

Original Article

Salivary Cotinine and Leptin as Biomarkers of Tobacco Exposure and Oral Carcinogenesis

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KEY WORDS

Enzyme-linked immunosorbent assay (ELISA);
Leukoplakia, Oral;
Oral Squamous Cell Carcinoma;
Tobacco smokers;

ABSTRACT

Background: Tobacco use is a major etiological factor in oral potentially malignant disorders (OPMDs) and oral cancer. Cotinine, a stable metabolite of nicotine, serves as a reliable biomarker of tobacco exposure and can be non-invasively detected in saliva. Leptin, an adipokine involved in immune and inflammatory pathways, demonstrates altered expression in neoplastic conditions and is reported to be reduced in smokers. In this context, evaluating salivary cotinine and leptin may provide valuable insights into tobacco exposure and oral carcinogenesis.

Purpose: This study aimed to quantify, compare, and correlate salivary cotinine and leptin levels among tobacco smokers (TS), oral leukoplakia (OL), and oral squamous cell carcinoma groups (OSCC).

Materials and Method: This observational analytical cross-sectional study comprised 63 male participants categorized into three groups: TS, OL, and OSCC, with 21 participants in each group. Unstimulated fasting saliva samples were collected and salivary cotinine and leptin levels were quantified using enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed using the Mann-Whitney U test, Kruskal-Wallis test, one-way ANOVA and Spearman's rank correlation test, with p Value < 0.05 considered statistically significant.

Results: Mean salivary leptin and cotinine levels were lowest in the OSCC group, followed by TS and highest in the OL group, with a statistically significant difference observed for leptin ($p = 0.001$). Mean cotinine levels among active smokers were significantly different within the OSCC, OL and TS groups ($p = 0.007$, 0.014 and < 0.001 , respectively). A significant positive correlation was observed between salivary leptin levels and body mass index (BMI) in OL group ($p = 0.008$).

Conclusion: Salivary leptin shows promise as a non-invasive biomarker in oral carcinogenesis, with elevated levels in OL and reduced levels in OSCC, along with a significant association with BMI. In contrast, salivary cotinine primarily reflects tobacco exposure and lacks discriminatory potential, indicating independent roles of these biomarkers.

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Introduction

Cancer remains a major global public health challenge,

with its burden continuing to increase despite substantial advances in diagnostic and therapeutic strategies [1].

Oral cancers including those affecting the floor of the mouth, palate, lips, tongue, gingiva, and oral mucosa account for approximately 250,000 new cases annually worldwide. These malignancies are frequently diagnosed at advanced stages, necessitating extensive treatment and often resulting in poor prognosis. Oral cancer is commonly preceded by precancerous lesions and conditions that manifest clinically as red or white patches within the oral cavity. These entities are collectively termed oral potentially malignant disorders (OPMDs), a group of conditions affecting the oral mucosa with an increased risk of malignant transformation [2-3].

Among OPMDs, oral leukoplakia (OL) is the most frequently encountered lesion and is defined as “a predominantly white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer” [4]. Its global prevalence ranges from 1.5% to 4.1%, with reported malignant transformation rates varying widely from 0.1% to 36.4%. This variability can be attributed to differences in diagnostic criteria, geographic distribution, study populations, risk factor exposure, reporting standards, duration of follow-up, and completeness of outcome assessment [3, 5].

Similarly etiological factors contribute, either individually or synergistically, to the development of both OPMDs and oral cancer. These include tobacco use in both smoked and smokeless forms, alcohol consumption, dietary influences, biological agents such as viruses and genetic alterations involving oncogenes and tumor suppressor genes [6-8]. The observed reduction in OL prevalence following smoking cessation highlights the strong etiological role of tobacco, with smoking increasing the risk of leukoplakia and its malignant transformation through the generation of reactive oxygen species and consequent oxidative damage to the oral mucosa, thereby establishing it as a major epidemiological risk factor [7-8]. Cancers attributable to tobacco use account for a significant share of the global cancer burden, with the World Health Organization (WHO) estimating that nearly half of individuals who begin smoking at a young age may ultimately develop tobacco-associated malignancies [9]. Tobacco smoke contains more than fifty established carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons [10]. Nicotine, the primary addictive component of to-

bacco in both smoked and smokeless forms, has a short half-life of approximately 2-3 hours; however, it is extensively metabolized in the liver to cotinine, with nearly 80% undergoing this conversion via C-oxidation. Due to its longer half-life of around 20 hours and slower elimination, cotinine accumulates in body fluids and serves as a reliable biomarker for assessing recent tobacco exposure [11].

