Original Research Article

Clinical, Cytological and Histopathological Correlation of Oral Mucosal Changes in Gutka Chewers - A Prospective Study

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Abstract

Background: Gutka chewing is the most and popular form of smokeless tobacco use in India. Epidemiological studies showed a strong association between smokeless tobacco use and oral precancerous lesions, carcinoma and other oral mucosal lesions.

Aim and objectives: To know the patterns of clinical and pathological manifestations of oral mucosal lesions in Gutka chewers (patients) attending out-patient department of oral Medicine and Radiology at Govt. Dental college/ Hospital, Hyderabad, To assess the efficacy of the oral brush cytology in the detection of precancer and cancerous conditions of the buccal mucosa.

Materials and methods: Oral mucosal lesions diagnosed in patients of both sexes with Gutka chewing habit were taken at Dept. of Oral Medicine and Radiology, Govt. Dental College, Hyderabad. Total 800 cases were studied from 2006 to 2017 (10 years study), Oral Brush cytology Procedure with papanicolaou staining procedure was used. Brush cytology results were divided into 3 groups: Negative brush cytology (normal or inflammatory smear), Positive brush cytology (Dysplasia positive smear), Atypical brush biopsy (Suspicious smear).

Results: Out of 800 patients, males (80%) outnumbered females. Total no of controls 2246 with 51% were males. 75% were below 30 years of age. Higher percentage of cases (60%) belonged to the

lower socio economic groups. Clinical diagnosis of sub mucosal fibrosis and carcinoma corresponding to histopathological confirmation is almost 100%.

Conclusion: Oral brush cytology is useful in early detection of precancerous and cancerous conditions of the oral cavity lesions in Gutka chewers.

Key words

Gutka, Oral brush cytology, Buccal mucosa.

Introduction

Smokeless tobacco 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 other constituents in tobacco or added to tobacco [1, 2].

Worldwide variety of smokeless tobacco products like chewing Tobacco (plug, loose –leaf , twist , and pan known as betel quid, snuff dry for the nasal passages or moist for holding in mouth) have been in use. A link between betel quid chewing and oral cancer was suspected as early as 1908, and by the late 1960's several studies demonstrated the association between betel quid chewing and other forms of tobacco use with oral cancer in India.

In India tobacco use is estimated to cause 800000 deaths annually. There are 23 major tobacco growing districts in the country. Chewing tobacco is grown in Tamil Nadu, West Bengal, Uttar Pradesh and Orissa. India is the third largest consumer of tobacco next to china and USA [3, 4]. Gutka was introduced in Indian market in early 1970s. Gutka 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 [5]. It usually contain three basic ingredients, tobacco, areca nut and slacked lime (calcium hydroxide) which are, sealed in small attractive, colourful and inexpensive plastic or foil pack [5, 6]. Other substances like cardamom coconut and saffron flavours and colours are also added to make them more attractive [3].

Gutka is placed in between teeth, buccal mucosa and below the tongue and is gently chewed or sucked over a period of several hours [7]. Young people have become gutka consumers in large numbers. Pan masala and gutka are very convenient to carry and are easily available at low cost even in the remotest villages. Wide variety of mucosal changes has been noted in habitual users of smokeless tobacco. These changes are due to the irritants, toxins and carcinogens found in cured or burned tobacco leaves or may also be due to the drying effect, increased temperature or ph changes of the mucosa or immuno compromised individuals with already existing fungal or viral infections.

The following are significant mucosal changes noted in tobacco users [8, 9, 10], chewing tobacco leads to buccal mucosal lesions (sub mucosal fibrosis and oral cancer), cardiovascular diseases [8], CNS depression, dental and gum diseases, DNA damage, erythroplakia, oesophageal cancer, gastric mucosal damage, hypertension, hypokalemia, hyper salivation, salivary gland hyperplasia, in pregnant woman causing pre term delivery and low birth weight babies.

