BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 9

DOI:10.58240/1829006X-2025.21.9-557

ORIGINAL ARTICALE

COMPARATIVE EXPRESSION OF TOLL-LIKE RECEPTOR 4 IN ORAL PAPILLARY AND CONVENTIONAL SQUAMOUS CELL CARCINOMA USING RT-QPCR AND IMMUNOHISTOCHEMISTRY (COMPARATIVE CROSS- SECTIONAL STUDY)

Nancy Abd Elrazik Abd Elhafiz Khedr[1], Awatef Ibrahim Draz[2], Noura Mohamed Kamal[3]

[1.]Assistant lecturer of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt. E-Mail: nancy.abdelhafiz@dentistry.cu.edu.eg

[2.]Professor of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt, E-Mail: *awatef.draz@dentistry.cu.edu.eg*

[3.]Lecturer of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo University, Cairo, Egypt, E-Mail: *noura.ali* @dentistry.cu.edu.eg

The corresponding author: Nancy Abd Elrazik Abd Elhafiz Khedr**;** assistant lecturer of Oral and Maxillofacial Pathology, Faculty of Dentistry, Cairo university, Egypt. **E-Mail:** nancy.abdelhafiz@dentistry.cu.edu.eg
Cell phone: 00201212686960

Received: *Aug 27. 2025;* ***Accepted:*** *Sep 29, 2025;* ***Published:*** *Oct, 23. 2025*

ABSTRACT

Background: Oral papillary squamous cell carcinoma (OPSCC) is a rare subtype of squamous cell carcinoma with debatable prognosis and metastatic potential. To better understand this neoplasm, it is essential to investigate its biological behavior, growth patterns, and tumor microenvironment. Our study aims to compare the expression of toll-like receptor 4 in papillary and various grades of conventional squamous cell carcinoma.

Materials & Methods: This comparative cross-sectional study included formalin-fixed paraffin-embedded samples of different grades of conventional squamous cell carcinoma, OPSCC, and normal epithelial tissue as a control group. TLR4 mRNA levels were evaluated using RT-qPCR, while protein expression was assessed by immunohistochemical staining. Statistical analysis was performed to compare expression between the groups.

Results: TLR4 exhibited cytoplasmic and variable nuclear expression in both OSCC and OPSCC, with the highest levels found in poorly differentiated SCC. RT-qPCR confirmed significantly elevated TLR4 mRNA in all cancer groups compared to controls. No statistically significant differences in TLR4 expression were observed between OPSCC and well-differentiated or moderately differentiated squamous cell carcinoma in either immunohistochemical or PCR analysis.

Conclusion: The results indicate that while OPSCC may display unique biological characteristics compared to conventional OSCC, its behavior appears to resemble that of OSCC with similar histological grading, regardless of connective tissue invasion.

Keywords*:*Cross-sectional study, Immunohistochemistry, Oral papillary squamous cell carcinoma, RT-qPCR, Squamous cell carcinoma and Toll-like receptor 4.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most prevalent cancers of the head and neck, with an increasing incidence in many countries. It predominantly affects males, with increased susceptibility in middle-aged and elderly men.[1] Oral papillary squamous cell carcinoma (OPSCC) is a rare, variant of squamous cell carcinoma that has a better prognosis.[2] It histologically and clinically resembles verrucous carcinoma or squamous cell papilloma due to the prominent papillary growth of squamous epithelium.[3]

Clinically, OPSCC appears as a soft, friable, polypoid, and exophytic mass with a papillary surface.[4] Histologically, two subtypes are recognized: the papillary variant, characterized by thin, finger-like projections with fibrovascular cores, and the exophytic type, featuring broad, bulbous projections.[5] Additionally, hyperparakeratinization and dysplastic

features, including increased mitosis, cellular and nuclear pleomorphism, nuclear hyperchromatism, individual cell keratinization, and basilar hyperplasia, are observed in the surface epithelium.[6]

Although OPSCC has been reported to have a relatively better prognosis than OSCC,[7] some studies suggest its invasive and metastatic potential.[8] Furthermore, certain studies have noted a high rate of recurrence and the development of secondary malignancies in some cases.[9-11]

Toll-like receptor 4 (TLR4) is a member of the TLR family that plays a key role in innate immunity by recognizing pathogen-associated molecular patterns and mediating inflammation. [12] Recent research has highlighted the role of innate immunity in carcinogenesis, positioning cancer immunotherapy as the emerging fifth pillar of cancer treatment, alongside surgery, chemotherapy, radiotherapy, and targeted therapy. [13]

