

RESEARCH ARTICLE

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Tobacco-Associated Dysregulation in *TAP-1* and *TAP-2* Expression in Oral Premalignant Lesions and Oral Squamous Cell Carcinoma

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Abstract

Background: *TAP-1*, and *TAP-2* are required for MHC class I-restricted antigen surveillance. Downregulated TAP expression in oral carcinogenesis might affect the antigen presentation system, leading to immune evasion. Tobacco is the most common aetiological risk factor for Oral Squamous Cell Cancer, and it could influence antigen-processing mechanisms throughout the stages of Oral Squamous Cell Cancer. **Materials and Methods:** This hospital-based case-control study included 60 cases of oral squamous cell carcinoma (OSCC), 60 oral premalignant lesions, and 60 age and sex-matched healthy controls. A standardised questionnaire was used to collect information about tobacco exposure patterns. Before beginning treatment, peripheral blood samples were taken. Total RNA was extracted and reverse-transcribed, and *TAP-1* and *TAP-2* gene expression was measured using SYBR Green-based real-time PCR, with relative gene expression calculated using the $2^{-\Delta\Delta Ct}$ method with normalization to the housekeeping gene. Non-parametric tests and multivariate linear regression was used for statistical analysis. **Results:** Combined tobacco users were most common among OSCC (50–55%), with about 88.3% of controls being non-users. *TAP-1* expression demonstrated a significant decrease in leukoplakia smokeless tobacco-related (mean = 0.80; $p = 0.048$), and with the OSCC dedifferentiation, which was even lower in poorly differentiated tumours (mean = 0.68; $p = 0.029$). Expression of *TAP-2* was not altered in premalignant conditions, whereas it was remarkably decreased in OSCC patients with mixed tobacco habit (mean = 0.85; $p = 0.044$). Multivariate analysis revealed an independent effect of mixed tobacco on lower TAP expression, as well as a role for grade ($p < 0.05$). **Conclusion:** Simultaneous tobacco exposure induces a modest but significant decline in *TAP-2* with differentiation-dependent reduction of *TAP-1*, suggesting that the subtle dysfunction in antigen processing machinery during oral carcinogenesis.

Keywords: Oral squamous cell carcinoma, Tobacco use, Antigen processing and presentation, *TAP-1*, *TAP-2*

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common oral cavity malignancy and is often preceded by clinical recognizable premalignant lesions of leukoplakia, oral submucous fibrosis (OSMF) and oral lichen planus (OLP). Nevertheless, OSCC remains a disease with high levels of morbidity and mortality, which is mostly related to late diagnosis and complicated biological processes that cause malignant cell transformation. One of the well-established etiological factors is tobacco use, whether

smoked or smokeless, and most frequently in combination with alcohol, which is the most significant pathogenic modifiable risk factor in oral carcinogenesis [1].

Historically, the role of tobacco-linked carcinogenesis has been seen as a direct genotoxic impact, such as DNA adduct formation, oxidative stress, and mutagenesis. Nonetheless, there are growing indications that risk factors associated with tobacco have deep immune regulation effects on the host. Prolonged exposure to tobacco and constituents of areca nuts triggers persistent inflammation and changes immune signalling in an

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immune-surveillance-defeating way [2, 3]. This type of immune deregulation allows the survival and growth of dysplastic and malignant epithelial cells to remain undetected and destroyed, which in turn promotes the maintenance of a lesion, its development and tumour growth [4].

The MHC-I antigen presentation pathway plays a crucial role in anti-tumour immune surveillance. Effective cytotoxic T lymphocyte-mediated immune responses require appropriate generation and presentation of endogenously synthesised antigens, which is limited by the actions of the transporter associated with antigen processing proteins (*TAP-1* and *TAP-2*) [5]. These transporters play a role in the translocation of antigenic peptides into the endoplasmic reticulum, in which they are bound to MHC-I complexes. Hence, relatively small changes in TAP expression can have a dramatic impact on peptide loading and tumor antigen presentation, and the sensitivity of CTLs to TAP inhibition constitutes an important mechanism for preventing immune evasion. Crucially, these differences might be early, perhaps initiating rather than complete loss of antigen processing and presentation [6]. The recent molecular evidence suggests that minor changes in *TAP-1* and *TAP-2* expression may be sufficient to significantly reduce, but not completely eliminate, MHC-I efficient presentation of antigens. Subtle shifts in immune escape were found during different stages of OSCC, or when the tumour differentiation status was taken into consideration [7, 8].