Chronic tobacco use is also associated with sustained inflammatory responses and increased lipid peroxidation, largely mediated by adipokines released from adipose tissue. Among these, adipocytokines such as leptin and adiponectin play key roles in regulating inflammation and metabolic processes [1, 12]. Leptin, a 16 kDa cytokine-like protein composed of 167 amino acids, is involved in the regulation of body weight, energy balance, and metabolism. Alterations in leptin levels can influence metabolic homeostasis and have been increasingly implicated in inflammatory and neoplastic conditions [13]. Notably, most studies report decreased leptin levels in various malignancies, including oral squamous cell carcinoma (OSCC), whereas elevated levels have been observed in OPMDs, suggesting a differential role in disease progression [14].

Leptin and cotinine can be detected in various biological fluids, including blood, serum, urine, cervical exudate, and saliva. Among these, saliva offers distinct advantages as a diagnostic medium due to its non-invasive, patient-friendly nature and prolonged stability, making it well-suited for large-scale and outreach-based studies [15].

A comprehensive review of the literature reveals a significant paucity of studies investigating the combined role of leptin and cotinine in OL and OSCC, with only limited evidence available regarding their individual significance in OL. Therefore, the present study was undertaken to evaluate the correlation between salivary leptin and cotinine levels among tobacco smokers, patients with OL and OSCC using enzyme-linked immunosorbent assay (ELISA).

Materials and Method

A prospective, observational, cross-sectional study was carried out in the outpatient Department of Oral Pathology at a dental teaching institution. A total of 63 male participants aged 35- 65 years were enrolled and equally

allocated into three groups ($n = 21$ each) based on tobacco-use history, clinical examination, and histopathological diagnosis where applicable. Group I (TS) comprised tobacco smokers without clinically evident oral lesions. Group II (OL group) included patients with clinically diagnosed OL, and Group III (OSCC group) consisted of patients with clinically suspected and histopathologically confirmed OSCC. Participants with a history of tobacco smoking for more than one year were included and further categorized as very light smokers (≤ 5 cigarettes/day) or heavy smokers (> 5 cigarettes/day) [16]. Only male participants were included to minimize sex-related variability in salivary cytokine levels, as sex hormones can influence immune responses and cytokine expression. This approach reduced potential confounding and improved group homogeneity [17].

Individuals with systemic diseases including metabolic, inflammatory, autoimmune, or other chronic systemic disorders were excluded from the study. Additional exclusion criteria included a history of chemotherapy, radiotherapy, or surgery; malignancies other than OSCC, obesity, bronchial asthma, drug allergies, alcohol consumption, tobacco chewing, use of medications known to influence inflammatory or metabolic biomarkers (e.g., corticosteroids, immunosuppressive agents or long-term anti-inflammatory drugs), and a body mass index (BMI) below 18kg/m^2 or above 25kg/m^2 .

The study protocol was approved by the Biomedical and Health Research Ethics Committee (PGIDS/BHRC/22/37) and written informed consent was obtained from all participants in accordance with the *Declaration of Helsinki*. Sample size estimation was performed using Open Epi software (USA), considering a 99% confidence interval and 95% study power, which yielded a minimum requirement of 21 participants per group.

Demographic and clinical details were recorded, and anthropometric measurements, including height and weight, were obtained for BMI calculation before sample collection. Participants were instructed to refrain from food, beverages, and smoking for 8-10 hours prior to saliva collection. Unstimulated fasting whole saliva samples were collected between 9:00 a.m. and 12:00 p.m. following a brief mouth rinse with water for 1-2 minutes. The passive drooling method was used, wherein participants expectorated saliva into sterile collection containers at 30-second intervals over a period of 5

minutes. Subsequently, saliva samples were stored under appropriate conditions for the estimation of leptin and cotinine using ELISA.

The collected saliva samples were centrifuged at 3000 rpm for 15 minutes at 4°C to remove debris and mucin. The supernatant was aliquoted and stored at -80°C until further analysis. Salivary leptin and cotinine levels were quantified using sandwich ELISA (Lisawash 4000) with commercially available kits [Human LEP ELISA Kit Code: EH0216, Fine Biotech Co., Ltd., Wuhan, China; Human Cotinine Catalogue No: E2043Hu, BT-LAB, Jiaxing Korain Biotech Co. Ltd., Zhejiang, China], following instructions of the manufacturer. Quantification was performed using standard calibration curves with concentration ranges of 3-48 (3, 6, 12, 24 & 48) ng/mL for cotinine and 0-2000 (0, 62.5, 125, 250, 500, 1000 & 2000) pg/mL for leptin.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., USA). Descriptive statistics were expressed as mean and standard deviation. The Shapiro-Wilk test demonstrated non-normal data distribution. Intergroup comparisons of leptin and cotinine levels were carried out using the Kruskal-Wallis test, followed by pairwise comparisons with the Mann-Whitney U test. One-way analysis of variance (ANOVA) was used to compare BMI and weight among the study groups. Correlations between leptin, BMI, and cotinine were assessed using Spearman's rank correlation coefficient. All statistical tests were two-tailed and a p Value < 0.05 was considered statistically significant.

