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Research Article

Prevalence and Clinicopathological Features with Oral Biological Correlates of Potentially Malignant Oral Disorders in Individuals with High-Risk Oral Habits: A Study from Pakistan

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ABSTRACT

Background: Potentially malignant oral disorders (OPMDs) are those that have the potential to develop into oral squamous cell carcinoma. Tobacco, areca nut, gutka, and related products are extensively used and play a major role in the burden of these disorders in Pakistan.

Objective: To assess the prevalence, clinic pathological features, and oral biological associations of OPMDs in patients with high-risk oral habits in Pakistan.

Methods: A descriptive cross-sectional study was conducted with 250 individuals with high-risk oral habits. A structured questionnaire and oral examination were used to record demographic characteristics, habit history, and clinical findings. Where indicated, histopathological assessment and biomarker analysis were performed, including p53 and Cyclin D1 expression. Data were analyzed with SPSS version 26.

Results: In 108 (43.2%) participants, OPMDs were identified. Oral submucous fibrosis (41.7%) and leukoplakia (31.5%) were the most frequent lesions. The most common histopathological changes were mild dysplasia (45.4%). Positive expression of p53 and Cyclin D1 was seen in 63.9% and 56.5% of the cases, respectively. There were significant relationships between the expression of biomarkers and dysplastic severity ($p < 0.05$).

Conclusion: High-risk oral habits are common among people with OPMDs in Pakistan. The strong correlation between p53 and Cyclin D1 expression and dysplastic severity indicates that these proteins may play a role in assessing risk and detecting early signs of malignant transformation.

Keywords: Oral Potentially Malignant Disorders, Oral Submucous Fibrosis, Leukoplakia, Tobacco Use, P53, Cyclin D1, Oral Cancer, Pakistan.

INTRODUCTION

Oral potentially malignant disorders (OPMDs) are a group of oral mucosal lesions and conditions with a higher risk of developing into oral squamous cell carcinoma (OSCC). Leukoplakia, erythroplakia, oral submucous fibrosis, and oral lichen planus are all important public health issues because they have the potential to become malignant. Recent studies suggest that the clinicopathological features of OPMDs vary significantly between tobacco users and non-users, suggesting that behavioral risk factors play a significant role in the occurrence and progression of OPMDs (1). In addition, cytological studies have revealed a significant incidence of human papillomavirus (HPV) infection in the oral cavity of patients with potentially malignant lesions (PML), indicating that viral involvement may be linked to the development of epithelial dysplasia and precancerous changes in the oral mucosa (2).

Oral mucosal diseases are complex diseases with multiple biological and environmental factors involved in their pathogenesis. Changes in the oral tissues may be indicative of underlying pathological processes that can predispose a person to malignant transformation. Clinicopathological variables that have been evaluated in research studies have shown strong correlations between tissue reaction and disease severity (3). Epidemiological studies in other parts of the world have reported an increasing disease burden of OPMDs and oral cancer, and there is a need to continue to monitor and investigate the situation in each region to understand better the patterns of disease and associated risk factors (4). Early detection of OPMDs is an important part of oral cancer prevention. Clinical examination and histopathological assessment for the recognition of suspicious oral lesions allow early intervention and better patient outcomes.

Improvements in the diagnostic techniques have improved the ability of the clinician to differentiate potentially malignant lesions from benign oral lesions, which has increased the opportunities for disease control before malignant transformation (5). Although these developments have occurred, knowledge of the risk factors and prevention strategies for oral cancer is still poor in many populations, including Pakistan, where research has shown that even those with educational backgrounds related to health care have significant gaps in knowledge (6). The use of molecular markers in the assessment of OPMDs has improved the understanding of their biological behavior and malignant potential. In this group of biomarkers, p53 has been identified as an important one because of its ability to control the cell cycle and preserve genomic stability.

