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Research Article

Association Between Tobacco Use And Histopathological Severity Of Oral Leukoplakia: A Cross-Sectional Study

Abdus Saboor[1], Sadaf Shakoor[2], Ilyas Rahman[3], Abbas Saleem Khan[4], Abdul Wali Khan[5], Mehwish Waris[6], Maria Ilyas[7], Shahzad Ahmad[8]

[1]Associate Professor & HOD Oral Pathology, Saidu College of Dentistry, Swat, Pakistan.
[2]Lecturer in Physiology, Sir Syed Collage of Medical Sciences, Karachi, Pakistan.
[3]General Dentist Reem medical Center , Doha, Qatar.
[4]Professor, Department of Oral Pathology, Peshawar Dental College, Riphah International University, Peshawar Campus, Pakistan.
[5]Assistant professor Oral and Maxillofacial Surgery, Saidu College of Dentistry, Swat, Pakistan
[6]KMU- Institute of Dental Sciences, Kohat, Pakistan.
[7]Assistant Professor, Department of Oral Pathology, CMH Lahore Medical College and Institute of Dentistry, Lahore, Pakistan.
[8]School of Medicine, The University of Buckingham, Buckingham, UK.

Correspondence author: Abdul Wali Khan
Assistant professor Oral and Maxillofacial Surgery, Saidu College of Dentistry, Swat, Pakistan.
Email: awali4450@gmail.com
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ABSTRACT

Background: Oral leukoplakia is the most common oral potentially malignant disorder, and tobacco use is accepted as the main etiological factor. In many developing countries, the relationship between various patterns of tobacco consumption and the severity of the disease is still poorly understood.

Objective: To determine the association between tobacco use and the histopathological severity of oral leukoplakia.

Materials and Methods: This analytical cross-sectional study was carried out at Oral Pathology, Saidu College of Dentistry, Swat for 6 months. A total of 85 patients with histopathologically confirmed oral leukoplakia were recruited using non-probability consecutive sampling. A structured proforma was used to record the demographic characteristics, tobacco use history, and histopathological findings. IBM SPSS 26.0 was used to analyze the data. Association was determined by the chi-square test, and $p < 0.05$ was considered statistically significant.

Results: The average age of participants was 46.8 ± 11.7 years, with 68.2% being male. The most common histopathological diagnosis was mild epithelial dysplasia (36.5%). A significant association was found between the type of tobacco use and the histopathological severity ($p = 0.018$). Also, duration of tobacco use was significantly associated with histopathological severity ($p < 0.001$), with moderate and severe dysplasia seen more frequently with tobacco use over 10 years.

Conclusion: There was a significant relationship between smoking and the histopathological severity of oral leukoplakia, especially when taken with tobacco over long periods. The risk of malignant transformation can be reduced with early diagnosis, histopathological evaluation, and successful tobacco cessation interventions.

Keywords: Oral Leukoplakia, Tobacco, Histopathology, Epithelial Dysplasia, Oral Potentially Malignant Disorders.

INTRODUCTION

Oral leukoplakia (OL) is defined as a predominantly white plaque or patch that cannot be clinically or histopathologically diagnosed as another known lesion and is the most common potentially malignant disorder (OPMD) of the oral cavity.[1] It is a spectrum of epithelial changes from simple hyperkeratosis (with no dysplasia) to varying degrees of epithelial dysplasia, and in some cases, early invasive squamous cell carcinoma.[2] Oral leukoplakia is one of the most clinically relevant precancerous lesions seen in dental and oral pathology practice and is a condition that potentially can become malignant.[3] Histopathological examination is still the most important procedure for diagnosis, risk stratification, and treatment planning; the severity of epithelial dysplasia is

the most reliable predictor of malignant progression.[4]

