

Original Article

CLINICAL AND CYTOLOGICAL EVALUATION OF ORAL MUCOSAL LESIONS IN PAN-CHEWERS FROM LAHORE, PAKISTAN

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ABSTRACT

Objective: The current study was performed to assess demographic, clinical and cyto-morphological features of oral mucosa in pan chewers.

Methods: This was a cross-sectional, survey. After taking relevant history and informed consent, a clinical examination was performed on 300 pan users from Lahore city. Cytological smears were obtained to be stained with Haematoxylin and Eosin, Giemsa and Pap's stain. All the smears were examined under light microscope to evaluate cyto-morphological changes. Data were analyzed with the help of SPSS.

Results: The average age for all subjects was 32.28 ± 0.59 and 95% were males. The majority were from poor social background and some of them gave history of smoking and alcohol etc. Many had discolored and sensitive teeth, bleeding gums and painful oral lesions. The mean duration of pan chewing was 11.47 ± 0.39 years and majority of subjects used to chew 1-5 pans /day. On naked eye examination of oral cavity, leukoplakia, erythroleukoplakia, oral submucous fibrosis, erythroplakia, and growth were seen in 8.7%, 2.3%, 1.7%, 1%, and 1% subjects respectively. On cytological examination epithelial dysplasia, oral squamous cell carcinoma, keratosis, inflammation, bacterial and candidal loads were observed in 57.7%, 1%, 54.3%, 74.7%, 48.7%, and 7.7% subjects respectively.

Conclusion: Poor orodental hygiene, inflammatory conditions, pre-malignant lesions like leukoplakia, erythroplakia, epithelial dysplasia and malignancy of oral mucosa were alarmingly common in pan chewers from Lahore, Pakistan who belonged to the poor socioeconomic background and do not seek medical advice.

Key Words: Oral Mucosa, Leukoplakia, Keratosis, Oral submucous fibrosis

INTRODUCTION

Pan or betel quid chewing is widespread in many parts of Asia and the migrants from this area have transferred this abuse from Asia to all parts of the world.¹ It has been estimated that 10% of world's population (600 million) is addicted to betel quid chewing across the globe.² People around world are quite familiar with the name of betel quid. Unfortunately, inadequate attention is given to its varied components. The key ingredients of 'betel quid' (pan – a term more known to Southeast Asians) are betel leaf, areca nut, slaked lime, and tobacco.³

Additional constituents such as cardamom, aniseed, sweeteners (coconut, dried dates), and essences (rose petals, mint, menthol) are added to enhance its flavor.⁴ In Pakistan, pans are readily available at 'pan stalls'. Pan sellers often apply a mixture of slaked lime, areca nut and a variety of other flavoring ingredients to a betel leaf and wrap into a rough triangle shape 'pan'.⁵

Consumption of betel quid and a number of its constituents like areca nut and tobacco are detrimental to oral mucosa as they contain numerous toxic compounds, heavy metals and other hazardous by-products including reactive oxygen species which are established mutagens and carcinogens.^{6,7} A large number of lesions like leukoplakia, erythroplakia, oral submucosal fibrosis, epithelial dysplasia, and oral cancer are caused by habitual pan chewing.⁴ This study was conducted to describe the demographic

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attributes of chronic pan users and to demonstrate the variety of abnormalities found in them on clinical examination and cytological evaluation of their buccal mucosa.

METHODS

This was a cross-sectional descriptive type of study, conducted in Lahore city. The cytological evaluation of oral mucosal smears was carried out at the Department of Morbid Anatomy and Histopathology at the University of Health Sciences (UHS), Lahore from April 2011 and December 2012. The study was performed after approval by the Ethical Review Committee of the UHS Lahore. After taking informed consent, 300 subjects were included in the study, majority of them were approached at pan stalls/shops where they came to purchase betel quid. However, some of the samples were also obtained from Mayo Hospital and Punjab Dental Hospital Lahore. Thus, the sampling technique used in this study was a non-probability convenience sampling. The criteria for sample selection included both male and female habitual pan chewers of all ages, who have been chewing pan for at least the past 5 consecutive years. Occasional pan chewers and inadequate smears were excluded from study. Related socio-demographic information (name, age, gender, history of other risky habits, average number of pan consumption in a day and type of pan used, etc) was recorded. In order to make it easy to understand, all subjects were categorized (on the basis of frequency of intake) into light, moderate and heavy pan users. Those who were consuming upto 5 pans per day were categorized as 'mild pan chewer'. Similarly, more than 5 but not exceeding 10 per day were graded as 'moderate' whereas those exceeding 10 were kept in a separate category of 'heavy' pan users. After history taking, relevant examination of oral cavity and neck lymph nodes was performed. All subjects were requested to properly rinse their mouth. Oral buccal mucosa was scrapped with the help of a wooden spatula and smeared on to a

glass slide. The smears were fixed with ethyl alcohol (70 parts) and ether (30parts) solution for 30 seconds. After air-drying the slides, these were then subjected to staining procedures following the staining protocol of Eosin & Hematoxylin, Giemsa and Papanicolaou's stains. After staining, slides were ready to be viewed under light microscope. All relevant information was transferred to a specially designed proforma. After compilation, data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 18.0. Frequency and percentage were given for qualitative variables. Means \pm SD were given for quantitative variables.

