SCREENING OF TOBACCO USERS FOR POTENTIALLY MALIGNANT DISORDERS USING TOLUIDINE BLUE STAINING

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ABSTRACT

Objectives: To detect early signs of potentially malignant disorders (PMDs) in tobacco users through toluidine blue staining screening and to evaluate the diagnostic efficacy of toluidine blue staining in screening tobacco users for PMDs.

Methodology: This descriptive cross-sectional study was conducted at the Peshawar Dental College with effect from November 5, 2021, to September 25, 2022. A total of 300 tobacco users (either smokers or Naswar dippers) were included in the study, regardless of their age and gender. Whereas, those tobacco users who were both smokers and Naswar dippers were excluded. Every tobacco user was screened out for the presence of oral mucosal changes with a naked-eye oral examination followed by toluidine blue staining screening.

Results: The majority of tobacco users screened out for PMDs were male (92%) with a male-to-female ratio of 11.5:1, and most of them were between the ages of 51 and 70 years. Out of 300 tobacco users, more than half were Naswar dippers (63%). While the screening results showed that out of the total 300 tobacco users, 35 had positively stained, and more than two-thirds of them were Naswar dippers (68.6%),. On histological examination of 35 toluidine blue positive samples, we noted dysplastic changes in 29 (83%) cases, where 16 (55%) individuals showed moderately dysplastic changes and 11 (38%) displayed mildly dysplastic changes.

Conclusion: Tobacco users, especially Naswar dippers, were at high risk for PMDs. In addition, toluidine blue staining emerges as a useful diagnostic tool for the early detection of PMDs.

Keywords: Toluidine Blue; Tobacco; Naswar; Potentially Malignant Disorders; Oral Squamous Cell Carcinoma

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is becoming an increasingly emerging global health issue, with the occurrence of 355,000 new cases annually. It is the 16th most prevalent malignancy and the 15th leading cause of death worldwide.[1,2] Almost two-thirds of the new oral cancer cases are reported from developing countries like Pakistan, India, Sri Lanka, and Bangladesh. Oral cancer shows a very high incidence in Pakistan, representing the most prevalent type of cancer in males, whereas in females it stood at the 2nd position.

Social behaviors such as habits, literacy level, and access to healthcare facilities have a notable influence on the occurrence of oral cancer. The incidence of OSCC is greater in males than females. The major reason for the widespread prevalence of OSCC and other PMDs in Southeast Asian countries is the high consumption of smoked and smokeless tobacco.[4] More than 90% of global smokeless tobacco products are consumed in Southeast Asia. Smokeless tobacco is utilized in a variety of forms, including snuff (Naswar), paan, mava, khaini, maishiri, etc. Naswar is extensively used by the Pathan and Afghan communities. Naswar is made from ash, dry tobacco leaves, and lime. Most people place Naswar in their buccal sulcus, allowing the active agents to be absorbed via the oral mucosa. It is much cheaper than cigarettes.[5,6]

It has been estimated that almost sixty known carcinogens are present in smoked tobacco, while more than 20 carcinogens are found in smokeless tobacco.[1] OSCC can be preceded by PMDs, defined as morphologically altered tissue that has more potential to become malignant than its normal counterpart. Clinically, PMDs may present as white or red lesions, chronic indurated ulcers with raised margins, and exophytic or verrucous growths. The histological section shows varying degrees of dysplasia[8-10]. Early diagnoses of PMDs, especially in high-risk tobacco users, are considered an effective strategy for decreasing both mortality and morbidity associated with OSCC.[11] A 34% reduction in

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mortality rate was found in a randomized trial in India where the screening of high-risk tobacco and alcohol users was performed.[12]

Previous studies showed that a variety of methods are available for oral cancer screening, like a complete oral visual examination, toluidine blue staining, chemiluminescence (Vizilite), tissue fluorescence imaging (Velscope), tissue fluorescence spectroscopy, and brush biopsy.[13,14] The clinical application of toluidine blue was first reported in 1963 by Richart, who stained uterine carcinoma in situ. It is cost-effective with a lower false negative rate and also has the potential to be used as a tool for mass screening.[8,15] It has a sensitivity and specificity of about 86.36% and 76.9%, respectively.