Oral potentially malignant disorders (OPMDs) are seen in about 4-5% of the world population, and oral leukoplakia is the most common type of oral potentially malignant disorder.[5] The prevalence of oral leukoplakia has been estimated to be between 1–4% in the world but tends to be higher in South and south-east Asian countries where smoking and smokeless tobacco use are very common.[6] Oral leukoplakia is reported to be more common in males than females and primarily in those over 40 years old because of the higher incidence of tobacco and alcohol use in these groups.[7] Oral leukoplakia has been reported to have an annual malignant transformation rate of 1% to 3%, and lifetime transformation rates between 5% and 15%, depending on the characteristics of the leukoplakia, the site of involvement, and the histopathological grade.[8]

Smoking is known to be the major modifiable risk factor for the development of oral leukoplakia.[9] Tobacco smoke contains over 7,000 chemicals, many of which are known to cause cancer, oxidative stress, DNA damage, genomic instability, and chronic inflammation in the oral epithelium.[10] Likewise, smokeless tobacco products such as chewing tobacco, gutka, naswar, betel quid with tobacco, and snuff can remain in direct contact with the oral mucosa for extended periods, exposing epithelial cells to carcinogenic chemicals that lead to hyperkeratosis, epithelial atypia, and progressive dysplastic changes.[11] The cumulative effect of exposure to these carcinogens results in ongoing molecular changes affecting inflammatory mediators, cell cycle regulators, oncogenes, and tumor suppressor genes that can promote the development of potentially malignant epithelial changes.[12]

Tobacco-related oral disease is especially a major problem in low- and middle-income countries.[13] The World Health Organization (WHO) estimates that over 8 million people are killed every year by tobacco-related diseases around the world, while over 1.3 billion people use tobacco products.[14]

Histopathological evaluation is a crucial factor in the diagnosis of the biological behavior of oral leukoplakia.[15] Epithelial dysplasia is divided by the WHO into three groups: mild, moderate, and severe, depending on the degree of cytological and architectural disturbances.[16] Severe dysplasia and carcinoma in situ have the greatest risk of subsequent invasion, and thus early biopsy along with proper histopathological grading is important.[17]

Although tobacco has been implicated as a risk factor for initiation of oral leukoplakia, and little attention has been paid to the severity of the epithelial dysplasia as seen under a microscope; thus, there is a lack of understanding of the progression of disease among tobacco users. This association may be useful for clinicians to have as a prognosticator, to identify those who would benefit most from more intensive monitoring, and to support evidence-based tobacco cessation treatments in those who are most likely to develop malignancy. Hence, the present study was designed to assess the relationship between tobacco use and the histopathological severity of oral leukoplakia among patients with oral leukoplakia.

METHODOLOGY

The design of this study was an analytical cross-sectional study that was carried out in the Department Oral Pathology, Saidu College of Dentistry, Swat. The study was carried out over a period of six months, from July 2025 to December 2025. The number of participants in this study was the estimated sample size for a single population proportion from OpenEpi Version 3.01. The expected prevalence of 11% was assumed from the study of Kumar and Muniyandi with a margin of error of 6.6% and 95% confidence interval.[17] Using these parameters, the minimum sample size determined was 85 participants. Hence, there were 85 patients with histopathologically confirmed oral leukoplakia included in the study.

A non-probability consecutive sampling was used to recruit participants. During the study period all patients who were suspected to have oral leukoplakia were screened for inclusion in the study. Patients, who met the inclusion criteria and signed informed consent, were consecutively enrolled until the sample size of 85 patients was reached.

Patients of both sexes, aged 18 years or above, were included and those who had clinically diagnosed oral leukoplakia and confirmed by histopathological examination. Patients who have used tobacco in any form (smoked or smokeless or both) or non-tobacco users with a history of oral leukoplakia were included. The patients were recruited based on a written informed consent and were willing to participate in the study.

Patients with white lesions other than leukoplakia (e.g., oral lichen planus, oral candidiasis, frictional keratosis, other potentially malignant disorders) were excluded from the study. Those patients who had a previous oral squamous cell carcinoma, those who had undergone a treatment for oral leukoplakia prior to biopsy, patients with recurrent oral leukoplakia and inadequate or inconclusive biopsies were also excluded. Further, patients who refused to join the study or whose clinical and histopathological details were incomplete were excluded.