Results

A total of 63 male participants (21 per group) aged 35-65 years were included in the study. The mean age was comparable across the groups (TS: 45.25 ± 9.24 years; OL: 51.04 ± 9.88 years; OSCC: 52.38 ± 9.12 years), with no statistically significant difference (p Value = 0.905). At the time of sample collection, the mean body weight was lowest among participants with OSCC (55.6 ± 10.6 kg), followed by those with OL (60.66 ± 10.39 kg) and TS (65.19 ± 7.44 kg). Similarly mean BMI was lowest in the OSCC group, followed by OL and TS groups (19.9 ± 3.60 , 21.52 ± 1.83 & 22.48 ± 1.74 kg/m^2 , respectively). One-way ANOVA revealed statistically significant differences in both weight and BMI across the

Table 1: Distribution of demographic characteristics, mean body weight, body mass index (BMI) and salivary levels of leptin and cotinine among the study groups

Parameters	TS, n-21	OL, n-21	OSCC, n-21	p Value	Inference
Age, Range (years)	35-65	35-65	35-65	-	-
Mean Age (years)	45.25±9.24	51.04±9.88	52.38±9.12	0.905	Not significant
Sex (All males)	21	21	21	-	-
Mean salivary leptin (pg/ml)	166.30 ± 107.74	237.51 ± 165.59	75.47 ± 71.02	0.000*	Highly significant
Mean salivary cotinine (ng/ml)	9.88 ± 4.99	12.90 ± 12.01	8.69 ± 3.82	0.822*	Not significant
Mean Weight (kg)	65.19±7.44	60.66±10.39	55.6±10.6	0.003 [#]	Significant
Mean BMI (kg/m ²)	22.48±1.74	21.52±1.83	19.9±3.60	0.001 [#]	Significant

n= Number of participants, TS= Tobacco smokers with no lesion; OL= Oral leukoplakia; OSCC= Oral squamous cell carcinoma; BMI= body mass index; pg/ml=picogram per milliliter; ng/ml=nanogram per milliliter; kg=kilograms; kg/m²= kilogram per meter square; Mean±standard deviation; *Kruskal Wallis Test^{*}; [#]One way ANOVA test[#] ($p < 0.05$ significant, > 0.05 non-significant)

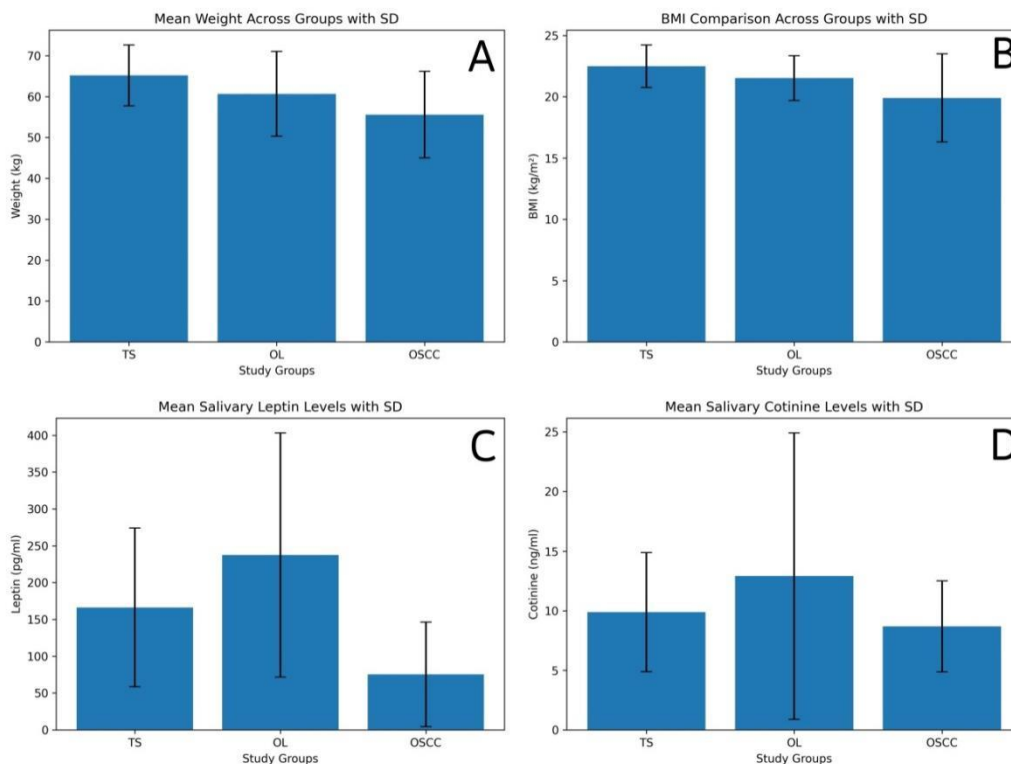
groups ($p = 0.003$ and 0.001 , respectively).