There is evidence of an association between alterations in p53 expression and an increased risk of malignant transformation, and both tissue p53 protein expression and serum p53 antibodies have been shown to predict progression from OPMDs to OSCC (7). These results highlight the growing relevance of combining molecular diagnostics with traditional histopathological analysis. Besides p53, Cyclin D1 has also received a lot of attention as a marker of cellular proliferation and neoplastic progression. Higher levels of Cyclin D1 have been found in oral squamous cell carcinoma and possibly in premalignant oral disorders than in normal oral mucosa, suggesting that molecular changes may occur during the early stages of carcinogenesis (8). The knowledge of these molecular changes is crucial to identify high-risk patients and to develop targeted preventive and therapeutic approaches.

Oral malignancies also present and progress differently across demographics. Comparative studies comparing clinicopathological features of OSCC in younger and older patients have shown significant differences in disease patterns, exposure to risk factors, and tumor behavior (9). Likewise, recent studies have reported a worrying rise in the incidence of oral cancer among young adults, leading to the search for new risk factors, indicators, and molecular markers to account for this new trend (10). These observations highlight the need for early detection and surveillance of OPMDs in all age groups. In addition to the behavioral risk factors, environmental exposures can play a role in oral carcinogenesis. There is emerging evidence that changes in serum levels of trace and toxic metals are related to the development of oral cancer and that interaction between environmental contaminants and established carcinogens may occur (11). In addition, the spread of oral malignancies is often associated with regional lymphatic spread, and it has been reported that a significant number of patients with clinically negative neck examinations have occult lymph node metastases, highlighting the need to prevent the progression of the disease at the premalignant stage (12).

The current knowledge acknowledges that OPMDs are dynamic disorders that are affected by a complex interaction of genetic, molecular, behavioral, and environmental factors. Modern reviews have highlighted the importance of better risk stratification models, which could combine clinical, histopathological, and molecular features to better predict malignant transformation (13). Tobacco exposure remains one of the most important factors in oral carcinogenesis in South Asian populations, and tobacco-related lesions have unique clinicopathological characteristics, necessitating targeted tobacco prevention and control efforts (14). There have been recent technological advancements that have brought new methods for screening and early detection of OPMDs.

Artificial intelligence (AI) based mobile screening applications have shown

encouraging diagnostic accuracy in detecting potentially malignant oral lesions in both urban and rural areas, providing a potential opportunity to improve access to oral health care services in resource-constrained countries like Pakistan (15). At the molecular level, the transcription factors that regulate EMT, such as ZEB1 and ZEB2, have been associated with the progression of tumors and the severity of the histopathological characteristics of oral cancers (16). Furthermore, diabetes mellitus and high BMI have been linked to poor survival in OSCC patients, and the oral carcinogenesis process is a multifactorial one that is influenced by patient-related factors (17). Therefore, detailed epidemiological studies of the prevalence, clinicopathological features, and biological associations of OPMDs are necessary to enhance the prevention efforts, early detection, and control of oral cancer in Pakistan.

Objective: To evaluate the prevalence of potentially malignant oral disorders, clinicopathological features, and oral biological correlates of high-risk oral habits in Pakistan, and to determine factors associated with disease severity.

MATERIALS AND METHODS

Study Design: Descriptive cross-sectional study.

Study Setting: In tertiary care dental hospitals in Pakistan, the Oral Medicine and Oral & Maxillofacial Surgery departments.

Study Duration: November, 2025 to April, 2026.

Sample Size: The total number of participants in the study was 250.

Sampling Technique: The non-probability consecutive sampling technique was used to select eligible participants who presented during the study period.

Inclusion Criteria: Those aged 18 years and above who had high-risk oral habits, such as smoking, smokeless tobacco use, chewing betel quid, gutka, naswar, areca nut, and others for a period of one year or more, were included. Patients with clinically apparent oral mucosal lesions suggestive of potentially malignant oral disorders who gave informed consent were recruited.

Exclusion Criteria: Patients with previously diagnosed oral squamous cell carcinoma, patients undergoing treatment for oral malignancies, patients with severe systemic illnesses affecting oral health, pregnant women, and patients who refused to participate were excluded. Participants with incomplete clinical records or lesions of traumatic or infectious origin not related to potentially malignant disorders were also excluded from the study.