Oral premalignant lesions provide a unique biological window to investigate immune alterations occurring at early stages of carcinogenesis, before the development of invasive disease. Variations in tobacco exposure across different premalignant conditions may result in lesion-specific patterns of immune modulation, which may intensify with malignant transformation and tumour dedifferentiation [9]. Despite this, data examining the relationship between tobacco exposure patterns and *TAP-1* and *TAP-2* expression across the spectrum of oral premalignant lesions and OSCC remain limited and inconsistent [10, 11]. While complete loss of antigen presentation has been described in advanced cancers, it remains unclear whether tobacco-associated premalignant lesions exhibit subtle yet biologically meaningful dysregulation of TAP-mediated antigen processing that may contribute to early immune escape. Addressing this gap, the present study investigates *TAP-1* and *TAP-2* gene expression across oral premalignant lesions and OSCC in relation to tobacco exposure. By integrating epidemiological risk assessment with corrected molecular expression analysis, this study aims to determine whether tobacco-related risk factors are associated with early alterations in antigen-processing pathways, thereby providing insight into immune escape mechanisms operative during oral carcinogenesis. We hypothesised that tobacco exposure is associated with progressive dysregulation of *TAP-1* and *TAP-2* expression across premalignant lesions and OSCC, reflecting early immune escape mechanisms

Materials and Methods

Study Design and Setting

It was a hospital-based case control study that was performed in the Ear, Nose, and Throat (ENT) outpatient department of the All India Institute of Medical Sciences (AIIMS), Gorakhpur, India to assess the correlation of the tobacco exposure and Transporter Associated with Antigen Processing genes (*TAP-1* and *TAP-2*) expression in oral premalignant lesions and oral squamous cell carcinoma (OSCC). The Institutional Ethics Committee of AIIMS, Gorakhpur, gave consent to the study (Ethical Clearance No.: (IHEC/AIIMS-GKP/BMR/118/2023). All the participants were enrolled with written informed consent.

Participants

In the present study, sixty histologically confirmed cases of oral squamous cell carcinoma (OSCC) were compared with sixty cases of oral premalignant lesions and sixty control subjects. Inclusion criteria included histologically confirmed OSCC or clinically and histologically diagnosed premalignant lesions, age above 18 years, and willingness to provide informed consent. Participants were recruited between September 2023 and March 2025, during which clinical assessment, exposure recording, and sample collection were performed. Cases and premalignant lesions were recruited consecutively from patients attending the ENT outpatient department, while controls were selected from hospital visitors and attendants after eligibility screening. The sample size was calculated with the assumption of 5.00% precision and an estimated prevalence of 2.70% [7], assuming an infinite population size. These parameters facilitated the calculation of a 95% confidence interval, yielding bounds of 0% to 7.7%, reflecting the estimated prevalence adjusted by the specified precision. Although the initial sample size estimate indicated that 41 participants would provide statistical reliability, 60 samples were ultimately collected to enhance the robustness of the study and accommodate practical considerations. The OSCC group included tumors that were well, moderately or poorly differentiated. The premalignant group consisted of patients with leukoplakia, oral submucous fibrosis (OSMF) and oral lichen planus (OLP). The control group comprised individuals who were age- and sex-matched but had no clinical evidence for oral premalignant or malignant disease nor any history at all of cancer. When available clinical records showed that patients with human papillomavirus (HPV)-associated oral lesions or Patients with HPV-associated oral lesions or HPV-positive OSCC were excluded from the study to avoid potential confounding related to viral-driven carcinogenesis.

Exposure Assessment and Clinical Data

Primary outcomes were relative expression levels of *TAP-1* and *TAP-2* genes, while primary exposure variables included tobacco use categories (smoked, smokeless, mixed, or none). Age, sex, and tumour differentiation were treated as potential confounders.