Consecutive sampling was used to identify eligible patients who attended the Department of Oral Pathology during the study period. All participants were informed of the purpose of the study and informed consent was obtained in writing prior to entering the study. A structured data collection proforma was used to gather demographic and detailed tobacco use data (type, duration of use, and frequency of use) from the respondents. Full clinical examination of the oral cavity was done, and incisional/excisional biopsy was done when clinically suspected in patients with oral leukoplakia following standard clinical guidelines. Biopsy specimens were fixed in 10% neutral buffered formalin, processed in a routine manner, embedded in paraffin, sliced into 4-5 µm pieces and stained with hematoxylin and eosin. The lesions were diagnosed by experienced oral pathologists and graded as hyperkeratosis without dysplasia, mild dysplasia, moderate dysplasia, and severe dysplasia based on the World Health Organization (WHO) criteria. The study proforma was used to document all clinical and histopathological findings and participants' confidentiality was maintained.

The data collected were entered and analysed in IBM SPSS Statistics version 26.0. The summary statistics for continuous variables, including age, are expressed as mean ± standard deviation (SD); and frequencies and percentages are used for categorical variables, such as gender, tobacco use, type of tobacco, duration of tobacco use, and histopathological severity of oral leukoplakia. The association of tobacco use with histopathological severity of oral leukoplakia was evaluated by the Chi-square test and/or Fisher's exact test as appropriate. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 85 patients with histopathologically confirmed oral leukoplakia were studied. The mean age of the participants was 46.8 ± 11.7 years, and the majority of participants were in the age group 46–60 years (37.6%). The study population consisted of 68.2% males, and 55.3% of the population lived in urban areas. The most commonly reported type of tobacco use was smokeless tobacco (40.0%) followed by smoked tobacco (31.8%) and mixed smoked and smokeless tobacco use (18.8%). The majority of tobacco users were more than 10 years of tobacco users (38.8%), and most of them also indicated that they used tobacco 5-10 times a day (38.8%) (Table 1).

The buccal mucosa was the most common site of lesions (43.5%), whereas homogeneous leukoplakia was the most common clinical presentation (63.5%). A histological examination of specimens revealed that 36.5% of the samples were mildly dysplastic, 29.4% moderately dysplastic, 17.6% severely dysplastic, and 16.5% hyperkeratotic (Table 2). A statistically significant association between the type of tobacco use and the severity of the oral leukoplakia in terms of histopathological diagnosis ($p = 0.018$). Epithelial dysplasia was more prevalent among participants who used both smoked and smokeless tobacco versus participants who used only one type of tobacco or no tobacco at all (Table 3).

Likewise, the length of tobacco use was significantly associated with histopathological severity ($p < 0.001$). The findings revealed that participants who had used tobacco for more than 10 years had more moderate and severe dysplasia than those who had used tobacco for less than 10 years (Table 4).

Table 1. Demographic and Tobacco-Related Characteristics of the Study Participants (N = 85)

Variable	Category	n (%)
Age (years)	18–30	13 (15.3)
	31–45	25 (29.4)
	46–60	32 (37.6)
	>60	15 (17.6)
Mean age (years)	46.8 ± 11.7	
Gender	Male	58 (68.2)

	Female	27 (31.8)
Residence	Urban	47 (55.3)
	Rural	38 (44.7)
Type of tobacco use	Smoked tobacco	27 (31.8)
	Smokeless tobacco	34 (40.0)
	Both smoked & smokeless	16 (18.8)
	Non-user	8 (9.4)
Duration of tobacco use	<5 years	16 (18.8)
	5–10 years	28 (32.9)
	>10 years	33 (38.8)
	Non-user	8 (9.4)
Frequency of tobacco use/day	<5 times/day	18 (21.2)
	5–10 times/day	33 (38.8)
	>10 times/day	26 (30.6)
	Non-user	8 (9.4)