RESULTS

This study included 300 pan chewers around various pan shops located within Lahore were included. Mean age for habitual pan chewing was calculated to be 32.28 ± 0.59 (Range: 17 – 70) years. A large number of them were males ($n= 285$, 95%) and only 15(5%) were females. The majority (53%) belonged to the lower socio-economic class. The largest percentage of pan chewers happened to be laborers as shown in Figure-1.

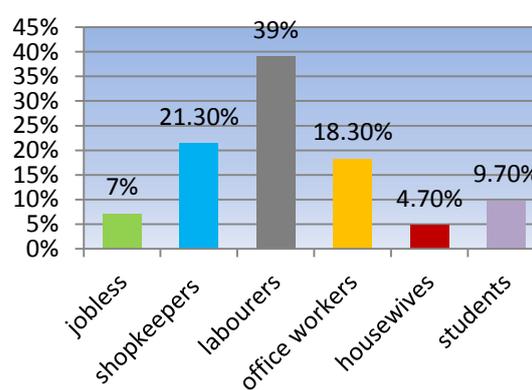


Figure-1 Occupations of the pan chewers

When the oral cavity was thoroughly examined, majority of the subjects ($n=148$, 49.3%) were found to have discolored teeth. 74 (24.7%) pan chewers complained of hot and cold sensation. Bleeding gums were observed in 71 (23.7%) and painful oral mucosal lesions in 7 (2.3%) individuals. A

large number of habitual pan chewers were also found to be indulged in cigarette smoking (n=103, 34.3%). Alcoholism was observed in 3.3% (n=10) as shown in Figure-2.

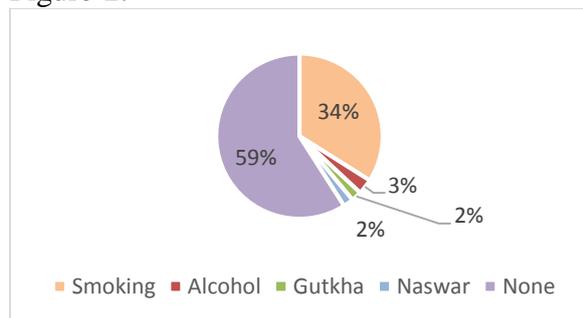


Figure 2. Additional habits among habitual pan chewers

When mean for the total duration of quid chewing was calculated among the study population, it was found to be 11.47 ± 0.39 (Range: 5 – 50) years. The average consumption of betel quid in a day was classified into mild (1 – 5 pans/day), moderate (6 – 10 pans/day) and severe (>10 pans/day). More than half of the study group fell into the mild class of pan users (n=157, 52.3%), the detail is shown in Table-1.

Category	Frequency	Percentage
Mild user (1 – 5 pans/day)	157	52.3%
Moderate user (6 – 10 pans/day)	100	33.3%
Severe user (>10 pans/day)	43	14.4%

Table-1. Pan consumption per day in 300 subjects

In our study, we found that majority of pan users were in the habit of adding tobacco to their pans (n=282, 94%), however there were only few (n=18, 6%) who used to get themselves simple pans without adding tobacco. Nearly half of the study population (n=138, 46%) were in the habit of keeping pans in their left oral vestibule whereas another large proportion (n = 125, 41.7%) used to place pan in their right vestibular

area. However, it has been observed that only a few persons were placing it on either (left/right) sides of their oral cavity. When oral cavities of these subjects were thoroughly examined, various oral mucosal lesions were encountered as given in Table-2 & 3.

Oral Mucosal Lesions on naked eye examination	Frequency	Percentage
White lesions	26	8.7%
Red-white lesions	07	2.3%
Fibrotic mucosa	05	1.7%
Red lesions	03	1.0%
Ulcerated growth / mass	03	1.0%

Table 2. Oral mucosal lesions on naked eye examination

Oral Mucosal Lesions on Microscopy / Cytology	Frequency	Percentage
Epithelial dysplasia	173	57.7
- Mild	65	21.7
- Moderate	75	25
- Severe	33	11
Squamous cell carcinoma	03	1.0
Keratosis	163	54.3
Inflammation		
- Lymphocytes predominant	224	74.7
- Lymphocytes and Eosinophils	76	25.3
- Mixed (acute and chronic)	70	23.3
- Eosinophils	56	18.7
- Neutrophils	41	13.7
- Neutrophils	09	3.0
Bacteria	146	48.7
- Minimal to moderate load	111	37
- Heavy load	35	11.7
Candida	23	7.7
- Minimal to moderate load	17	5.7
- Heavy load	06	2.0