Toluidine blue is chemically tolonium chloride. It stains only dysplastic cells but does not affect normal cells because of its staining quality in the acidic components of the tissue, like carboxylates, sulfates, and phosphate radicals, and stains only DNA and RNA.[8,17] Thus, it shows that malignant cells have greater DNA and RNA content.[17,18]

Oral cancer screening is defined as "a process through which a clinician accesses an asymptomatic patient to determine the risk of having a PMDs or malignant disease."[19] Screening is performed in three different ways. "Population-based screening," in which a specific community is evaluated for the existence of OSCC, "Opportunistic screening" is where those patients who come to health care providers for some other reason are screened out. "Targeted screening" occurs when only high-risk tobacco users are accessed.[20]

The rationale of the current study is to highlight the need for future public health efforts to intensify screening for high-risk individuals.

METHODOLOGY

This descriptive cross-sectional study was conducted at the Peshawar Dental College from 10th November 2021 to 25th September 2022. A total of 300 individuals who were tobacco users either in the form of smoking or Naswar dipping were included in the study regardless of age and gender. Those individuals who were both smokers and Naswar dippers were excluded. Every individual was screened for the presence of oral mucosal changes with naked eye oral examination followed by toluidine blue staining. The patient was asked to rinse the mouth twice for 20 seconds with water to remove debris. Then 1% acetic acid was applied for 20 seconds to remove saliva. After that 1% toluidine blue swab was applied all over the oral mucosa, waited for one minute and then the patient rinsed mouth twice again with 1% acetic acid. The interpretation may be dark blue stain (positive), pale blue (doughtful), no colour change (negative). Any lesion that appears positive on toulidine blue stain was confirmed with scalpel biopsy.

The collected data was recorded on a specially designed performa and analysed by using SPSS. The results were presented in the form of tables and charts.

RESULTS

Out of 300 tobacco users, majority were males (92%) with a male to female ratio of 11.5:1. The mean age of the patient recorded was 50 years and age range was from 31 to 78 years. Most of the individuals screened were the age of 51 to 70 years (Table 1).

Among these, more than half were Naswar dippers (63%) as compare to smokers. (Figure1). Regarding toluidine blue staining, only a minority i.e 35 individuals had positive stain and more than two third of these were Naswar dippers (68.6%) (Table 2).

According to our analysis, the histological examination of 35 toluidine blue positive samples indicated dysplastic changes in majority of cases 29 (83%). Out of these 29 cases,16 (55%) individuals showed moderate dysplasia while 11(38%) had mild dysplastic changes (Table 3).

DISCUSSION

In the ongoing study, the high risk individuals who were using tobacco (either smoked or smokeless) were screened for the presence of PMDs. Out of total 300 individuals, 276 were males while only 24 were females

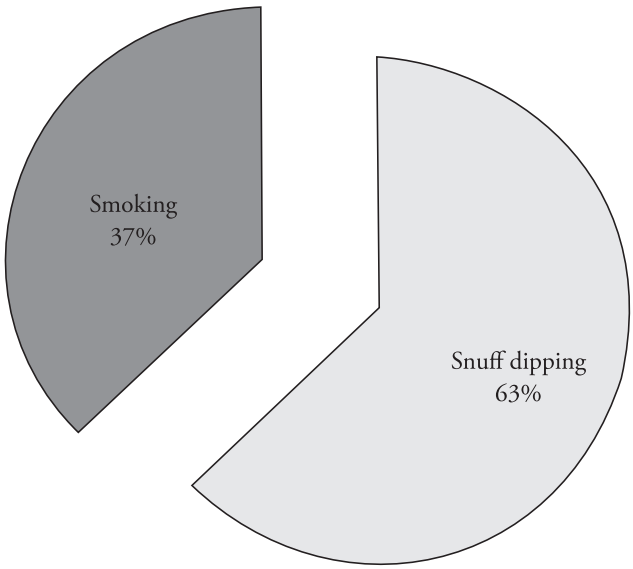

Figure 1: Habit pattern noted among tobacco users (n=300)

Table 1: Demographic distribution of tobacco users (n=300)

Age distribution	
Groups	N (Percentage)
30-50 years	142 (47.3%)
51-70 years	150 (50%)
Above 70 years	8 (2.7%)
Gender distribution	
Male	276 (92%)
Female	24 (8%)

Table 2: Distribution of life style risk factors and the concordance of toluidine blue results.