Methods

Ethical approval was obtained from the institutional review board, and eligible participants were recruited based on the predetermined inclusion and exclusion criteria. All subjects gave written informed consent prior to data collection. A structured questionnaire was used to gather the demographic information, medical history, and information on high-risk oral habits such as smoking, smokeless tobacco, chewing of betel quid, gutka, naswar, and areca nut. A thorough oral examination was carried out to detect clinically suspicious lesions. The size, appearance, and location of each lesion and clinical features were recorded. Biopsy and histopathological examination were performed on cases indicated for potentially malignant oral disorders. Available tissues were used to evaluate oral biological correlates such as selected molecular and histopathological markers. The data was entered and analyzed using SPSS version 26. The prevalence and clinicopathological characteristics were obtained by descriptive statistics, and the associations between variables were evaluated by inferential tests.

RESULTS

The sample consisted of 250 people with high-risk oral habits. Table 1 shows the demographic characteristics of the participants. The majority of participants were in the age group 25 to 34 years (76, 30.4%) and 35 to 44 years (64, 25.6%). Participants aged 18–24 years accounted for 58 (23.2%) cases, while those aged 45–54 years and ≥55 years represented 34 (13.6%) and 18 (7.2%) cases, respectively. The majority of the study population were males (176, 70.4%) and females (74, 29.6%). The results indicated that the high-risk oral habits were more prevalent among males and young to middle-aged adults.

Table 1. Demographic Characteristics of Study Participants (N = 250)

Variable	Frequency (n)	Percentage (%)

Age Group (Years)		
18–24	58	23.2
25–34	76	30.4
35–44	64	25.6
45–54	34	13.6
≥55	18	7.2
Gender		
Male	176	70.4
Female	74	29.6
Total	250	100.0

Table 2 displays the distribution of high-risk oral habits among the participants. The most common habit was the use of smokeless tobacco (98, 39.2%). The second most prevalent habit was chewing areca nuts (72, 28.8%), followed by cigarette smoking (46, 18.4%). 21 (8.4 %) respondents reported Consumption of gutka, and 13 (5.2 %) respondents reported chewing of betel quid. The high prevalence of the use of smokeless tobacco and areca nut is indicative of the high exposure of the study population to known risk factors for oral potentially malignant disorders.

Table 2. Distribution of High-Risk Oral Habits among Participants (N = 250)

High-Risk Oral Habit	Frequency (n)	Percentage (%)
Smokeless Tobacco	98	39.2
Areca Nut Chewing	72	28.8
Cigarette Smoking	46	18.4
Gutka Use	21	8.4
Betel Quid Chewing	13	5.2
Total	250	100.0

A total of 108 individuals were diagnosed with oral potentially malignant disorders (OPMDs) out of the study population. The most prevalent lesion was oral submucous fibrosis (OSMF), which accounted for 45 (41.7%) cases as presented in Table 3. The second most common lesion was leukoplakia, which was present in 34 (31.5%) participants. Oral lichen planus and erythroplakia were found in 17 (15.7%) and 12 (11.1%) participants, respectively. The high prevalence of OSMF and leukoplakia suggests a possible association with chronic use of areca nut and tobacco-containing products.

Table 3. Prevalence and Types of Potentially Malignant Oral Disorders (N = 108)

Type of OPMD	Frequency (n)	Percentage (%)
Oral Submucous Fibrosis (OSMF)	45	41.7
Leukoplakia	34	31.5
Oral Lichen Planus	17	15.7
Erythroplakia	12	11.1
Total	108	100.0

The results of histopathological evaluation and biomarker assessment are summarized in Table 4. The most common histopathological changes were mild epithelial dysplasia (49, 45.4%). Moderate dysplasia was recognized in 34 (31.5%) and severe dysplasia was detected in 15 (13.9%) lesions. Positive p53 expression was observed in 69 (63.9%) lesions, and positive Cyclin D1 expression was observed in 61 (56.5%) lesions. The results of statistical analysis showed a significant correlation between the expression of the biomarkers and the severity of dysplastic, where p53 expression had a p-value of 0.002, and Cyclin D1 expression had a p-value of 0.004. The results indicate that higher levels of these biomarkers could correlate with disease progression and a higher risk of malignant transformation.