Sample Collection and RNA Isolation

Tissues total RNA was extracted by an improved TRIzol approach, providing for high yield and quality suitable for subsequent gene expression analysis, a method that has been previously validated in cancer tissues [12]. Peripheral venous blood (3 mL) was collected before initiation of treatment. Peripheral blood samples were selected as a minimally invasive and clinically feasible source for assessing systemic gene expression changes. This approach enables evaluation of circulating molecular alterations associated with disease progression and may provide potential biomarkers for early detection and monitoring. However, it may not fully represent tumor microenvironment-specific gene expression.

Of this, 400 µL was transferred into TRIzol reagent for RNA isolation, and 2.6 mL was used for serum separation. The leukocytes of peripheral blood were used to extract total RNA using TRI-Reagent and commercial kits (Qiagen and G-Biosciences). The purity and concentration of RNA were measured spectrophotometrically (260/280 nm), and the integrity of RNA was confirmed by electrophoresis using a gel made up of agarose [13].

Exposure Assessment and Clinical Data

Detailed clinical history, including tobacco use (smoked and/or smokeless forms), was recorded for all participants using structured questionnaires and medical records where available. Tobacco exposure categories were predefined as smoked tobacco, smokeless tobacco/areca nut, mixed exposure, and no exposure based on self-reported habitual use. To minimise measurement bias, gene expression analysis was performed in triplicate using standardised protocols, and exposure data were collected using structured questionnaires to reduce recall variability.

Quantitative Real-Time PCR

Reverse transcription kits were used to synthesise complementary DNA (G-Biosciences and Takara). Quantitative real-time PCR was conducted on SYBR Green chemistry on a QuantStudio 5 platform with primers in *TAP-1*, *TAP-2*, and housekeeping genes (*GAPDH*, β -*actin*, and 2 microglobulin). Melting curve and agarose gel electrophoresis were used to confirm amplification specificity. Triplicate reactions were done. Expression of genes was normalized with a housekeeping gene, and relative changes in the level of mRNA were determined using the $2^{-\Delta\Delta Ct}$ method [14].

Gene Expression Analysis

Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method as described by Livak and Schmittgen (2001), with normalization to the housekeeping gene *GAPDH* [15, 16]. The calibrator was healthy controls, which were normalized to 1.00.

Biochemical Analysis

Centrifugation of serum at 3000 rpm was done at room temperature for 15 minutes. Internal and external quality control that measured levels of C-reactive protein, Creatinine, Ferritin, and Erythrocyte Sedimentation Rate Commercial kits (Erba Mannheim, Germany)

were measured on a full automated analyzer (XL-1000, Transasia Bio-Medicals Ltd.), after internal and external quality control.

Statistical Analysis

Gene expression values obtained from the $2^{-\Delta\Delta Ct}$ method were analysed as continuous variables; no arbitrary categorisation was applied to preserve biological variability. Statistical tests were conducted with the help of the IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). The Chi-square test or Fisher's exact test was used to compare the categorical variables. The Shapiro-Wilk test was used to assess normality. The Mann-Whitney U test was used to compare two-group analysis with continuous gene expression data, and Kruskal Wallis test examines many groups, including the Dunn post hoc test where necessary. Multivariate linear regression analysed independent associations, adjusted by age and sex. The p-value of less than 0.05 was statistically significant. All the participants were enrolled with written informed consent.