In the quantitative analysis of salivary biomarkers, both mean salivary leptin and cotinine levels were lowest in the OSCC group, followed by TS and highest in the OL group (leptin: 75.47 ± 71.02 , 166.30 ± 107.74 and 237.51 ± 165.59 pg/mL; cotinine: 8.69 ± 3.80 , 9.88 ± 4.99 and 12.90 ± 12.01 ng/mL, respectively). The difference in leptin levels was highly significant ($p < 0.001$), whereas cotinine levels did not show a statistically significant difference ($p = 0.822$) using the Kruskal-Wallis test (Table 1). The graphical representation of the study parameters is illustrated in Figures 1a-d.

Intergroup comparison of median salivary leptin levels demonstrated a statistically significant difference

between OSCC and OL ($p = 0.000$) as well as between OSCC and TS ($p = 0.002$). However, no significant difference was observed between OL and TS ($p = 0.191$). In contrast, median salivary cotinine levels did not show any statistically significant differences between the groups, including OSCC vs. OL ($p = 0.940$), OL vs. TS ($p = 0.521$) and OSCC vs. TS ($p = 0.678$) (Figure 2).

Study participants were further categorized as light and heavy smokers based on their smoking habits. Stratification by smoking intensity revealed no statistically significant differences in mean salivary leptin levels between light and heavy smokers across all groups, including OSCC ($p = 0.522$), OL ($p = 0.456$) and TS ($p = 0.201$). In contrast, mean salivary cotinine levels were

**Figure 1: A-D:** Comparison of body weight, BMI, mean salivary leptin, cotinine across study groups (TS, OL, OSCC)

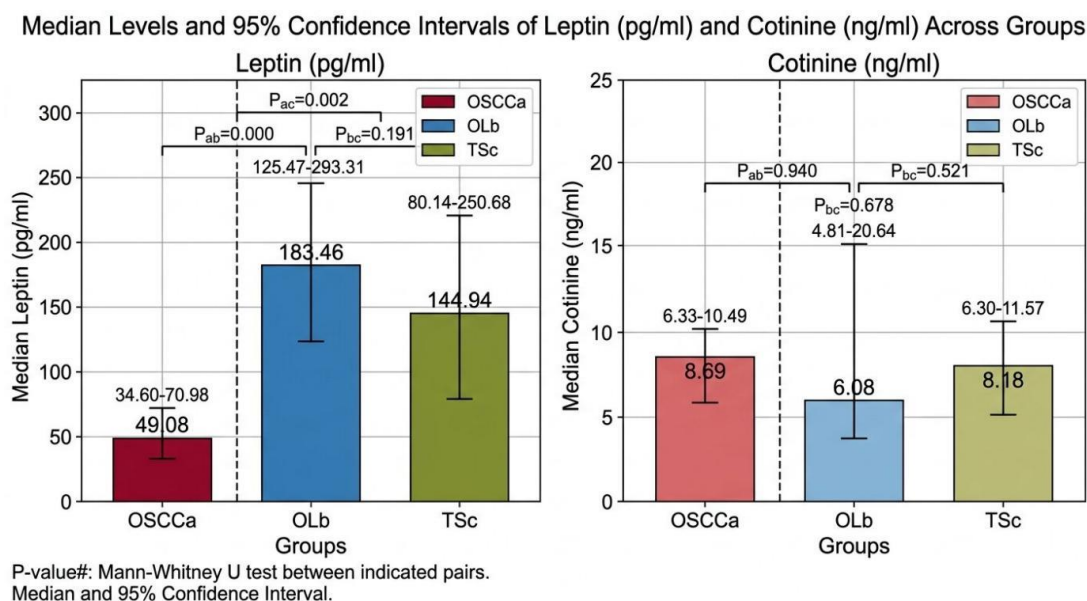


Figure 2: Comparison of median salivary leptin and cotinine levels with 95% confidence intervals among the study groups

significantly higher in heavy smokers compared to light smokers across all groups, including OSCC ($p=0.007$), OL ($p=0.014$), and TS ($p<0.001$) (Table 2).

