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Oral cytomorphological study of chronic tobacco chewers in rural area: A prospective study

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Abstract
Aims and objectives:
1. To study the cytomorphological changes in oral cavity of different pathological lesions of chronic tobacco chewers in the rural population.
2. Assess the significance of the oral scrape cytology in oral mucosa of tobacco chewers.
Materials and methods: A total number of 100 patients with oral mucosal lesions with Gutkha chewing habit were studied. The distribution of patients included both males and females of all age groups and socioeconomic status compared with the controls comprising of 50 persons with no history of tobacco chewing.
Results: On cytological diagnosis, 53% lesions were inflammatory followed by 30% dysplastic and 12% were positive/suspicious for malignant cells. Out of 16 clinically suspected carcinoma cases, 12 came out positive on cytology and 13 were confirmed on histopathology.
Conclusion: This study supports the hypothesis that tobacco chewing elevates the risk of oral submucous fibrosis, leukoplakia and cancer.
Oral scrape cytology has been found to be very helpful to detect precancer and cancerous conditions; hence we recommend the use of oral scrape cytology as a screening tool in all suspicious intraoral lesions for rapid diagnosis.

Keywords: Tobacco, Oral submucous fibrosis, Oral scrape cytology.

1. Introduction
Tobacco use kills millions of people worldwide, cause immense suffering and social coasts, with one person dying every nine seconds from tobacco related cause [WHO, 1998] [1]. Smokeless tobacco (ST) is tobacco that is not burned when it is used. It may be used alone or in combination with other substances and used at any site that permits the absorption into the human body of nicotine and/or other constituents in tobacco or added to tobacco [2]. Gutkha was introduced in Indian market in early 1970s.
Gutkha is a mixture of substances that is placed in mouth, where it is sucked or actively chewed and thus remains in contact with the mucosa over an extended period.[3] It usually contains three basic ingredients, tobacco, areca nut and slacked lime (calcium hydroxide), which are sealed in small, attractive, colourful and inexpensive plastic or foil pack, which appeal to young generations [3].
Tobacco use is one of the chief preventable causes of death in the world. A wide variety of mucosal changes have been noted in habitual users of smoked and smokeless tobacco. These changes most likely result from the many irritants, toxins, and carcinogens found naturally in cured or burned tobacco leaves, may also arise from the mucosal drying effects, the high intraoral temperature, intraoral pH changes, local alteration of membrane barriers and immune responses, or altered resistance to fungal and viral infections [4].
The dentists, oral surgeons and physicians therefore bear a great responsibility with regards to early diagnosis of cancer of the oral cavity. The only diagnostic technique that fits in admirably with this scheme, is the use of exfoliative cytology. The first modern use of cytology for head and neck cancers was in 1949, when Morrison et al successfully used exfoliative smears for nasopharyngeal cancers. In 1963, the American Dental Association's House of Delegates passed resolutions that acclaimed oral cytology as an 'excellent measure in prolonging life' and endorsed training for dentists in cytology technique. In a 1967 editorial in the Journal of the American Dental Association, it was recommended that oral cytology should be a part of every oral examination in which the dentist detects even the least suspicious lesion. Given that these recommendations were published approximately 30 years ago.

Aims and Objectives

1. To study the cytomorphological changes in oral cavity of different pathological lesions of chronic tobacco chewers in the rural population.
2. Assess the significance of the oral scrape cytology in oral mucosa of tobacco chewers.

Materials and Methods

A total number of 100 patients with oral mucosal lesions were screened taken at Department of Oral Medicine and Radiology, Swami Ramanand Teerth Rural Government Medical College and Hospital, Ambajogai. Patients with good oral hygiene without any history of tobacco chewing and without any pathological lesions were taken as controls (no. 50) to compare the clinical and socioeconomic status.

1. The selected patients included both males and females of all age groups and socioeconomic status.
2. The distributions for age, sex and socioeconomical conditions were examined for cases and controls.

The following data were also recorded for each patient.

1. Complete examination of the oral mucosa.
2. Measurement of interincisor/interarch distance (IID)/(IAD): The distance between upper and lower central incisors when the patient opens his mouth to the maximum extent and is measured by a Vernier calipers and recorded [5].