Results

Distribution of Tobacco Exposure Across Study Groups

Table 1 shows the distribution of patterns of tobacco exposure among oral squamous cell carcinoma (OSCC), oral premalignant lesions and control subjects. A clear difference in the exposure profile was found in non-lesional groups, premalignant groups and malignant groups. Mixed tobacco habits (smoked and smokeless tobacco with or without alcohol) were the most common type of exposure in OSCC cases. Mixed habits were found in 55 percent of well differentiated, 55 percent of moderately differentiated and 50 percent of poorly differentiated cases of OSCC. The second most common pattern of exposure was exclusive use of smokeless tobacco, and isolated use of smoked tobacco was relatively low. In non-exposure to tobacco, OSCC patients were rare especially in well and moderately differentiated tumors. On the other hand, oral premalignant lesions showed different distributions of exposure. The use of smokeless tobacco/areca nut was the most common in oral submucous fibrosis (OSMF) (45.5%), and leukoplakia (33.3%). The pattern of exposure to oral lichen planus (OLP) was heterogenous, as 50 percent had no tobacco exposure, which is indicative of contributing factors other than tobacco use. The control group mainly comprised of non-exposed people (88.3%), which proves its appropriateness as the reference population. In general, these results indicate that combined tobacco exposure between controls and premalignant lesions and eventually OSCC is on a gradual escalating pattern, which is in line with an epidemiological gradient as indicated in oral carcinogenesis.

Association Between Tobacco Exposure and TAP-1 Gene Expression

The $2^{-\Delta\Delta Ct}$ method was used was used to measure *TAP-1* gene expression with the control group against which calibration was performed being 1.0. Table 2. summarizes corrected relative expression values according

to lesion types and categories of tobacco exposure.

The controls and the majority of the OLP cases did not show any signs of decrease in *TAP-1* expression (mean = 1.00), which suggests that the individuals have preserved antigen-processing activity. In the case of leukoplakia in exposure to smokeless tobacco, there was a borderline decrease (mean = 0.80) in the expression of *TAP-1* which was statistically significant ($p = 0.048$). There was a small deviation of the baseline *TAP-1* expression in OSMF cases (mean = 0.88; $p = 0.21$) and the finding indicates that *TAP-1* remained relatively unaltered even with the chronic exposure to smokeless tobacco. *TAP-1* expression also showed a statistically significant but minor decrease in cases of mixed tobacco use in OSCC (mean = 0.74; $p = 0.032$). Tumor differentiation stratification demonstrated a progressive decrease, with the minimal *TAP-1* expression in poorly differentiated OSCC (mean = 0.68; $p = 0.029$). Notably, the degree of the mentioned differences was rather small, which is a sign of minor modulation instead of a significant inhibition of *TAP-1*.

Association Between Tobacco Exposure and *TAP-2* Gene Expression

Changes in the expression of *TAP-2* gene were in

the same although slightly decreased pattern (Table 2). TLAP - *TAP* (mean = 1.00) was stable in controls and OLP cases, indicating the intact antigen-processing activity. Premalignant lesions exhibited minor and insignificant variation and leukoplakia (mean = 0.93) and OSMF (mean = 0.96) did not have any statistically significant differences with controls. On the contrary, mixed tobacco exposure in OSCC cases showed a marginal decrease in *TAP-2* expression (mean = 0.85; $p = 0.044$). There was a weak correlation between the negative change in *TAP-2* expression and tumor differentiation, where poorly differentiated OSCC exhibited the lowest levels of *TAP-2* (mean = 0.80; $p = 0.041$). Effect sizes were again small and biologically subtle as in the case of *TAP-1*.

Lesion-Specific Stratified Patterns of *TAP* Expression

The clear patterns of *TAP* modulation when examined by the type of lesion as opposed to pooled exposure categories were different. The selective, borderline loss of *TAP-1*, but relative preservation of *TAP-2*, indicated an early immune regulation in Leukoplakia. ASMF - OSMF showed almost basement levels in the expression of both of the genes, but OSCC showed simultaneous yet slight expressions of *TAP-1* and *TAP-2* yet most prominently in the poorly differentiated tumor.