Correlation analysis using Spearman's rank test demonstrated a statistically significant positive correlation between salivary leptin levels and BMI in the OL group ($p=0.008$). However, no significant correlation was observed between leptin and BMI in the OSCC ($p=0.223$) and tobacco smoker groups ($p=0.647$) (Table 3). Further analysis revealed no significant association between salivary leptin and cotinine levels across the study groups. A weak negative correlation was observed between OSCC and TS ($r=-0.093$, $p=0.557$), while no correlation was noted between OL and TS ($r=-0.002$, $p=0.988$) (Table 4).

Discussion

The present study evaluated the diagnostic potential of salivary cotinine and leptin in tobacco smokers, OL, and OSCC with an emphasis on their role in tobacco exposure and oral carcinogenesis. Saliva has emerged as a

promising, non-invasive biofluid for biomarker-based screening owing to its ease of collection, accessibility, and suitability for large-scale population screening [1]. Although the primary objective of this study was to quantify salivary cotinine and leptin levels in tobacco smokers, OL and OSCC using ELISA, and to explore potential correlations between these markers, the selection criteria included only male participants. This was done because women, even with comparable BMI, generally have higher adipose tissue and experience hormonal variations that can influence leptin levels [17].

Furthermore, in the selected population, men exhibited higher prevalence and frequency of tobacco use, whereas tobacco consumption among women was often underreported. A similar strategy was employed by Jaleel *et al.* [12], who included only male participants to assess leptin and blood lipid levels in smokers and non-smokers.

The present study demonstrated that both the mean body weight and BMI differed significantly among the study groups ($p=0.003$ and 0.001 respectively). The

Table 2: Comparison of mean salivary leptin and cotinine levels between light and heavy smokers among the study participants

Groups	Type of Smokers	Leptin (pg/ml)	p Value #	Cotinine (ng/ml)	p Value #
OSCC	LS (n-12)	95.69±88.80	0.522	7.13±3.74	0.007
	HS (n-9)	48.49±18.24		10.77±2.96	
OL	LS (n-7)	181±91.24	0.456	4.77±0.81	0.014
	HS (n-14)	265.61±189.19		16.97±12.98	
TS	LS (n-12)	191.87±117.49	0.201	6.52±2.07	0.000
	HS (n-9)	132.22±88.05		14.36±4.13	

n= number of participants; TS= tobacco smokers with no lesion; OL=oral leukoplakia; OSCC= oral squamous cell carcinoma; LS: light smokers; HS: Heavy smokers; #Mann Whitney U Test ($p<0.05$ significant, >0.05 non-significant); Mean ±SD = Mean±standard deviation

Table 3: Correlation between salivary leptin levels and body mass index (BMI) across the study groups

Groups	Mean salivary leptin (pg/ml)	Mean BMI (kg/m ²)	r	p Value	Inference
OSCC	75.47 ± 71.02	19.9±3.60	1.00	0.223	Not significant
OL	237.51 ± 165.59	21.52±1.83	1.00	0.008	Significant
TS	166.30 ± 107.74	22.48±1.74	1.00	0.647	Not significant

TS= tobacco smokers with no lesion; OL=oral leukoplakia; OSCC= oral squamous cell carcinoma; BMI=body mass index; pg/ml=picogram per milliliter; kg/m²= kilogram per meter square; Spearman's rho correlation (r) test (p Value:<0.05 significant,>0.05 non-significant)

Table 4: Correlation between salivary leptin and cotinine levels across the study groups

Groups	Correlation coefficient between Leptin and Cotinine *	p Value
OSCC versus TS	-0.093	0.557
OL versus TS	-0.002	0.988

TS= tobacco smokers with no lesion; OL=oral leukoplakia; OSCC= oral squamous cell carcinoma; *Spearman's rho correlation test (p Value < 0.05 significant,>0.05 non-significant)

observed progressive decline in weight and BMI from tobacco users to OL and ultimately to OSCC may reflect the systemic impact of disease progression. This trend suggests that advancing pathological changes are accompanied by metabolic alterations, potentially driven by tumor-associated factors. One plausible explanation for this decline is the role of pro-inflammatory cytokines, which are known to be elevated in malignancy. These cytokines can disrupt normal metabolic processes, leading to reduced appetite, increased energy expenditure, and subsequent weight loss, eventually culminating in cancer-associated cachexia [18]. Thus, the reduction in BMI and body weight observed in OSCC patients may not only serve as a clinical indicator of disease severity but also highlight the underlying biological interplay between tumor progression and systemic metabolism.