Oral scrape cytology was performed for all the 100 cases. It is important to scrape the edges of an ulcer as well as the floor, in order to obtain diagnostic material (Fig. 1) with blunt metal spatula. The process was repeated from additional areas if necessary. As soon as the specimen was obtained on the spatula, it was transferred to the glass slides which were kept readily labelled and numbered. The smears were prepared by spreading the material thinly and uniformly with a circular motion over the middle third of the slide. Then the glass slide was placed immediately in the fixative that was 95% ether alcohol mixture. Smears were stained with PAP stain.

Fig 1: Oral scrape cytology procedure

Scrape cytology results were divided in to three categories [6]:

1. *Negative scrape smear (normal or inflammatory smear)*: No epithelial abnormality was detected. If the patient reports resolving of the lesion (Fig. 2); no treatment was referred.
2. *Positive scrape smear (dysplasia positive smear):* Indicates that definitive cellular evidence of epithelial dysplasia or carcinoma may be present (Fig. 3). Referred for biopsy (excisional or incisional; scalpel, punch or needle; do not repeat scrape cytology) and histology to grade and stage the lesion.
3. *Atypical scrape biopsy (suspicious smear):* Indicates abnormal epithelial changes are present (these abnormal cells originate most often from a precancerous or cancerous lesion, although they may also develop in a benign inflammatory lesion, such as lichen planus) (Fig. 4). Referred for biopsy (excisional or incisional; scalpel, punch or needle) and histopathological examination to grade and stage the lesion or rule out cancer.

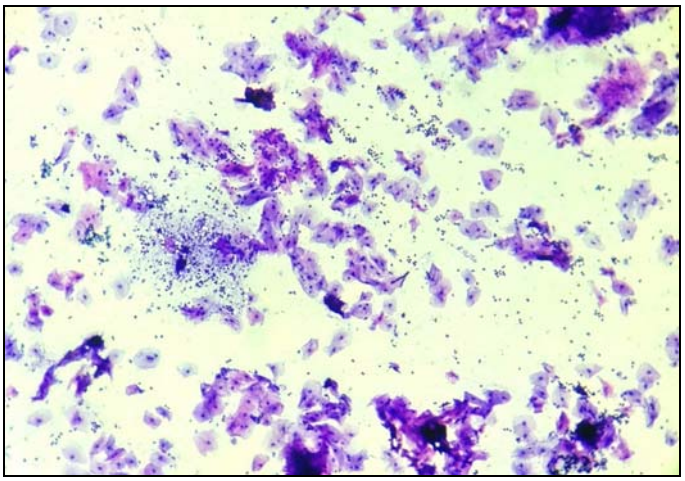

Fig. 2: Inflammatory buccal smear of OSMF case.

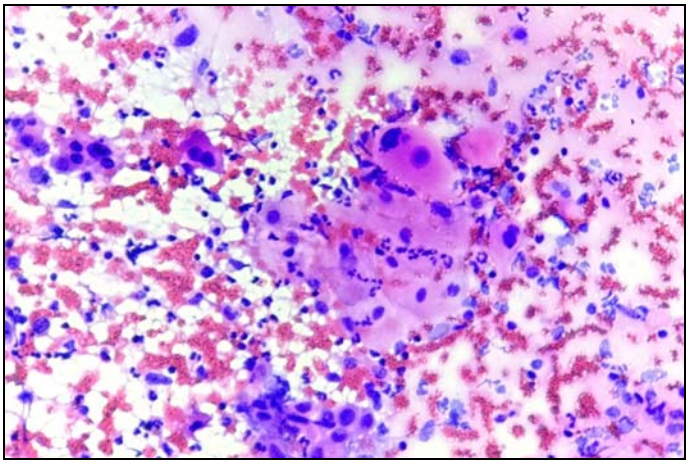

Fig. 3: Dysplasia.