Table 1. Distribution of Tobacco Exposure Patterns Across Oral Squamous Cell Carcinoma, Oral Premalignant Lesions, and Control Groups

Study Group	Smoked Tobacco n (%)	Smokeless Tobacco / Areca n (%)	Mixed Habits* n (%)	No Exposure n (%)	Total
OSCC – Well differentiated	2 (10.0)	7 (35.0)	11 (55.0)	0 (0)	20
OSCC – Moderately differentiated	2 (10.0)	6 (30.0)	11 (55.0)	1 (5.0)	20
OSCC – Poorly differentiated	2 (10.0)	4 (20.0)	10 (50.0)	4 (20.0)	20
Oral Lichen Planus (OLP)	0 (0)	14 (23.3)	16 (26.7)	30 (50.0)	60
Oral Submucous Fibrosis (OSMF)	0 (0)	27 (45.0)	21 (35.0)	12 (20.0)	60
Leukoplakia	0 (0)	20 (33.3)	25 (41.7)	15 (25.0)	60
Controls	1 (1.7)	3 (5.0)	3 (5.0)	53 (88.3)	60

Table 2. Association of Tobacco Exposure and Lesion Type with Borderline Changes in *TAP-1* and *TAP-2* Gene Expression ($2^{-\Delta\Delta Ct}$)

Lesion Type	Predominant Tobacco Exposure	<i>TAP-1</i> Relative Expression ($2^{-\Delta\Delta Ct}$)	<i>TAP-1</i> p-value	<i>TAP-2</i> Relative Expression ($2^{-\Delta\Delta Ct}$)	<i>TAP-2</i> p-value	Interpretation (Conservative)
Controls	No tobacco exposure	1.00 (Reference)	—	1.00 (Reference)	—	Baseline antigen-processing activity
Oral Lichen Planus (OLP)	No tobacco / mixed	0.97	0.41	0.98	0.44	Preserved <i>TAP</i> expression
Leukoplakia	Smokeless tobacco	0.8	0.048	0.93	0.19	Borderline, minor downregulation of <i>TAP-1</i>
Oral Submucous Fibrosis (OSMF)	Smokeless tobacco	0.88	0.21	0.96	0.33	Minimal, non-significant variation
OSCC (overall)	Mixed tobacco $\pm$ alcohol	0.74	0.032	0.85	0.044	Minor, borderline coordinated reduction
OSCC – Well differentiated	Mixed	0.79	0.067	0.88	0.081	Near-baseline expression
OSCC – Moderately differentiated	Mixed	0.72	0.041	0.84	0.049	Borderline reduction
OSCC – Poorly differentiated	Mixed	0.68	0.029	0.8	0.041	Most evident yet still modest decrease

Table 3. Multivariate Linear Regression Analysis of Factors Associated with *TAP-1* and *TAP-2* Expression

Predictor	<i>TAP-1</i> β (95% CI)	p-value	<i>TAP-2</i> β (95% CI)	p-value
Smokeless tobacco	-0.083 (-0.164 to -0.002)	0.047	-0.052 (-0.119 to 0.015)	0.127
Mixed tobacco	-0.126 (-0.233 to -0.019)	0.021	-0.091 (-0.174 to -0.008)	0.032
OSCC	-0.148 (-0.271 to -0.025)	0.019	-0.109 (-0.207 to -0.011)	0.029
Histological grade	-0.062 (-0.118 to -0.006)	0.031	-0.049 (-0.096 to -0.002)	0.041

Multivariate Regression Analysis

Multivariate linear regression analysis was conducted to test independent associations in which *TAP-1* and *TAP-2* expression were dependent variables (Table 3).

In the case of *TAP-1* mixed tobacco exposure, a slight decrease in expression was significantly related ($\beta = -0.126$; C.I = -0.233 to -0.019; $p = 0.021$) but smokeless tobacco use was borderline ($\beta = -0.083$; $p = 0.047$). Also, histological grade increase was linked with the reduced expression of *TAP-1* ($\beta = -0.062$; $p = 0.031$). In the case of *TAP-2* a borderline non-significant independent relationship was found between mixed tobacco exposure and lower expression ($\beta = -0.091$; $p = 0.032$), and tumor dedifferentiation ($\beta = -0.049$; $p = 0.041$). *TAP* expression was not independently related to age and sex. These results together indicate that there is an important association of tobacco exposure and tumor dedifferentiation with small, marginal decreases in the expression of *TAP-1* and *TAP-2*. The agreement between epidemiological patterns of exposure and corrected molecular information evolves a model of minor antigen-processing modification in oral premalignant transformation and malignant progression, but shows no indication of deep immune suppression. Figure 1. demonstrates an increasing trend of mixed tobacco exposure across the oral carcinogenesis spectrum, accompanied by inverse, modest declines in *TAP-1* and *TAP-2* expression, while *GAPDH* remains stable, indicating subtle antigen-processing modulation associated with tobacco exposure.