In the present study, mean salivary leptin levels were highest in the OL group, followed by tobacco smokers and lowest in OSCC, with a statistically significant difference among all the groups. This pattern suggests a potential role of leptin in the early stages of oral carcinogenesis, characterized by elevated levels in premalignant conditions and a subsequent decline with malignant transformation. The increased leptin levels in OL may reflect its involvement in inflammatory pathways and cellular proliferation, whereas the reduced levels in OSCC may reflect tumor-associated metabolic alterations, depletion of adipose tissue secondary to cancer-associated cachexia and dysregulation of adipokine signaling [19].

Leptin is primarily synthesized by adipocytes, and its circulating concentration is closely associated with adipose tissue mass and energy homeostasis. Progressive weight loss and cancer-associated cachexia, which are common in patients with OSCC, may reduce leptin production through depletion of adipose tissue. In addition, chronic systemic inflammation and tumor-derived cytokines, including interleukin-6 and tumor necrosis factor- α , contribute to metabolic dysregulation and negative energy balance, further influencing leptin secretion. Although leptin has been shown to promote cellular proliferation, angiogenesis and inflammatory signaling through pathways such as JAK/ STAT, PI3K/Akt and MAPK, systemic or salivary leptin concentrations may decline in advanced malignancy owing to these metabolic alterations. This apparent discrepancy, often referred to as the leptin paradox, suggests that local leptin signaling within the tumor microenvironment may not parallel circulating or salivary leptin concentrations [18-20]. The biological mechanisms underlying reduced salivary leptin levels in OSCC require further investigation, as the present study did not evaluate leptin receptor expression or downstream signaling pathways. Therefore, the proposed mechanisms should be considered biologically plausible rather than definitive. Nevertheless, the observed pattern is supported by the limited available literature. To the best of our knowledge, salivary leptin has been scarcely investigated in oral cancer, with only one previous study by Kaur *et al.* [17] reporting significantly lower salivary leptin levels in patients with OSCC than in healthy controls (55pg/mL vs. 178 pg/ mL), consistent with the findings of the present study.

A similar trend has been reported in serum-based studies, demonstrating reduced leptin levels in OSCC compared to tobacco smokers (Kamboj *et al.* [1]; 3.62± 1.11 ng/ml <6.27± 2.36 ng/ml), in OSCC compared to healthy controls (Gharote *et al.* [13]; 2.67±1.98 ng/ml < 5.71±3.58ng/ml; Kaur *et al.* [17] 3.54ng/ml <5.06 ng/

ml). This reduction may be explained by decreased body fat mass and weight loss commonly seen in cancer patients, affecting leptin levels in both serum and saliva [1].

Furthermore, earlier studies in head and neck SCC have demonstrated lower serum leptin levels in malignant conditions compared to premalignant lesions, which aligns with the present salivary findings [13-14]. As salivary leptin has not been extensively evaluated in OL, the elevated levels observed in this study provide a valuable reference point for the future research. The higher leptin levels in OL may be attributed to increased pro-inflammatory mediators, such as interleukins (IL-6, IL-17) and leptin itself, along with a relative decrease in anti-inflammatory mediator adiponectin. This imbalance may contribute to the inflammatory microenvironment characteristic of premalignant lesions, whereas reduced levels in OSCC may reflect altered tumor biology and systemic metabolic changes [14].

Age, sex, and BMI-matched tobacco smokers without clinically evident oral lesions were included as the control group to minimize potential confounding by demographic and anthropometric factors. In this study, salivary leptin levels in tobacco smokers were higher than in OSCC patients but lower than in the OL group. This trend aligns with previous serum-based study, which reported higher leptin levels in tobacco smokers compared to OSCC (TS: 6.27 ± 2.36 ng/mL vs. OSCC: 3.62 ± 1.11 ng/mL) [1]. Similarly, a study comparing serum leptin in tobacco smokers and ex-smokers observed lower leptin levels in current smokers (9.76 vs. 29.76 ng/dL) [12]. This pattern may reflect the combined effects of systemic metabolic and inflammatory changes associated with tobacco use and disease progression. In tobacco smokers without oral lesions, leptin levels are relatively elevated, possibly due to ongoing adipose tissue-mediated inflammatory activity and nicotine-induced modulation of adipokine secretion. Conversely, leptin levels decline in OSCC, likely as a consequence of cancer-associated weight loss, reduced adipose tissue mass and metabolic dysregulation, including cachexia and altered cytokine signaling. Likewise, lower leptin levels in current smokers compared to ex-smokers suggest that chronic tobacco exposure may suppress leptin production, whereas cessation allows partial recovery of adipose tissue function and leptin

secretion. These findings indicate that leptin reflects a complex interplay between adiposity, inflammation, and disease status rather than tobacco exposure alone.