Table 2. Clinical and Histopathological Characteristics of Oral Leukoplakia (N = 85)

Variable	Category	n (%)
Site of lesion	Buccal mucosa	37 (43.5)
	Tongue	22 (25.9)
	Gingiva	11 (12.9)
	Floor of mouth	7 (8.2)
	Palate	8 (9.4)
Clinical type	Homogeneous	54 (63.5)
	Non-homogeneous	31 (36.5)
Histopathological severity	Hyperkeratosis (without dysplasia)	14 (16.5)
	Mild dysplasia	31 (36.5)
	Moderate dysplasia	25 (29.4)
	Severe dysplasia	15 (17.6)

Table 3. Association Between Type of Tobacco Use and Histopathological Severity of Oral Leukoplakia (N = 85)

Type of tobacco use	Hyperkeratosis n (%)	Mild dysplasia n (%)	Moderate dysplasia n (%)	Severe dysplasia n (%)	p-value
Smoked tobacco	5 (18.5)	12 (44.4)	8 (29.6)	2 (7.4)	0.018*
Smokeless tobacco	6 (17.6)	12 (35.3)	10 (29.4)	6 (17.6)	
Both smoked & smokeless	1 (6.3)	3 (18.8)	6 (37.5)	6 (37.5)	
Non-user	2 (25.0)	4 (50.0)	1 (12.5)	1 (12.5)	
Chi-square test; p < 0.05 considered statistically significant.					

Table 4. Association of Duration of Tobacco Use with Histopathological Severity Among Tobacco Users (N = 77)

Duration of tobacco use	Hyperkeratosis n (%)	Mild dysplasia n (%)	Moderate dysplasia n (%)	Severe dysplasia n (%)	p-value
<5 years (n=16)	5 (31.3)	8 (50.0)	3 (18.8)	0 (0.0)	<0.001*
5–10 years (n=28)	5 (17.9)	13 (46.4)	8 (28.6)	2 (7.1)	
>10 years (n=33)	2 (6.1)	6 (18.2)	13 (39.4)	12 (36.4)	
Chi-square test; p < 0.05 was considered statistically significant.					

DISCUSSION

This is an analytical cross-sectional study that aimed to assess the association between

tobacco use and the severity of oral leukoplakia by histopathological examination. The average age of the participants was 46.8 ± 11.7 years, and the highest proportion was in the age group 46–60 years. The most common form of tobacco consumption was smokeless tobacco, and the most common histopathological findings were mild epithelial dysplasia. In addition, there was a statistically significant association between the type of tobacco use and histopathological severity (p-value 0.018) and also a significant association between the duration of tobacco use and the degree of epithelial dysplasia (p-value < 0.001).

This study had a predominance of males and a majority of lesions were in the middle age group. The results are in line with Porto et al. (2024) who also found that male patients aged 40–49 years had a predominance of oral leukoplakia.[18] The higher prevalence among males may be due to the higher prevalence and length of time individuals use tobacco, including smoking and smokeless tobacco usage, which exposes the oral mucosa to carcinogenic substances for longer periods.

In our study, the most commonly affected area was the buccal mucosa, the tongue was the second most common area, and the most common clinical type was homogeneous leukoplakia. Likewise, Adamczyk et al.2026 reported that the buccal mucosa is the most common site in smokers due to direct and prolonged contact of the oral mucosa with tobacco smoke and other carcinogenic substances.[19] Both studies indicate that the sites of the mouth that are most directly exposed to tobacco are the most vulnerable to changes in the epithelium that can result in leukoplakia.

Histopathologic evaluation in this study showed that the most common diagnosis was epithelial dysplasia in the mild stage, while the moderate and severe stages of epithelial dysplasia together combined for almost 50% of the cases. Similar results were observed by Șerban et al. 2025 in which mild dysplasia was seen in both smokers and non-smokers, but there was a significant number that also had moderate to severe dysplasia.[20] The results point to the broad histopathological spectrum of OL and stress the need for regular biopsy to grade them, irrespective of whether the patient smokes or not.