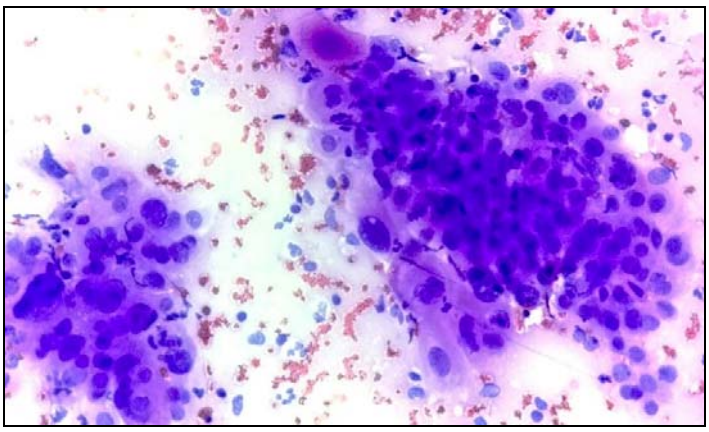

Fig. 4: Malignant smear.

Results

A total number of 100 patients with oral mucosal lesions were screened. In that the number of controls was 50 with no history of tobacco chewing. The 100 cases of our study were having the lesions, out of which 73 were males and 27 were females (Fig. 5). Overall picture showed male predominance.

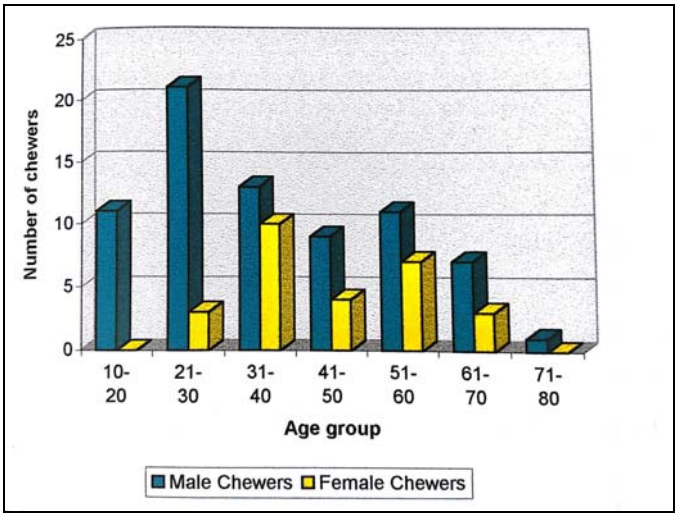

Fig. 5: Age-sex distribution of cases.

Majority (58/100) of the cases are below 40 years (Table 2) age group compared to the least number 11 cases were above 60 years age group, which indicates that the younger generation consuming more Gutkha than the older generation. The similar interpretation was expressed by other authors too [7]. Oral submucous fibrosis is four times more common in males than in females. It is more common in persons chewing ghutaka, pudi or having mixed habits [Ghutaka + pudi] i.e 23 (48.93%) cases followed by tobacco and lime users i.e 17 (36.17%) cases. The maximum cases were of oral submucous fibrosis [47%] followed by other benign lesions like stomatitis, glossitis and benign ulcers [32%] followed by clinically suspected malignant lesions [16%]. Five cases of leukoplakia were reported [Table 1].

Table 1: Effect of chewing methods on various clinical lesions.

Tobacco preperation	Oral submucous fibrosis			Leukoplakia			Malignancy		
	M	F	Total	M	F	Total	M	F	Total
Tobacco + Lime	10	7	17 [36.17%]	2	1	3 [60%]	9	3	12 [75%]
Ghutaka	3	-	3 [6.38%]	-	-	-	-	-	-
Mixed [Tobacco + Gutkha+ Pudi]	16	-	16 [34.04%]	-	-	-	-	-	-
Pudi [Tobacco + betel nut + lime]	4	-	4 [8.51%]	-	-	-	-	-	-
Tobacco + pan + lime	5	2	7 [14.90%]	1	1	2 [50%]	3	1	4 [25%]
Total	38	9	47 [100%]	3	2	5 [100%]	12	4	16 [100%]

Inflammation was the prominent cytological findings in 39 patients having duration of exposure up to 10 years. Maximum number of (8) malignancy cases were seen in patients having history of tobacco chewing for more than 20 years [Table 2].

Table 2: Relation of duration of tobacco chewing and cytological findings.