Table 4. Association of Histopathological Findings and Biological Markers in OPMD Cases (N = 108)

Variable	Frequency (n)	Percentage (%)	p-value
Histopathological Grade			
Mild Dysplasia	49	45.4	
Moderate Dysplasia	34	31.5	
Severe Dysplasia	15	13.9	
Hyperkeratosis Without Dysplasia	10	9.2	
p53 Positive Expression	69	63.9	0.002
Cyclin D1 Positive Expression	61	56.5	0.004

Statistical significance was considered at $p < 0.05$.

DISCUSSION

The present study aimed to determine the prevalence, clinicopathological features, and oral biological correlates of potentially malignant oral disorders (OPMDs) in people with high-risk oral habits in Pakistan. The results showed that 43.2% of the participants had been diagnosed with OPMDs, which is a significant burden of premalignant oral lesions among this population. These findings are similar to earlier studies that showed a significant link between tobacco-related behaviors and the development of OPMDs. In the study by Khan et al., tobacco users showed significantly higher incidence of potentially malignant lesions and abnormal clinicopathological features of the oral cavity than non-users, indicating the carcinogenic effects of chronic tobacco use on the oral cavity (1). Biological factors are also involved in the development of oral premalignant lesions, as well as traditional risk factors. Javed et al. showed a significant presence of Human Papillomavirus (HPV) in potentially malignant oral conditions and proposed that HPV infection could be a factor in the development of early epithelial dysplasia (2).

The high prevalence of dysplastic lesions seen in the present study suggests that other biological and environmental factors may play a role in the progression of the disease, though HPV status was not assessed. Most of the respondents in this study were male and in the younger and middle-aged groups. They are typical of the demographic profile that is seen in populations that use tobacco and areca nut products. However, the role of demographic and clinicopathological factors on oral disease outcomes has been highlighted in previous investigations in head and neck cancer patients (3). Likewise, epidemiological studies from Saudi Arabia have indicated that oral potentially malignant disorders are especially common among those who have been exposed to known risk factors for a long period of time, such as tobacco and related products (4). The demographic distribution in the present study is consistent with regional and international trends.

Early diagnosis is always one of the best ways to prevent malignant transformation of OPMDs. Zarraa discussed the significance of the correct clinical recognition and early diagnosis of potentially malignant oral lesions to ensure a good prognosis and decrease the risk of oral cancer progression (5). Rehman et al. reported that the knowledge of the dental students about prevention and risk factors for oral cancer was found to be significant, which indicated the need for increased educational and preventive efforts (6). The results of this study highlight the need for regular oral screening programs, especially for high-risk groups. Oral submucous fibrosis (OSMF) was seen as a significant finding in the present study, followed by leukoplakia. These lesions have always been linked to the consumption of areca nut, gutka, and smokeless tobacco products. Histopathological examination also showed that the most common was mild dysplasia, while moderate and severe dysplasia were also detected. The clinical significance of such observations is that the risk of malignant transformation increases with the severity of epithelial dysplasia.

Molecular biomarkers can also contribute to a more accurate evaluation of the malignant potential. Khan et al. showed that the expression of p53 in tissue and the presence of p53 antibodies in the serum could be useful biomarkers for the progression of OPMDs to oral squamous cell carcinoma (7). These findings are confirmed by the strong correlation between p53 expression and dysplastic severity noted in the present study. Cyclin D1 expression was another important biological correlate that was investigated in this study. Increased Cyclin D1 positivity was

noted in lesions of increasing dysplasia, indicating a possible role in disease progression. Bakhtiar et al. also reported that Cyclin D1 expression was significantly higher in OPMDs and oral squamous cell carcinoma than in normal oral mucosa (8). The observed link between the expression of Cyclin D1 and dysplastic progression suggests that this marker could be used to detect lesions with a higher likelihood of malignant transformation. There have been recent publications that have emphasized the evolving epidemiology of oral cancer, especially in the younger population. Raina et al. reported significant clinicopathological differences between younger and older patients with oral squamous cell carcinoma (9).

