows that mild dysplasia was the prominent cytological finding in 28 patients (58.33%) out of 41 cases having history of duration of exposure up to 10 years. Out of total 50 cases of positive /suspicious cases of malignancy maximum number of 24 cases were seen in patients having history of tobacco chewing more than 25 years (48.00%), followed by 15 cases having duration of exposure up to 21 - 25 years (30.00%).

Table – 7: Cytological findings related with duration of tobacco use.

Duration of tobacco use (Years)	No of cases	cytology					Suspicious/ positive for malignancy
		Negative	Inflammatory	Mild	Moderate	Severe	
1 -5	13	1	2	9	-	1	-
6 – 10	28	-	2	19	4	3	-
11 – 15	23	-	-	9	10	2	2
16 – 20	15	-	-	4	2	-	9
21 – 25	25	-	1	5	2	2	15
>25	31	-	-	2	2	3	24
Total	135	1	5	48	20	11	50

Table – 8: Cytological findings correlated with histopathological findings in 59 proved malignant cases in chronic tobacco users.

Histopathology Malignant cases	Cytology for malignant cells		Percentage Accuracy
	Positive/ suspicious	Negative	
59	50	9	84.75%

As per **Table – 8**, in 59 cases histologically diagnosed malignant cases; only 50 were diagnosed on cytology. In the 9 negative cases, 4 cases were diagnosed as severe dysplasia, 2 as moderate dysplasia and 3 as mild dysplasia. The percentage accuracy of cytological diagnosis in relation to histopathological diagnosis was 84.75%.

Discussion

The present study comprises study of cytologic smears taken from oral cavities of 135 chronic tobacco users. Histopathological examination was done in cases of clinically suspected malignant oral lesion whenever possible.

Age and sex

In the present study age range was 15 - 80 years. The youngest patient with oral lesion was 15

years old and the oldest patient was 80 years old. The mean age is 40.79 years. Other authors have similar observations regarding the age incidence of oral lesions as given in the **Table – 9**.

Table – 9: Age incidence of oral lesions in chronic tobacco users in different studies.

Author	Age incidence
Wahi 1968 [11]	35-70
Folsom, et al. 1972 [3]	15- 84
A. Singh 2010 [12]	20 - 80
Present study 2013	15 - 80

Our findings are similar to findings of Folsom, et al. (1972) [3] and A. Singh (2010) [12].

Site of lesion

The present study shows the commonest site of lesion occurrence as buccal mucosa in 76 cases (56.30%), followed by tongue in 23 cases

(17.04%), gingivoalveolar sulcus 22 cases (16.30%), lip 4 cases (2.96%), palate 4 cases (2.96%), retromolar region 2 cases (1.48%), angle of mouth 2 cases (1.48%), floor of mouth 1 case (0.74%) and alveolus 1 case (0.74%) (**Table – 2**).

In present study and Patil P B, et al. (2013) [13] study buccal mucosa was found to be the most common site of involvement while in present study tongue (17.04%) and gingivobuccal sulcus (16.30%) were 2nd and 3rd most common sites of involvement, Patil P B, et al. (2013) [13] observed that labial mucosa and palatal mucosa were more frequently involved, following buccal mucosa. In his study tongue and gingiva were involved in 3.6% and 2.90% cases respectively.

Clinical diagnosis

In the present study of 135 cases, 59 cases (43.70%) presented as clinically suspected malignancy, 30 cases (22.22%) as oral submucosal fibrosis, 5 cases (3.70%) of leukoplakia and rest 41 cases (30.37%) of benign inflammatory lesions. The commonest presenting symptom in present study was ulceration in oral cavity seen in 69 cases (51.11%), followed by swelling with ulceration in 30 cases (22.22%), difficulty in opening mouth in 30 cases (22.22%), whitish patch 12 cases (8.89%) and dysphagia in 2 cases (1.48%).