Chewing tobacco has been linked to colorectal cancer, gastric cancer, prostate cancer, endocrinological disorders, fetal malformations, and placental damage. According to the global health report 65% of all men use some form of tobacco (35% smoking, 22% smokeless tobacco, 8% is both). There are several recent reports, predicting and increasing oral cancer incidence in India. The prediction is based upon the observation of an increasing prevalence of oral sub mucosal fibrosis, especially in younger

individuals in 1963 the American dental associations house of delegates passed resolutions that acclaimed oral cytology as an excellent measure in prolonging life, and recommended oral cytology should be a part of every oral examination, in which the dentist detects even the least suspicious lesion.

There has been a reduction in no. of cervical cancer deaths in US, and most experts feel that this decline is due to the widespread use of the cervical pap smear, given the similarity in mucosal pathology between the oral cavity and the cervix it is predicted that the use of cytology in dentistry can also reduce the no.of oral cancers.

Aim and objectives

- To know the patterns of clinical and pathological manifestations of oral mucosal lesions in Gutka chewers (patients) attending out-patient Dept. of Oral Medicine and Radiology at Govt. Dental college/ Hospital, Hyderabad.
- To assess the efficacy of the oral brush cytology in the detection of precancer and cancerous conditions of the buccal mucosa.

Materials and methods

Oral mucosal lesions diagnosed in patients with gutka chewing habit were taken at Dept. of Oral Medicine and Radiology at Govt. Dental College, Hyderabad. Total 800 cases were studied from 2006 to 2017 (10 years study). The selected patients included both males and females of all age groups and socioeconomic status. The selection of cases for the present study is based on clinical examination of the patient and the clinical findings are confirmed by brush cytology and histopathological examination.

Patients with chronic mucosal lesions without history of Gutka chewing were taken as controls. Oral Brush cytology Procedure with papanicolaou staining procedure was used to prepare the cyto smears and correlated with histopathological examinations after performing biopsy.

Oral Brush cytology procedure:
The identified abnormal area in the oral mucosa is gently scraped and quickly, evenly spread over the glass slides and immersed in the fixative and stained with PAP method. After fixing the smears for 1 hour, washed with running tap water and stained with Harris' Hematoxylin for 4-6 minutes. Then subjected to washings with distilled water and dipped in 70% alcohol for two changes, followed dipping into 3% soln. of Ammonium hydroxide in 70% ethanol until the smear changes to blue color, again rinsed in 70% alcohol followed by 95% alcohol drippings' 5-10 times and stained with Orange G for 11/2 minutes, stained with EA50 after subjected to two dips in 95% alcohol. Kept for 2 minutes and again dipped in 95% alcohol two changes and mounted in DPX after clearing with xylene (**Figure – 1 to 5**).

Figure - 1: 23/M, white patches and thick plaques on dorsum of the tongue with 6 years gutka chewing history. Micro photograph showing thickened mucosa with inflammatory cells in connective tissue. Sub mucosal fibrosis.
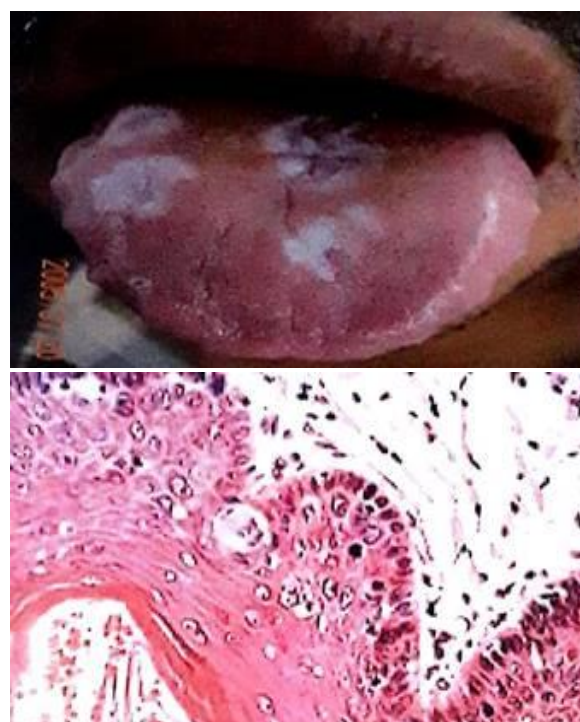
Figure - 2: 31/M, diffuse fibrosis of cheek – 2 years of Gutka chewing. Microphotograph showing sub mucosal deposition of densened avascular collagenous tissue with minimal inflammatory cells.