Duration of tobacco chewing	No of cases	Cytology					
		Negative	Inflammatory	Dysplasia			Suspicious/ Positive
				Mild	Moderate	Severe	
1-5	31	4	26	1	-	-	-
6-10	22	-	13	8	-	1	-
11-15	17	1	9	5	-	1	1
16-20	10	-	3	2	-	2	3
21-25	7	-	1	2	-	1	3
>25	13	-	1	3	2	2	5
Total	100	5	53	21	2	7	12

Table 3: Distribution of clinically suspected malignant lesions to anatomical sites.

Site	Male	Female	Total
Right cheek	4	1	5
Left cheek	3		3
Tongue	3	2	5
Floor of mouth	1	1	2
Palate	1		1
Total	12	4	16

Table 3 shows distribution of clinically suspected malignant lesions to anatomic sites. The maximum number of cases were of either right or left cheek i.e. in 8 [50%] cases followed by 5 cases [31.25%] of tongue and 2 [12.5%] cases of floor of mouth.

The 53% cases showed inflammatory lesion followed by 30% cases showing dysplasia of mild to severe grade. The 12 cases were suspicious or positive for malignant cells [figure 6].

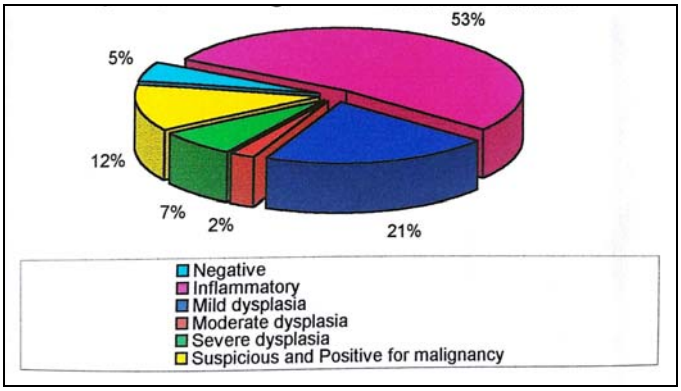

Fig 6: Cytological findings in chronic tobacco chewers [n= 100 cases]

On cyto-histo correlation of 13 proved malignant cases one case of severe dysplasia, the histological diagnosis came out as squamous cell carcinoma. The percentage accuracy of cytological diagnosis in relation to histopathological diagnosis

was 92.30%.

In our study, 48 (32%) oral submucous cases out of 150 cases of Gutkha chewers comprised 47, compared to 1 (02%) in controls (p-value 0.0005), which strongly supports the role of Gutkha etiology in OSMF.

Next common lesion was carcinoma with 16 (10.6%) cases and 0 (0%) in controls (p-value, 0.00005), which strongly supports the role of Gutkha etiology in carcinoma cases.

Leukoplakia occupied five (3.3%), lichen planus, nonspecific ulcers and moderate-severe dysplasia cases are amongst the others.

Discussion

Tobacco use is a common habit in India. It is practicised in variety of ways. This habit is more common in males than in females [8]. Gutkha chewing is the most common and popular form of ST (smokeless tobacco) use in India. In the present study this habit was more common in males [73%] than in females [27%] as observed by Sanghavi and Khanolkar, 1955.

In the present study scrape cytological smears from chronic tobacco chewers showed moderate to heavy inflammatory infiltrate with presence of bacteria and necrotic debris. Average of superficial cells was decreased in number in chronic tobacco chewers [46.2%] and increase in population of intermediate cells [50.1%] and there was average increase in the denucleated cells [16.3%] in chronic tobacco chewers. Similar observations were noted by Lahiri in 1971 [9].

As stated earlier the maximum cases were of oral submucous fibrosis [47%] followed by other benign lesions like stomatitis, glossitis and benign ulcers [32%] followed by clinically suspected malignant lesions [16%]. Five cases of leukoplakia were reported. Similarly Robert Ingram [10] in 1963 observed clinical presentation in 422 cases of leukoplakia [36.25%], chronic inflammation [28.20%], keratosis [10%] and ulcers [19%], malignancy [4.03%] and others benign.

In 1967 Pindborg, Gupta, Chawla studied 10,000 peoples from Lucknow, India and showed that males are more commonly affected than females and maximum number of cases occurred in age group of 20- 40 followed by 41-60. Our findings are similar with Pindborgs [11] study.