As compared to studies of Robert Ingram [14] and A. Singh (2010) [12], our study showed higher percentage of patients presenting as clinically suspected malignancy. Presentation at advanced stage may be due to unawareness among the people about presentation of premalignant conditions as mostly they are asymptomatic.

Oral submucous fibrosis

In present study maximum number of cases 22 (73.33%) of oral submucosal fibrosis, observed in 21- 40 years of age group who were using using gutkha or kharra with tobacco since 5 to 10 years duration.

Our observations are similar with Sinor P.N., et al. (1990) [15] and Hazare V.K., et al. (1998) [16] observations.

Leukoplakia

In the present study out of 5 cases of leukoplakia. Males were more commonly affected, 4 cases (80.00%) being males and 1 (20.00%) was female. It was more commonly seen in age group of 31-40 years.

Findings of present study are similar with studies of Pindborg, Gupta Chawala (1967) [17], Minati M, et al. (2005) [18] and A Singh (2010) [12].

Clinically suspected malignant lesions

The present study shows the maximum number of suspected malignant cases (59 cases) in age group of 41-60 years. The youngest patient was of 30 years and oldest was of 80 years

Henry Sandler [4] in 1962 who studied 208 cases of malignancy and observed that the proportion of malignant lesions increased with aging to a maximum 21.9% in 55 to 59 years and 33.8% in 60 to 69 years.

As compared to his study, incidence of malignancy is seen a decade earlier in present study. The comparative findings observed by of different workers regarding age incidence of malignant cases are given in **Table – 10**.

Table – 10: Age incidence of malignant cases in chronic tobacco users in different studies.

Worker	Age incidence
Henry Sandler (1962) [4]	55-69
K Pande (1973) [19]	45-65
A Singh (2010) [12]	41-60
Present study (2013)	41-60

Our findings are similar with studies of Karuna Pande (1973) [19] and A. Singh (2010) [12].

Tobacco habits

Present study shows oral submucous fibrosis is more common in persons chewing gutkha (30.00%) followed by persons using tobacco and

kharrā (20.00%) and with use of tobacco and gutkha (16.67%). Similar observations were made by Babu, et al. [7] in 1996. Pan masala and gutkha is associated with oral submucous fibrosis after 2.7 ± 0.6 years of use whereas the betel quid users presented with oral submucous fibrosis reported 8.6 ± 2.3 years. Use of pan masala or areca nut products shows earlier presentation of oral submucous fibrosis. Hazare VK, et al. (1998) [16] observed a dose response relationship with use of areca nut, kharrā and pan masala per day. In the present study all gutkha and betel nut users presented with oral submucous fibrosis.

In present study leukoplakia is seen in persons using tobacco lime (40.00%) and tobacco gutkha and persons with mixed habits of tobacco chewing and smoking (40.00%). Leukoplakia is more common in males than females. Similarly Pindborg, Gupta Chawala (1967) [17] and Minati M (2005) [18] also observed that leukoplakia was more common with habits of gutkha, pan masala and betel nut use and bidi smoking. Condition was more common in males.

In present study malignant lesions were seen higher in persons with mixed habit of tobacco chewing and smoking (45.76%) followed by habit of tobacco, betel nut (paan) and areca nut use (16.95%). Males outnumbered females. Similarly Balram P, et al. (2002) [20] observed that among men 35% of oral cancer is attributable to the combination of smoking and alcohol drinking and 49% to pan - tobacco chewing. Among women, chewing and poor oral hygiene explained 95% of oral cancer.

Duration of tobacco use

In present study out of 135 chronic tobacco users 41 cases were having duration of tobacco use less than 10 years. The most common cytological changes on smears were mild dysplasia in 28 cases (68.29%), followed by inflammatory smear in 4 cases (9.76%), moderate dysplasia in 4 cases (9.76%), severe dysplasia in 4 cases (9.76%) and 1 negative smear (2.44%) which was within normal limits.