Moreover, TLR4 is expressed in the basal cells of the oral epithelium, with a progressive increase in both the intensity and proportion of immunoexpression observed from normal oral epithelium to different grades of OSCC. [14- 15] High cytoplasmic TLR4 expression in OSCC has been associated with aggressive tumor behavior and poorer prognosis. [16]

TLR4 activation in tumor cells initiates signaling cascades that promote tumor invasion, chemoresistance, immune evasion, and metastasis through the release of cytokines and chemokines. [17] In addition to increasing tumor cell survival, TLR4 activation leads to the expansion of pro-vascular progenitors in the bone marrow and spleen and their recruitment to the cancer microenvironment to reconstruct epithelial, stromal, and vascular structures.[18] It also upregulates matrix metalloproteinases and integrins, facilitating epithelial–mesenchymal transition and metastasis. [19]

Despite advances in our understanding of these malignancies, there remains a critical need for reliable biomarkers to predict prognosis and guide treatment strategies. Our study aimed to compare the expression of TLR4 in OSCC and OPSCC to evaluate its potential as a prognostic biomarker. By examining the differences in TLR4 expression between these tumor variants, we hope to gain insights into their biological behaviors, metastatic potentials, and therapeutic responses.

To the best of our knowledge, this is the first study to directly compare conventional OSCC and papillary OSCC regarding TLR4 expression, offering new perspectives on their molecular profiles.

MATERIAL AND METHODS

Study design and sample size calculation

This comparative cross-sectional study was approved by the Research Ethical Committee of the Faculty of Dentistry, Cairo University (No. 4623), and was conducted in compliance with the Helsinki Declaration. The study took place from June 2023 to February 2025. The sample size was calculated based on the proportions reported by Pakdel et al., where TLR4 expression was 72.4% in squamous cell carcinoma and 12.7% in normal mucosa. [20] A two-sided Fisher's exact test was used, with an alpha level of 5%, power of 80%, and effect size of 0.0049. Sample size calculation was performed using G*Power version 3.1.9.2.

A total of 30 formalin-fixed paraffin-embedded tumor tissue blocks were retrieved from the archives of the Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Cairo University. Of the 30 cases, 12 were OPSCC, 6 were well-differentiated squamous cell carcinoma (WDSCC), 6 were moderately differentiated squamous cell carcinoma (MDSCC), and 6 were poorly differentiated squamous cell carcinoma (PDSCC). Additionally, 12 cases of normal oral mucosa archival blocks were collected from raised gingival flaps removed during surgical procedures for impacted third molars as a control group. Clinical information was gathered from histopathological reports.

Inclusion criteria:

- OSCC and OPSCC archived blocks from the last 10 years.
- Accurately fit the histopathological criteria set by WHO (2022).
- Normal oral mucosa archival blocks removed for surgical purposes such as gingival flaps raised during the removal of impacted third molars.

Exclusion criteria:

- SCC from other sites and origins outside the oral cavity.
- Cases that received treatment.
- Inadequate or missing tissue blocks.

Staining:

The specimens were stained histologically with H&E and immunohistochemically with TLR4 at the Faculty of Medicine, Cairo University.

Immunohistochemical (IHC) Staining:

All samples underwent IHC analysis for TLR4 expression. Blocks were selected based on histological confirmation and tissue adequacy.

First, sections (4 µm) were deparaffinized in xylene and rehydrated with graded alcohols for 10 minutes. Next, antigen retrieval was performed by boiling the tissue sections in 10 mM citrate buffer, pH 6.0 for 10-20 minutes followed by cooling at room temperature for 20 minutes. Sections were then incubated in 0.3%

hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. After washing, 400 µL of TLR4 antibody diluted 1:100 was applied and incubated at 30 °C for 60 minutes. The secondary antibody was then applied for 30 minutes at room temperature. Diaminobenzidine was applied for 15 minutes at room temperature after washing off the secondary antibody. The slides were counterstained with hematoxylin, dehydrated, and mounted.

Positive and Negative Controls:

Normal gastric mucosa was used as a positive control for TLR4 expression. The primary antibody was replaced with a solution of BSA in phosphate-buffered saline for the negative control.

Evaluation:

Two independent, blinded oral pathologists evaluated the slides using a transmitted light microscope to detect positive immunoreaction and localization of the immunostain within the tissues. Staining of the tumor cell cytoplasm, nucleus, or both was one of the criteria used to identify antigen-positive regions.

We examined the immunohistochemically stained sections using the Leica Qwin 500 software on the image analyzer computer system at the Faculty of Dentistry, Cairo University. Additionally, ImageJ software was used for measuring area percent and automated nuclear count of TLR4 immunoexpression, performed in a standard frame area of 5.04 x 10^6 µm2.