Discussion

The current study investigated the link between tobacco exposure patterns and the expression of the antigen-processing genes *TAP-1* and *TAP-2* in oral premalignant lesions and oral squamous cell carcinoma (OSCC). Habitual exposures such as opium have been reported to modulate inflammatory pathways [15], and since inflammation represents a key component of immune defence, tobacco exposure may similarly influence genetic expression and the immune microenvironment, potentially through alterations in *TAP-1* and *TAP-2* expression; therefore, their expression was evaluated in the present study. Integrating epidemiological exposure data with $2^{-\Delta\Delta Ct}$ -based gene expression analysis sheds light on how identified risk factors can lead to immune dysregulation during oral carcinogenesis [9]. A distinct gradient of tobacco exposure was identified from controls to premalignant lesions and then to OSCC, with mixed tobacco habits predominating in malignant cases. This distribution is consistent with current epidemiological evidence indicating that combined smoked and smokeless tobacco use has a higher carcinogenic risk than isolated exposure. Importantly, the control group was largely non-exposed, supporting its suitability as a biological baseline for evaluating exposure-related molecular changes [17].

The majority of instances with oral lichen planus (OLP) and non-tobacco-exposed persons showed mostly maintained antigen-processing activity, according to analysis of *TAP-1* expression. These lesion-specific

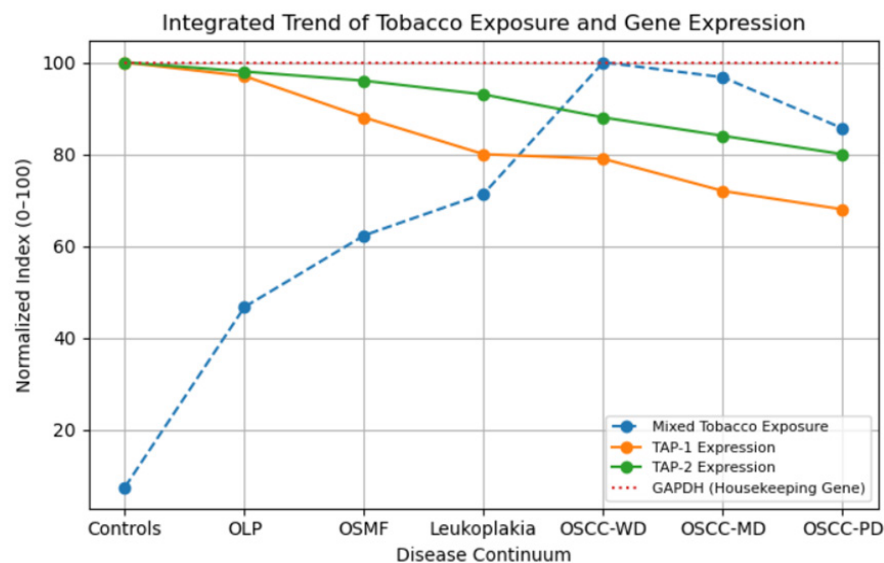


Figure 1. Integrated Trend of Tobacco Exposure and Gene Expression Across Disease Continuum

differences in TAP expression may reflect variations in the local immune microenvironment and stromal composition. For instance, OLP is characterized by a predominantly immune-mediated inflammatory response, whereas leukoplakia and OSMF are more strongly associated with chronic carcinogen exposure and fibrosis. Such differences in immune cell infiltration, cytokine milieu, and stromal remodeling may contribute to the observed heterogeneity in antigen-processing activity. This finding supports the idea that OLP may include immune processes different from traditional carcinogen-induced pathways and is not predominantly caused by tobacco use, despite its inflammatory nature. On the other hand, leukoplakia linked to exposure to smokeless tobacco showed a marginally significant but statistically significant decrease in *TAP-1* expression. This result implies that early immune regulation may take place at the premalignant stage, especially in lesions with high exposure to smokeless tobacco and areca nut, which are known to cause long-term mucosal fibrosis and inflammation [18].