Incidence of malignancy was seen increasing with increased duration and frequency of tobacco use. Out of total 59 cases of histologically confirmed cases of malignancy, maximum were seen with use of tobacco for more than 25 years. Similarly in 1960, Irving Chapman and Reddish [21] observed that severity of epithelial proliferation was directly related to the length of smoking time and amount of tobacco smoked. Wahi PN (1968) [11] also observed that risk of oral cancer increases with increases in frequency and duration of tobacco chewing or keeping quid in mouth while sleeping.

Cytological findings

Present study of 135 chronic tobacco users cytology findings shows 35.55% mild dysplasia, 25.92% positive for malignancy, 14.81% moderate dysplasia, 11.11% suspicious for malignancy, 8.15% severe dysplasia, 3.70% inflammatory lesions and 0.74 % negative smears.

Findings observed by Vijay L. Lahiri [22] in 1974, in 150 chronic tobacco chewers of which 42.00% were negative, 23.33% cases showed inflammation, 18.67% mild dysplasia, 10.67% moderate dysplasia and 3.33% severe dysplasia and 0.67% positive for malignancy. Similar observations also noted by Henry Sandler [4] 1962, Tyler Folsom 1972 [3] and Sol Silverman 1977 [23].

As compared to all above studies, present study showed more number of cases with mild dysplasia and less number of inflammatory cases. In present study there are also more number of cases positive and suspicious for malignancy, as compared to Vijay L. Lahiri (1974) [22] who observed only 1 case (0.67%) positive for malignancy. These differences in findings may be because of more number of cases presented as clinically suspected malignant lesion in present study.

Diagnostic accuracy

In the present study biopsies were taken from clinically suspected malignant cases whenever

possible. 59 cases in which biopsy shows squamous cell carcinoma, cytologically 50 were positive or suspicious for malignancy and 9 were negative. Diagnostic accuracy was 84.75%.

The 9 cases which were negative showed severe dysplasia in 4 cases, moderate dysplasia in 2 cases and mild dysplasia in 3 cases.

False negative smears might be because of faulty technique or due to necrotic debris and

inflammation present over lesion because of which lesion site could not have been scraped properly.

Similar findings were observed by many workers and comparative diagnostic accuracy and false negativity is as per **Table – 11**.

Our findings are nearly similar to findings of Cawson R.A. (1960) [25], Folsom (1972) [3], A Singh (2010) [12] and M Babashet (2011) [26].

Table – 11: Diagnostic accuracy of cytological findings of oral lesions in chronic tobacco users in various studies.

Worker and year	False / Negative (%)	Diagnostic Accuracy (%)
H. Peters, 1956 [24]	4	96
Cawson R.A., 1960 [25]	19	81
H. Sandler , 1962 [4]	9	91
Folsom, 1972 [3]	13.9	86.1
Sol Silverman , 1977 [23]	1	99
A Singh,2010 [12]	15	85
M Babashet, 2011 [26]	18	82
Present study 2013	15.25	84.75

Conclusion

Tobacco chewing and smoking habit is more common in males as compared to females. Most common clinical presentation is malignant lesion associated more commonly with habit of both tobacco chewing and smoking, followed by benign lesions (benign ulcers, glossitis, chelitis), oral submucous fibrosis and leukoplakia. Dysplasia is a prominent cytological finding when the duration of tobacco chewing was less than 10 years whereas malignancy is associated with duration of tobacco chewing more than 20 years. The oral cytological study of chronic tobacco users showed that dysplasia was the commonest finding, followed by malignancy and inflammation. The percentage accuracy of cytologic diagnosis in oral cancer when compared with histopathological diagnosis was 84.75%. Thus cytology is a reliable method for diagnosis of oral cancer but histological examination remains the gold standard. Squamous cell carcinoma was the common type of malignancy seen in chronic tobacco users,

predominantly in males more than 40 years of age.

Acknowledgement

We are thankful to Dept. of ENT and Dept. of Dentistry, IGGMC, Nagpur for their kind co-operation.

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