Table 3. Oral mucosal lesions on microscopy/cytology

DISCUSSION

The current study discusses some initial cyto-morphological transformations that arise within the oral cavity of chronic pan users. Our study comprised of 300 pan users from different areas within Lahore. The majority of them were males while only few

were females. This prevalence of males in this study was attributed to the fact that the samples taken from various pan shops that are usually seen crowded with males. Women in this part of world, are usually reluctant to visit these pan shops. The reason behind this hesitancy is certainly religious, social and cultural norms. Secondly, males population is more inclined towards habitual pan and tobacco chewing. Several previously published studies have also supported the preponderance of male pan users which is in accordance with our study.⁸

Misra and colleagues reported that their study population mainly consisted of those pan users, who have been daily consuming a minimum of 10 or more than 15 pans, for longer durations of time. They found that chronically pan chewing habits could predispose oral mucosa to early dysplastic changes, which can then act as a precursor for precancerous and frank malignant lesions.⁹ Pan war and friends conducted a study with similar findings. They confirmed that oral dysplastic changes are more frequently observed in those individuals who had been practicing tobacco pan chewing continuously for more than 5 years.¹⁰ Rubinstein conducted his study on United States population. He observed that habitual tobacco chewing is becoming prevalent in his study population and is gradually on rise. This continuous practice of tobacco chewing results in oral epithelial dysplasia that later on can take the form of epithelial malignancy.¹¹ Wen and colleagues showed that habitual chewing of betel quid may cause irritation of the oral mucosa. In addition chronic inflammation, oxidative stress, and cytokine production due to pan chewing may further damage the mucosa. This damaged mucosa can then harbor changes induced by carcinogens produced by long term use of betel quid.¹² Their study also confirmed the role of genes in malignant and premalignant lesions. Carcinogens produced by betel quid components may compromise the genomic stability of tissues, the key for malignant

transformation in oral epithelial cells. Another study using Next-Generation sequencing revealed a novel single nucleotide polymorphism (SNP) in multiple cancer-related genes (AR, BRCA1, IL8, and P53). Yadav and his colleagues reported the strong correlation of areca nut derived arecoline with single nucleotide polymorphisms in these above-mentioned genes. Similarly, novel deletions in APC, BRMS1, CDK2AP1, CDKN2B, GAS1, IGF1R and RB1 and novel insertions in BRCA2, MSH6, RASSF1, IGF1R, BARD1, and CCND2 were also found associated with areca nut.⁽¹³⁾ There are many other studies which also support the notion as well as provide clear evidence for the carcinogenic and pathogenetic role of betel-quid and its components.¹⁴⁻¹⁷

In our study, the habit of pan chewing was found to be 19 times more prevalent in males individuals as compared to women and the young adults were the commonest age group as depicted by the average age of 32 years. More than half of the pan chewers were from low social background. A few of them gave a history of cigarette smoking, alcohol, and other similar additions. Many of them had discolored teeth which were sensitive to hot and cold and they also had bleeding gums and painful oral lesions despite that majority of them were not concerned with their oral health. They were having pan chewing habits for more than a decade on the average and nearly half of them used to chew 1-5 pans /day. The remaining half had been chewing more than 5 pans/day. Although the calculations of pan chewing/day were based on subject's recall yet can be somewhat arbitrary. Subjects could not mention exact number of pans taken in last 3 days or last 24 hours. So the authors asked them whether they were taking 1-5, 6-10 or more than 10 pans/day and all of the subjects could segregate themselves into one of these categories. That's why we assume it as one of our study limitations and suggest that exact quantification of pan consumption may

require more strenuous efforts in the form of a prospective study like Little et al.¹⁸

The findings of clinical examination of oral cavity like white lesions, mixed red white, fibrotic, red lesions and ulcerated growth/mass were seen in a few of the subjects whereas cytological examination revealed epithelial dysplasia, keratosis, inflammation, bacterial and candidal loads were observed in 57.7%, 54.3%, 74.7%, 48.7%, and 7.7% subjects respectively. Last but the most important was the incidental diagnosis of 3 cases of oral squamous cell carcinoma in so-called 'healthy' pan chewers. Our findings are consistent with those of previous investigators.^{6, 19-23} Nonetheless, these findings are ominous, most of the pan chewers neither took notice of them and nor sought medical advice. This fact is even more worrisome and needs international, national and volunteer efforts by organizations and individuals to embark on effective campaigns against this deadly addiction leading to bans and legislation and ultimately reduction in the pan consumption.

CONCLUSION

Poor orodental hygiene, inflammatory conditions, white & red lesions, epithelial dysplasia and malignancy of oral mucosa were alarmingly common in pan chewers from Lahore, Pakistan who belonged to the poor socioeconomic background and do not seek medical advice.

ACKNOWLEDGMENT

Authors are grateful to all the pan shop owners, administration of Mayo Hospital Lahore and University of Health Sciences and Mr. Sameer Anjum, (Laboratory Technician at the Department of Morbid Anatomy and Histopathology), UHS Lahore.

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