Despite significant exposure to smokeless tobacco, oral submucous fibrosis (OSMF) displayed near-baseline *TAP-1* and *TAP-2* expression. The fibrotic and stromal-dominant character of OSMF, where carcinogenic progression is sluggish and immune escape mechanisms may not yet be completely activated, may be reflected in this relative preservation. Alternatively, it might suggest that lesion biology and exposure have an impact on antigen-processing impairment, which is not consistent across all premalignant situations [19, 20].

Both *TAP-1* and *TAP-2* expression demonstrated slight but statistically significant decreases in OSCC, especially in those with mixed tobacco habits. Although the present findings demonstrate reduced expression of *TAP-1* and *TAP-2*, the study remains observational and does not include functional validation of antigen-processing activity. As TAP proteins are essential for peptide transport into the endoplasmic reticulum and subsequent MHC class I antigen presentation, reduced expression may impair effective immune recognition. However, functional assays such as MHC class I surface expression analysis or peptide transport studies were not performed in the current study. Therefore, the direct impact of TAP downregulation on antigen presentation and cytotoxic T-cell response could not be established and warrants further investigation. A progressive decrease in expression from well-differentiated to poorly differentiated tumors was shown by stratification by histological grade. This gradual reduction in TAP expression may also be interpreted within the framework of tumor immunoediting, where early-stage tumors adopt partial impairment of antigen presentation to evade immune detection while avoiding complete loss of immunogenicity. These results corroborate the idea that as tumors develop and dedifferentiate, immune evasion mechanisms become more potent. The reduction's small magnitude, however, suggests that the antigen-processing machinery was only slightly modulated rather than severely suppressed. Even modest reductions in TAP expression may have important immunological consequences, as antigen presentation is highly sensitive to peptide transport efficiency. Partial

impairment of TAP function can reduce the repertoire of peptides presented on MHC class I molecules, thereby weakening cytotoxic T-cell recognition while still maintaining sufficient antigen presentation to avoid complete immune elimination. This subtle modulation may represent an adaptive tumor strategy to achieve immune escape without triggering strong immune activation. While avoiding total immunological invisibility, a tactic that is becoming more widely acknowledged in tumor immune escape, such modest downregulation might be adequate to hinder efficient cytotoxic T-cell identification [8, 19].

TAP-2 changes were less noticeable but followed a similar pattern to *TAP-1*, indicating that these transporters are differently sensitive to tobacco-related and tumour-associated factors. *TAP-2* changes were less pronounced but followed a similar trend to *TAP-1*, suggesting coordinated yet potentially differential regulation of these transporters. Although *TAP-1* and *TAP-2* function as a heterodimer for peptide transport, their expression may be independently regulated at the transcriptional or epigenetic level. Previous studies have indicated that *TAP-1* is more frequently downregulated in malignancies, possibly due to selective immune pressure or promoter-specific regulatory mechanisms. In contrast, relatively preserved *TAP-2* expression may reflect partial maintenance of antigen-processing capacity. Such differential modulation may influence the efficiency and selectivity of peptide presentation, thereby contributing to tumor immune evasion. Epigenetic regulatory mechanisms may also contribute to the observed alterations in TAP expression; DNA methylation, histone modifications, and microRNA-mediated suppression have been reported to influence antigen-processing machinery during early carcinogenesis.

A hypothesis of gradual attenuation of MHC-I-mediated antigen presentation rather than abrupt pathway failure is supported by the coordinated but minor reduction of both TAP components in OSCC [21]. These findings were further supported by multivariate regression analysis, which showed that, even after controlling for potential confounders, mixed tobacco exposure and rising histological grade were independently linked to slight reductions in *TAP-1* and *TAP-2* expression. The primary importance of exposure and tumor biology in causing these alterations is shown by the absence of independent correlations with age and sex. All of these findings point to tobacco's dual roles as a mutagenic agent and an immunomodulatory factor that modifies the body's ability to handle antigens during oral carcinogenesis [22].