A correlation was also identified between mean salivary leptin levels and BMI across the study groups, with a statistically significant association observed in the OL group ($p = 0.008$). This finding is in agreement with previous research demonstrating an inverse dose-response relationship between BMI and the risk of OL, even after adjusting for potential confounders such as age, gender, educational status, duration of tobacco use, smoking, and alcohol consumption. These observations suggest that individuals with lower BMI may be at a higher risk of developing leukoplakia. The underlying mechanisms may involve complex interactions between nutritional status, metabolic regulation, and hormonal factors. In particular, hormonal influences such as estrogen receptor activity and leptin signaling could be implicated, although their precise role in premalignant lesions and oral carcinogenesis remains to be fully elucidated [21]. These findings are also consistent with earlier investigations, which have established a strong association between serum and salivary leptin levels and BMI [13, 17]. Further studies are therefore necessary to clarify the association between hormonal profiles and leukoplakia risk.

From a clinical perspective, the observed association indicates that salivary leptin is influenced by systemic metabolic status in addition to disease-related changes. Therefore, BMI should be considered when interpreting salivary leptin concentrations and adjustment for anthropometric parameters may improve the diagnostic accuracy and clinical utility of leptin as a salivary biomarker for screening and risk assessment of OPMDs and OSCC.

Mean salivary cotinine levels were lowest in the OSCC group, followed by tobacco smokers and highest in OL, although the differences were not statistically significant. To date, no study has specifically evaluated the risk of OSCC based on salivary cotinine levels; however, lung cancer studies have demonstrated a strong dose-response relationship between blood cotinine levels and cancer risk [22-23]. The lower cotinine levels observed in OSCC patients may be attributable to the presence of ulceroproliferative oral lesions, which likely impaired regular smoking [24]. Tobacco use re-

mains a well-established risk factor for non-communicable diseases, including oral mucosal lesions and oral cancer. In particular, higher cotinine levels have been associated with increased frequency of oral mucosal lesions, such as leukoplakia, which can serve as an early indicator of malignant transformation and smoking is also clearly linked to nicotine stomatitis [25]. In continuation with these findings, the number of cigarettes smoked within the preceding 24 hours showed a positive correlation with salivary cotinine levels, reinforcing its reliability as a short-term biomarker of recent tobacco exposure. Although preliminary observations of salivary cotinine levels in OSCC and OL were documented in the present study, these findings require further validation through larger and more rigorous investigations to establish their consistency and diagnostic relevance.

Furthermore, the present study made a preliminary attempt to evaluate the correlation between salivary leptin and cotinine levels. The analysis revealed a non-significant negative correlation in both OSCC ($p=0.557$) and OL ($p=0.988$) groups when compared to controls. This lack of statistical significance suggests that leptin and cotinine may operate through distinct and independent biological pathways within the context of tobacco exposure and oral carcinogenesis. While leptin, an adipokine, is primarily involved in energy homeostasis, inflammation and tumor biology, cotinine serves as a biomarker of nicotine exposure, reflecting recent tobacco use rather than disease progression. Importantly, despite the absence of a significant correlation, leptin independently demonstrated a promising ability to differentiate between study groups, indicating its potential utility as a biomarker in oral carcinogenesis. In contrast, cotinine did not exhibit statistically significant discriminatory capacity in the present study. This disparity further reinforces the notion that while cotinine is valuable for assessing tobacco exposure, it may have limited applicability in distinguishing disease stages.

Overall, the present findings suggest that leptin and cotinine may play distinct roles in the pathophysiology of tobacco-associated oral lesions. Nevertheless, these observations are derived from a relatively limited sample size and therefore warrant cautious interpretation. Further studies incorporating larger cohorts, longitudinal follow-up and integrated biomarker panels are nec-

essary to validate the findings and to provide deeper insight into the complex interplay among metabolic, inflammatory and tobacco exposure-related markers in oral carcinogenesis.