Category	Smokers	Naswar dipping	Total
Toluidine blue Positive	11 (31.4%)	24 (68.6%)	35 (11.7%)
Toluidine blue Negative	100 (37.7%)	165 (62.3%)	265 (88.3%)

Table 3: Relationship between positive toluidine blue test and histological diagnosis

Dysplasia			No dysplasia	Total
29 (83%)			06 (17%)	35 (100%)
Mild dysplasia	Moderate dysplasia	Severe dysplasia		
11 (38%)	16 (55%)	02 (7%)		

with a male to female ratio of 11.5: 1. Abnormal tendency was found more in males. This finding is in accordance with other studies.[6,10, 21]

In relation to age, the majority of individuals screened (39%) were above the age of 50 years. This corresponded positively with the results of studies done worldwide.[3,6] With increasing age, the susceptibility of an individual to have genetic mutations also become high due to increased exposure of a person to behavioral and environmental risk factors.[3]

The present study revealed that the major proportion of tobacco users were Naswar dippers (63%) as compare to smokers (37%). The reason is that our sample included population who belonged mostly to Khyber Pakhtonkhwa Province of Pakistan. The habit of Naswar dipping is considered a cultural practice in this region.[5] Moreover, Naswar is much cheaper than cigarette due to its exemption from net tax and is readily available everywhere. Regarding toluidine blue staining, the stain was positive in 35 out of 300 participants screened. Among those 35 individuals having positive toluidine blue stain, the majority were Naswar dippers (68.8%). These results are in accordance with other studies done in this part of the world.[6,22] An explanation of more carcinogenic potential of Naswar may be due to the fact that Naswar contains lime and ash which increases its toxicity. Lime increases the pH (alkalinity) of Naswar. Higher nicotine level combined with high pH produces more cravings that lead to repeated use of Naswar and hence higher carcinogenic exposure. Furthermore, Naswar causes erosion of the tissues which make them more susceptible to undergo malignant change.[22]

Allegra et al[23] conducted a comparison between the clinical and histological outcome of toluidine blue stain and found that dysplasia was present in 96.2% cases who gave positive stain with toluidine blue. In another study performed by Kumbhare and Taralekar[24], a comparison was done between Vizilite and toluidine blue for PMDs, 87% cases were stained positive for toluidine blue showing mild to moderate dysplasia. In the present study we found 83% dysplastic changes in toluidine blue stain positive cases, which are closely related to above studies.

CONCLUSIONS

Our study findings indicated that toluidine blue is a useful diagnostic adjunct for the early detection of PMD and OSCC in high risk population especially in developing countries like Pakistan as it is economical and easily available. Screening provides not only the advantage of early diagnosis but it also improves the patient's awareness about the relationship between oral cancer and its risk factors like tobacco and alcohol. Moreover, patients with oral cancer symptoms usually visit medical doctors rather than dentists. Therefore, doctors should also be trained in oral cavity examination and toluidine blue

screening of high risk population.

RECOMMENDATIONS

It is highly recommended that toluidine blue screening should be routinely performed in patients having high risk factors and belonging to regions where use of smokeless tobacco particularly Naswar is very high. Studies with a larger sample size should be performed in future so that the use of toluidine blue becomes more widespread.

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Author's Contribution

NA conceived the idea, designed the study and performed data analysis. SM, MAK helped in designing the study, performed data analysis and helped in the write up of the manuscript. ZN, MHA and AS helped in the write up of the manuscript. All authors made substantial intellectual contributions to the study.

Conflict of Interest

Authors declared no conflict of interest

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None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.