The relatively small sample size of this study may have hampered the statistical power to identify tiny effect sizes and the generalizability of the results. Additionally, care should be taken when interpreting subgroup analyses across tumor grades and lesion types. However, the biological significance of the findings is reinforced by the cautious analytical strategy and steady exposure-expression patterns. Additionally, the hospital-based design may introduce selection bias, and peripheral blood-based expression analysis may not fully reflect tumour microenvironment-specific antigen processing changes; however, it provides a minimally

invasive approach to assess systemic immune alterations and may serve as a potential biomarker source for disease monitoring. Also, the lack of functional validation of TAP-mediated antigen presentation limits the ability to directly correlate gene expression changes with immunological outcomes. Additionally, tobacco exposure was categorized qualitatively, and the lack of quantitative measures such as duration or cumulative exposure limits the ability to establish precise exposure–response relationships with TAP expression.

Crucially, this study's conservative interpretation emphasises that immune dysregulation in oral cancer may be gradual and biologically complex. These minute alterations could have an impact on lesion persistence, progression risk, and response to immune-based treatments, making them especially important in the early stages of the disease. Furthermore, the absence of tumor tissue-based validation limits direct correlation between systemic and local gene expression patterns. Future studies should focus on functional validation of TAP-mediated antigen presentation using assays such as MHC class I surface expression analysis and peptide transport studies to better understand the immunological consequences of TAP downregulation.

In conclusion, this study shows that exposure to tobacco, especially mixed tobacco use, is linked to slight but significant decreases in *TAP-1* and *TAP-2* gene expression over the range of OSCC and oral premalignant lesions. Leukoplakia displayed early, borderline *TAP-1* downregulation, but OSMF mainly maintained baseline expression. Premalignant lesions displayed lesion-specific patterns. Modest and coordinated decreases in *TAP-1* and *TAP-2* expression were seen in OSCC; these decreases were more noticeable as tumour dedifferentiation increased. The lesion-specific patterns of TAP expression observed in leukoplakia and oral submucous fibrosis further suggest that immune dysregulation during oral carcinogenesis is heterogeneous and influenced by both exposure type and underlying molecular alterations, including microRNA-mediated regulation reported in earlier studies [23]. Tobacco negatively impacts serum concentrations of retinol, alpha-tocopherol, selenium, and zinc levels which are important component of anti-oxidant and immune system. These results lend credence to a paradigm in which risk factors associated with tobacco use modulate antigen-processing pathways subtly rather than significantly suppressing the immune system. Immune escape during premalignant transformation and malignant progression may be facilitated by such gradual immunological dysregulation. Risk assessment, early identification, and the creation of immunopreventive or immunotherapeutic approaches for oral cancer may all be impacted by an understanding of these early and mild changes in immune machinery. Understanding these early and subtle immune alterations may have implications for risk stratification, immunoprevention, and the development of adjunctive therapeutic strategies targeting immune modulation and nutritional or micronutrient-based interventions [23-26].

Author Contribution Statement

Study conception and design: Shailendra Dwivedi, Vijay Singh; Data collection: Vijay Singh, Neeraja Suresh, Anushka Rathore; Analysis and interpretation: Vijay Singh, Ruchika Agrawal, Akash Bansal; Manuscript drafting: Vijay Singh, Shailendra Dwivedi; Critical revision: Shailendra Dwivedi, Shashank Shekhar, Deepika Gupta; Final approval: All authors reviewed and approved the final manuscript

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Scientific Approval / Thesis Statement

This study was conducted as part of an academic research work. The study forms a component of the postgraduate (MSc) research work conducted at the Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Gorakhpur.

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Ethical Approval

This case-control research study was commenced after the acquisition of ethical approval from the Institutional Human Ethics Committee (reference number: IHEC/AIIMS-GKP/BMR/118/2023). The research study selected a convenience sample of case subjects from the ENT OPD of our Institute, All India Institute of Medical Sciences, Gorakhpur

Availability of Data

The datasets generated and/or analyzed during the current study

Conflict of Interest

The authors declare no conflicts of interest relevant to the content of this manuscript

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