Despite these important findings, certain limitations of the present study should be considered. Patients with OSCC often experience painful oral ulcerations, which may reduce their ability or willingness to continue tobacco use. Consequently, salivary cotinine levels may underestimate actual long-term tobacco exposure in this group. The relatively small sample size may have limited the statistical power of the study and reduced the generalizability of the findings. Furthermore, only male participants were included to minimize gender-related biological variability in salivary biomarkers; therefore, the findings may not be directly generalizable to females, in whom adipose tissue distribution, hormonal regulation and leptin metabolism differ. Finally, the absence of an age-matched, non-smoking healthy control group precluded the establishment of baseline salivary cotinine and leptin concentrations in individuals without tobacco exposure or oral lesions. Inclusion of such a control group in future studies would facilitate more robust comparisons and strengthen the interpretation of biomarker alterations associated with tobacco use, OL, and OSCC.

Additionally, clinical TNM/AJCC staging data were not available for the OSCC cases; therefore, stage-specific analyses could not be performed. Future studies incorporating standardized tumor staging are needed to determine whether salivary biomarker levels vary with disease stage and prognosis.

Importantly, the findings of the present study suggest that salivary leptin demonstrates comparable potential to serum leptin in predicting oral cancer risk, offering the advantage of a non-invasive and easily accessible diagnostic approach. Notably, salivary leptin levels were significantly higher in OL compared to OSCC, indicating its potential utility as an early diagnostic biomarker for identifying premalignant lesions prior to malignant transformation.

Furthermore, the significant correlation observed between salivary leptin levels and BMI in OL reinforces its potential relevance in disease risk assessment. Collectively, these findings suggest that salivary leptin may serve as a feasible and non-invasive alternative to serum

leptin estimation for risk prediction, offering a patient-friendly approach for both clinical practice and future research in oral carcinogenesis.

Future research should focus on large-scale, multi-centric studies including participants of both genders, passive smokers, and a broader spectrum of OPMDs stratified according to the presence and severity of epithelial dysplasia to further validate the present findings and establish the clinical utility of these salivary biomarkers. Longitudinal studies are warranted to explore the predictive potential of salivary leptin in the malignant transformation of OL. Moreover, the development and standardization of salivary biomarker assessment protocols are essential to support their integration into routine clinical practice and community-based screening initiatives.

Conclusion

The present study highlights the potential utility of salivary leptin as a non-invasive biomarker in oral carcinogenesis. The significantly elevated leptin levels observed in OL, followed by a notable decline in OSCC, suggest its possible involvement in early inflammatory and proliferative events, with subsequent reduction during malignant progression. The positive correlation between leptin levels and BMI further emphasizes its association with metabolic alterations and disease-related changes, including cancer-associated cachexia. In contrast, salivary cotinine predominantly reflected recent tobacco exposure and did not exhibit significant discriminatory ability among the study groups. Moreover, the absence of a significant correlation between leptin and cotinine suggests that these biomarkers may function through independent biological mechanisms. Collectively, these findings suggest that salivary leptin may serve as a promising adjunctive biomarker for the early detection and risk assessment of OPMDs and OSCC. However, given the influence of systemic metabolic factors on leptin concentrations, it is unlikely to function as a stand-alone diagnostic marker and may be better utilized as part of a multimarker panel in conjunction with other clinical and molecular biomarkers to improve diagnostic accuracy.

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Ethical approval

Biomedical and Health Research Ethics Committee (PGIDS/BHRC/22/37)

Author Contribution

Conceptualization- MK, JY, AN, AD; Methodology- MK, JY, AN, AD; Validation- MK, JY, AN, PSG, AD, AB; Formal Analysis- MK, JY, AN, AD, AB; Investigation- MK, JY, PSG; Writing-original draft- MK, JY; Supervision- MK, JY, AN, PSG, AD, AB; Project administration-MK; Funding acquisition-JY.

Research Highlights

- Salivary leptin and cotinine were evaluated as non-invasive biomarkers in tobacco smokers, oral leukoplakia and oral squamous cell carcinoma groups.
- Salivary leptin levels showed significant intergroup variation, with highest levels in oral leukoplakia and lowest levels in OSCC ($p=0.001$).
- Salivary cotinine levels effectively reflected active tobacco exposure across all study groups but did not demonstrate discriminatory value for oral carcinogenesis.
- A significant positive correlation was observed between salivary leptin levels and body mass index in the oral leukoplakia group ($p=0.008$).
- The findings suggest distinct biological roles of leptin and cotinine, highlighting the potential utility of leptin as a biomarker for early oral carcinogenic changes.

Conflicts of Interest

The authors deny any conflicts of interest related to this.

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