The present study revealed that there was a significant association between tobacco use and the histopathological severity, which was the main finding of this study. Patients who used both smoked and smokeless tobacco had more moderate and severe epithelial dysplasia than did those who used only smoked tobacco and non-users. These results are similar to the findings of Becker et al. 2024, who found that dysplastic changes were found in both smokers and non-smokers, and that smokers had more advanced epithelial changes and a higher risk of progression.[21] Oxidative stress, chronic inflammation and genetic changes are likely to be associated with tobacco-induced carcinogenic products, causing an increase in the severity of histopathological changes due to cumulative exposure to such products.

The other finding that warranted significant attention in the present study is the strong association between the length of tobacco use and the progression of the histopathological severity. The dysplasias were moderate and severe, with the highest frequency occurring in participants with tobacco exposure of more than 10 years, while hyperkeratosis and mild dysplasia were the most common, with shorter durations of tobacco exposure. While traditionally smokers and non-smokers have been compared, the study by Muto et al., 2022 also noted that there are progressive epithelial dysplastic changes with continued tobacco exposure and the importance of early cessation and frequent follow-up of chronic tobacco users.[22]

In conclusion, the results from the present study confirmed the previous evidence linking tobacco smoking to the histopathology of oral leukoplakia. The link between tobacco use, duration of use, and severity of epithelial dysplasia stresses the need for widespread tobacco cessation programs, early biopsy of any suspicious lesion, and periodic review of high-risk individuals. Rapid diagnosis of patients with advanced dysplasia may help to prevent the transition to oral squamous cell carcinoma and allow timely intervention.

Limitations

There are some limitations to this study. First, the cross-sectional design did not allow a causal link between tobacco use and the histopathological grade of oral leukoplakia. Second, the findings may not be generalizable to the larger population, given the relatively small sample size and single-center study. Thirdly, data on tobacco use such as duration and frequency, were self-reported and thus were subject to recall bias. Furthermore other potential confounding variables, including alcohol intake, nutritional status,

socioeconomic factors, human papillomavirus (HPV) infection, and genetic susceptibility, were not assessed. Larger, more prospective, multicenter studies with follow-up are suggested in order to better define the role of tobacco exposure in the progression and malignant transformation of oral leukoplakia.

CONCLUSION

In the present study, there was a significant association between tobacco use and the histopathologic severity of oral leukoplakia. The combined tobacco smoking and smokeless tobacco users and the longer-term tobacco users had more moderate and severe epithelial dysplasia, indicating the cumulative negative effect of tobacco on the oral mucosa. Early detection, regular histopathological assessment, and full tobacco smoking cessation counseling in those who present with oral leukoplakia are very important, as these are the findings that highlight this. The introduction of regular screening and prompt biopsy of suspicious lesions, especially in chronic tobacco users, could help facilitate early intervention, risk stratification, and hopefully decrease the burden of malignant transformation to OSCA.