The present study shows the maximum number of suspected malignant cases [15] in the age group of 41-70 years. Similarly Henry Sandler [12] in 1962 studied 208 cases of malignancy. The proportion increased with ageing to a maximum 21.9% in 55-59 years and 33.8% in 60-69 years.

The maximum number of clinically suspected malignant lesions i.e. 5 [31.25%] over right cheek, 3 [18.75%] over left cheek followed by 5 [31.25%] in tongue, 2 [12.50%] at floor of mouth and 1 [6.25%] over palate. Similar findings were noted by many workers as given in table 4.

Table 4: Location of malignant lesions.

Study	Tongue %	Cheek %	Floor of mouth %	Palate %	Gingiva %
Sandler [12], 1962	20.00	8.00	21.00	14.00	11.00
R. Hutter [13], 1966	35.00	7.14	14.28	7.14	14.28
Shanta V. [14], 1959	22.10	51.60	19.50	3.00	3.00
R. Brown [15], 1965	10.00	42.00	-	-	15.00
Present study, 1998	31.25	50.00	12.50	6.25	-

Cytologically in present study, the 53% cases showed inflammatory lesion followed by 30% cases showing dysplasia of mild to severe grade. The 12 cases were suspicious or positive for malignant cells and 5% were negative smears. Similar findings were noted by Lahiri in 1971. Similar observation was also noted by Henry Sandler 1962, Hannah Peter[16] 1956, Cawson[17] 1960, Salvator Allegra[18] 1968, Tyler Folsom[19] 1972.

On cyto-histo correlation of 13 proved malignant cases one case of severe dysplasia, the histological diagnosis came out as squamous cell carcinoma. The percentage accuracy of cytological diagnosis in relation to histopathological diagnosis was 92.30%. The one case which was negative [7.70%] showed severe dysplasia. Similar findings were observed by many workers and comparative diagnostic accuracy and false negativity is as given in table 5.

Study	False negative [%]	Diagnostic Accuracy [%]
H. Peter [16], 1956	4	96
Cawson R.A. [17], 1960	19	81
H. Sandler, 1962	9	91
Folsom [19], 1972	13.9	86.1
Allegra [18], 1968	1	99
Sol Silverman [20], 1977	1	99
Present study, 1998	7.7	92.3

In socioeconomic status, a higher percentage of cases were in the lower income groups than the controls, with 79% of cases in the lower income group. The availability and cost of ghutkha or pudi chewing habits were more prevalent amongst the middle class peoples and younger age group. Income is inversely associated with precancer and cancerous conditions in Gutkha chewers [7]. High-income group, education and income were associated with decreased risk of oral premalignant lesions in our study.

Habitual chewing of pan masala, Gutkha is associated with earlier presentation of oral submucous fibrosis than betel quid use. Factors that may be responsible for these differences are the tobacco content, the absence of the betel leaf and its carotenes and the much higher dry weight of pan masala, Gutkha [21].

Despite the small number of negative cases, oral cytology can improve the accuracy of histology, and may be a useful screening tool for the diagnosis of oral neoplasia/dysplasia. Most false negatives have been associated with leukoplakia (hyperkeratosis) lesions. Therefore, in a persistent oral lesion, even though a cytological scraping may not be suspicious or characteristic of malignancy, a biopsy should still be strongly considered.

Summary and Conclusion

Our study supports the hypothesis that Tobacco chewing elevates the risk of oral submucous fibrosis, leukoplakia, cancer and oral precancers.

Oral scrape cytology has been found to be very helpful to detect precancer and cancerous conditions.

In comparison to the cervical Pap, oral scrape cytology system had a sensitivity of 85% and specificity of 89.6 to 100% in identifying precancer lesions.

Despite the fact that false negative reports are possible, it is the best and most useful screening tool for the diagnosis of oral neoplasia/dysplasia.

Cervical Pap smear is used as screening tool for diagnosing cervical malignancy. Early oral scrape cytology should be used for early diagnosis of oral neoplasms and dysplasia. Our study strongly recommends the use of oral scrape cytology as a screening tool in all suspicious intraoral lesions for rapid diagnosis.

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