Figure - 3: Oral Brush Cytology. Cytological features of Dysplasia. Some of the cells at the centre show increased N/C ratio. Cluster of malignant cells with pleomorphism and hyper chromatic, irregular nuclei.
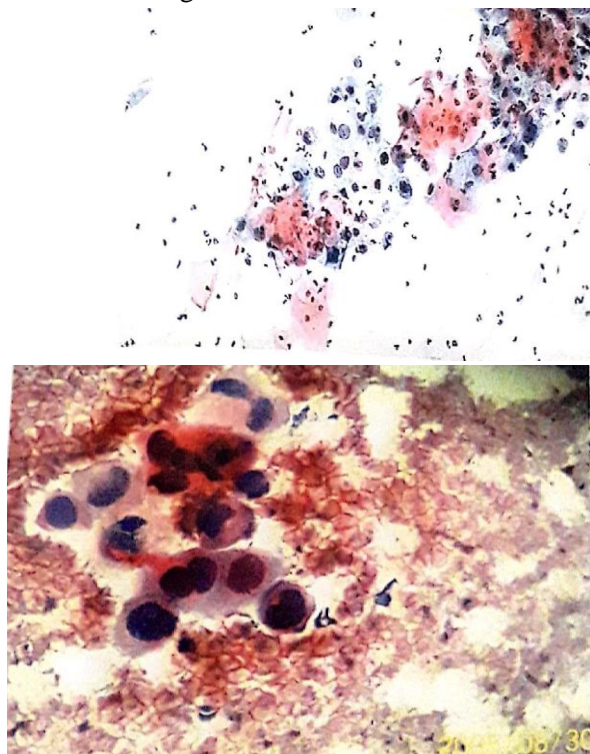

Figure - 4: Oral brush cytology procedure. Inflammatory Buccal smear of oral sub mucous fibrosis.

Figure - 5: 40/M ulcer in right lower alveolar border H/o Gutka chewing for 8 years.

Brush cytology results were divided into 3 groups

- Negative brush cytology (normal or inflammatory smear)

- Positive brush cytology (Dysplasia positive smear) referred to biopsy.
- Atypical brush biopsy (Suspicious smear) Referred to biopsy.

Results

In our study 62% are oral sub mucous cases, compared to 02% in controls (p- value0.0005) which strongly support role of gutka aetiology in Oral sub mucosal fibrosis (OSMF) (**Table – 1**). Next common lesion is carcinoma with 20% in cases and 0.1% in controls (p-value 0.00005) Leucoplakia 06%, lichen planus, 4%.

Table - 1: Individual lesion distribution.

Lesion	% in selected cases	% of controls
Oral sub mucosal Fibrosis	62%	02%
Oral carcinoma	20%	01%
Leukoplakia	06%	2.4%
Lichenplanus	04%	08%
Non-specific ulcer	04%	30%
Moderate to severe Dysplasia	04%	0.7%
Others	-	60%
Total	800	2246

Out of 62% of patients clinically suspected OSMF, 90% are inflammatory by brush cytology, 61%are confirmed by HPE.

Remaining were diagnosed as leucoplakia and moderate dysplasia (**Table – 2**). All the clinically suspected diagnosed carcinoma cases are confirmed by HPE, brush cytology could show severe dysplasia in 80%.

In our study, the minimum duration for oral sub mucosal fibrosis (OSMF) establishment in Gutka chewers is 1.5 years compared 2-5 years reported by Pindburg, et al. [5]. Majority of the lesions are identified in buccal mucosa as compared to other studies [5].