REFERENCES

1. Kumari, P., P. Debta, and A. Dixit, *Oral potentially malignant disorders: etiology, pathogenesis, and transformation into oral cancer*. Frontiers in pharmacology, 2022. **13**: p. 825266.
2. Rodriguez, I., et al., *Pathological features, differential diagnosis, prognosis, and diagnostic challenges in the classification of penile squamous neoplasia*. Histopathology, 2026. **88**(1): p. 265-300.
3. Zahid, E., et al., *Overview of common oral lesions*. Malaysian family physician: the official journal of the Academy of Family Physicians of Malaysia, 2022. **17**(3): p. 9.
4. Bates, T.J., A. Richards, and M. Pring, *Oral potentially malignant disorders: a practical review for the diagnostic pathologist*. Diagnostic Histopathology, 2023. **29**(4): p. 208-224.
5. Lonni, N., et al., *Global Prevalence of Oral Potentially Malignant Disorders: An Updated Systematic Review and Meta-Analysis*. Journal of Oral Pathology & Medicine, 2026.
6. Ranganathan, K. and L. Kavitha, *Clinical aspects of oral cancer and potentially malignant disorders in South and Southeast Asia*. Oral Diseases, 2025. **31**(5): p. 1406-1419.
7. Sharma, A.D., et al., *PREVALENCE OF ORAL LEUKOPLAKIA, ERYTHROPLAKIA, AND ORAL SUBMUCOSUS FIBROSIS IN PATIENTS CONSUMING TOBACCO AND ARECANUT: A HOSPITAL-BASED OBSERVATIONAL STUDY*. PRZEGL EPIDEMIOL, 2025. **79**(2): p. 241-249.
8. Guan, J.Y., et al., *Malignant transformation rate of oral leukoplakia in the past 20 years: A systematic review and meta-analysis*. Journal of Oral Pathology & Medicine, 2023. **52**(8): p. 691-700.
9. Zhang, C., et al., *The global prevalence of oral leukoplakia: a systematic review and meta-analysis from 1996 to 2022*. BMC oral health, 2023. **23**(1): p. 645.
10. Stanfill, S.B., et al., *From cultivation to cancer: formation of N-nitrosamines and other carcinogens in smokeless tobacco and their mutagenic implications*. Critical reviews in toxicology, 2023. **53**(10): p. 658-701.
11. Karshiyeva, D.R., *Changes in the organs and tissues of the oral cavity of people who smoke tobacco*. Журнал гуманитарных и естественных наук, 2023(1): p. 145-149.
12. Bhat, A.S., et al., *Cancer initiation and progression: a comprehensive review of carcinogenic substances, anti-cancer therapies, and regulatory frameworks*. Asian J Res Biochem, 2024. **14**(4): p. 111-25.
13. Carneiro, P.H., et al., *The impact of oral health disparities on smoking prevalence*. Frontiers in Public Health, 2025. **13**: p. 1667261.
14. Organization, W.H., *WHO global report on trends in prevalence of tobacco use 2000–2030*. 2024: World Health Organization.
15. Evren, I., et al., *Associations between clinical and histopathological characteristics in oral leukoplakia*. Oral diseases, 2023. **29**(2): p. 696-706.
16. Khoury, Z.H., M. Sultan, and A.S. Sultan, *Oral epithelial dysplasia grading systems: a systematic review & meta-analysis*. International Journal of Surgical Pathology, 2022. **30**(5): p. 499-511.
17. Alabdulaaly, L., et al., *Characterization of initial/early histologic features of proliferative leukoplakia and correlation with malignant transformation: a*

multicenter study. Modern Pathology, 2022. **35**(8): p. 1034-1044.
18. Porto, U.N., et al., *Leukoplakia and erythroplakia in youngers versus older individuals: a clinicopathological retrospective study*. Medicina Oral, Patología Oral y Cirugía Bucal, 2024. **29**(5): p. e665.
19. Adamczyk, A., et al., *The Impact of Cigarette Smoking on the Oral Mucosa*. Journal of Education, Health and Sport, 2026. **90**: p. 70553-70553.
20. Șerban, A.-E., et al., *Comparative Clinical and Histopathological Study of Oral Leukoplakia in Smokers and Non-Smokers*. Diagnostics, 2025. **15**(4): p. 502.
21. Becker, A.S., et al., *Diagnosis of differentiated dysplasia as a variant of oral epithelial dysplasia*. Oral Diseases, 2024. **30**(7): p. 4185-4194.
22. Muto, M., et al., *Field effect of alcohol, cigarette smoking, and their cessation on the development of multiple dysplastic lesions and squamous cell carcinoma: a long-term multicenter cohort study*. Gastro Hep Advances, 2022. **1**(2): p. 265-276.