Similarly, Lenoci et al. reported that oral cancer is a growing problem in young adults and that molecular biomarkers play a key role in understanding disease progression (10). Therefore, the current study may represent an emerging public health issue, which should be targeted for preventive interventions and early intervention strategies. Oral carcinogenesis could also be affected by systemic and environmental factors. Shaikh et al. found that the oral cancer patients had significantly higher levels of trace and toxic metals than healthy controls, which may indicate that environmental exposures play a role in the development of oral cancer (11). Moreover, Mahmood et al. showed that lymph node metastasis can be present even in clinically negative necks in OSCC patients, which means that it is important to detect the presence of premalignant lesions before the onset of invasive disease (12).

The available evidence suggests a multi-dimensional approach for the management of OPMD based on clinical assessment, histopathological evaluation, and molecular analysis. Future progress in OPMD research should be directed toward developing more accurate risk prediction models that can identify lesions most likely to become malignant (13). Chaudhary et al. pointed out that tobacco exposure is one of the major factors associated with oral carcinogenesis and emphasized the need for more effective prevention policies and tobacco-control strategies in populations of South Asia, where tobacco-related oral cancer is highly prevalent (14). New technologies could enable even earlier detection of oral lesions. Sadiq et al. showed that an AI-driven mobile screening application was effective in diagnosing potentially malignant oral lesions both in urban and rural areas of Pakistan (15).

In areas with limited resources, these technologies can be especially beneficial because access to specialized oral care is limited. Furthermore, the discovery of the molecular mechanisms involved in EMT has led to the identification of EMT regulators, including ZEB1 and ZEB2, that are related to disease progression and tumor aggressiveness (16). These biomarkers were not investigated in the present study, but their reported role in oral carcinogenesis highlights the emerging importance of molecular profiling in oral pathology. Disease outcomes can also be affected by patient-related factors. Adnan et al. reported that diabetes mellitus and higher BMI were associated with poor survival in patients with OSCA (17). The observations indicate that a thorough patient evaluation must not only focus on oral lesions, but also consider systemic health factors that may impact disease course and prognosis.

The results of this study indicate that there is a high burden of OPMDs among high-risk oral habits in Pakistan. The high prevalence of oral submucous fibrosis and leukoplakia, along with the strong correlation of the expression of p53 and Cyclin D1 with dysplastic grades, emphasizes the need for a combination of clinical, histopathological, and molecular methods to diagnose and assess the risk of oral dysplasia. Improved public knowledge, tobacco cessation programs, regular screening programs, and biomarker assessments can play a major role in reducing the impact of oral cancer on high-risk groups.

CONCLUSION

The present study revealed that tobacco, areca nut, gutka, and related products have a significant effect on oral health, and potentially malignant oral disorders (OPMDs) were highly prevalent among individuals with high-risk oral habits in Pakistan. Oral submucous fibrosis and leukoplakia were found to be the most prevalent lesions and were strongly associated with chronic exposure to oral carcinogens. The histopathological evaluation showed different degrees of epithelial dysplasia, which suggests that there were lesions with a high degree of malignant transformation potential. Additionally, higher expression of the biological markers p53 and Cyclin D1 was significantly correlated with dysplastic severity,

indicating their potential use for the detection of high-risk lesions and for the prediction of disease progression. The results highlight the need for regular oral screening, early diagnosis, histopathological assessment, and biomarker assessment in high-risk individuals. Educating the public, encouraging quitting habits, and providing preventive measures could play a significant role in the burden of oral cancer in Pakistan.

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