In clinically suspected cases of carcinoma low income group occupied 50%. 48% are below 30 years of age. Night quid habit and duration of chewing and dose quantity also showed significant effects on oral cancer occurrence (p-value 0.0005), 80% show strong brush cytology (chi square 98.86, p-value0.0005) histopathological correlation revealed 60%were well differentiated squamous cell carcinomas, 11% were moderately differentiated squamous cell carcinomas, 12% were poorly differentiated squamous cell carcinomas 105 were diagnosed as Verrucous carcinomas. 68% of the patients were unaware of the health hazards caused by Gutka chewing, 17% were having full knowledge, remaining 15 % was having inadequate knowledge.

Table - 2: Cyto-Histopathological correlation.

Clinical Diagnosis	Histopathology Diagnosis	Cytology diagnosis
Oral sub mucosal fibrosis - 496	496	Inflammatory:469 Inconclusive:26
Carcinoma-160	160	Inflammatory:21 Dysplasia:133 Inconclusive:6
Leukoplakia-48	48	Inflammatory:32 Dysplasia:16
Lichenplanus-32	32	Inflammatory:21 Non-specific:11
Non-specific ulcer-58	32 Dysplasia:32	Inflammatory:32 Dysplasia:32
Total-800	800	80

Discussion

Gutka chewing is the most common and popular form of ST (Smokeless tobacco) use in India. Extensive investigations in several cross sectional and prospective epidemiological studies showed a strong association between many forms of ST use and oral cancer [10]. Habitual chewing of pan masala / Gutka is associated with earlier presentation of oral sub mucosal 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 contents and much higher dry weight of pan masala/gutka [9, 10].

The finding that oral cancer is generally preceded by pre cancer facilitates the early detection of cancer. Although leukoplakia and sub mucosal fibrosis are demonstrated to be a high risk lesion and condition respectively, the difficulty lies in identifying which particular lesion would progress to cancer. The conventional approach of using epithelial dysplasia as a marker has been very helpful. In this aspect oral brush cytology can be used to detect such pre cancerous conditions in advance. False negative reports are possible with the oral brush biopsy technique. Persistent lesions should undergo tissue biopsy for definitive diagnosis [10].

In case of oral brush cytology and biopsy system the sensitivity of detection of oral epithelial dysplasia or squamous cell carcinoma was 77%,to 865 while the specificity was 89% to100%. The positive predictive value of an abnormal brush biopsy result (positive or atypical) was 44%, while the negative predictive value is 60%. Higher – income group (HIG) education and income were associated with decreased risk of oral premalignant lesions in our study [2, 3, 4]. Despite the small number of negative cases, oral cytology can improve the accuracy of histopathology and may be useful screening tool for the diagnosis of oral neoplasia/dysplasia. Most false negatives have been associated with leukoplakia (hyper keratotic) lesions, therefore in a persistent oral lesion, even though a cytologic scraping may not be suspicious or characteristic of malignancy, a biopsy should be still be strongly considered.

Our study supports the hypothesis that gutka chewing elevates the risk of oral sub mucosal fibrosis, leukoplakia, and oral cancer. Our study strongly recommends the use of oral brush cytology as a screening tool in all suspicious intra oral lesions for rapid diagnosis of pre cancerous lesions and cancers.

Conclusion

Gutka chewing is the most common and popular form of smokeless tobacco use in India. Epidemiological studies showed a strong association between smokeless tobacco (Gutka) use and oral pre cancerous lesions, carcinoma and other oral mucosal lesions.

Our study when compared with other studies have shown that oral sub mucous fibrosis a rare condition found mainly among young individuals in India.

The prevalence of dysplasia in our study was 45 studies have shown that prevalence of epithelial dysplasia in tobacco chewers ranged from 2-18%

Oral cancer is generally preceded by sub mucous fibrosis, epithelial dysplasia and leukoplakia. Oral brush cytology has been found to be very helpful to detect precancerous and cancerous conditions.

In comparison to the cervical PAP, oral brush cytology had a sensitivity of 85% and specificity89% to100% in identifying precancerous lesions.

Oral brush cytology can be used for early diagnosis of oral neoplasms and dysplasia.

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