RT-PCR:

To detect TLR expression at the mRNA level, RT-qPCR was performed at Science Way Laboratory in Nasr City, Cairo ,Egypt. Sections of paraffin-embedded tissue (25 to 30 µm thickness) underwent deparaffinization, ethanol wash, RNA extraction, and RNA transformation into complementary DNA. PCR machine settings included denaturation, reverse transcription, enzyme inactivation, and cooling. The denaturation temperature was 95 °C for 45 seconds, the annealing temperature was 60 °C for 45 seconds, and the extension temperature was 72 °C for 1 minute. These steps were repeated for approximately 30 cycles, with mRNA for GAPDH was used as a normalization control in RT-PCR.

The following primer pair sequences were used in the PCR test to detect the TLR4 gene:

Forward Sequence**:** AGCTTCTCCAATTTTTCAGAACTTC

Reverse Sequence: TGAGAGGTGGTGTAAGCCATGC

Statistical Methods:

The Kolmogorov-Smirnov test was used to examine the data. The results indicated that most of the data were normally distributed; therefore, one-way analysis of variance (ANOVA) was used to compare the groups. Tukey's post-hoc test was then applied for multiple pairwise comparisons.

The significance level was set at $P \leq 0.05$. SPSS 16.0 for Windows was used to perform statistical analyses.

RESULTS

Clinical Data:

Clinical information for all studied cases was gathered and summarized in Table 1.

Table 1. showing the clinical findings of the studied cases.

Variables	**Conventional OSCC (n=18)**	**OPSCC (n=12)**
Age (years)		
≤50 years	3(16%)	4(33.3%)
>50 years	15(84%)	8(66.6%)
Gender		
Male	9(50%)	9(75%)
Female	9(50%)	3(25%)

Site		
Buccal mucosa	9(50%)	3(25%)
Alveolar mucosa	2(11.11%)	6(50%)
Tongue	6(33.3%)	1(8.3%)
Palate	1(5.5%)	0(100%)
Lip	0(0%)	2(16.6%)

Immunohistochemical Findings

In the control group, only 16% of normal mucosa samples showed positive TLR4 expression (Figure 1a), while 84 % were negative (Figure 1b). In contrast, all cases of OSCC (100%) exhibited positive immunoexpression in epithelial cells. Well-differentiated SCC displayed positive cytoplasmic immunoexpression (Figure 2a), while moderately differentiated SCC showed positive cytoplasmic immunoexpression with some nuclear immunoexpression (Figure 2b, 2c). Conversely, poorly differentiated SCC demonstrated both nuclear and cytoplasmic immunoexpression (Figure 2d). Additionally, all OPSCC cases (100%) demonstrated diffuse cytoplasmic immunoexpression,with nuclear staining observed in some instances (Figures 3a, 3b).

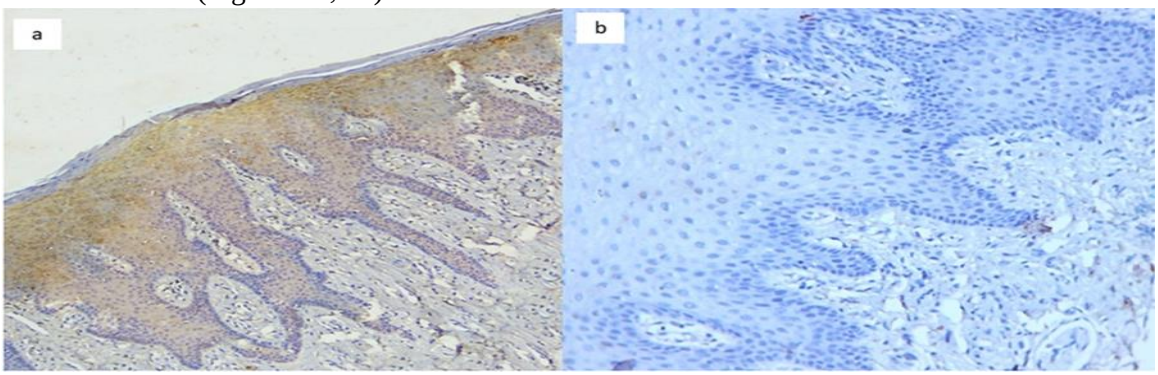

Figure. 1 (a) Photomicrograph of normal mucosa showing cytoplasmic expression of TLR4 limited to basal and parabasal layers. (x200). (b) Photomicrograph of normal mucosa showing negative immunoexpression of TLR4. (x200)

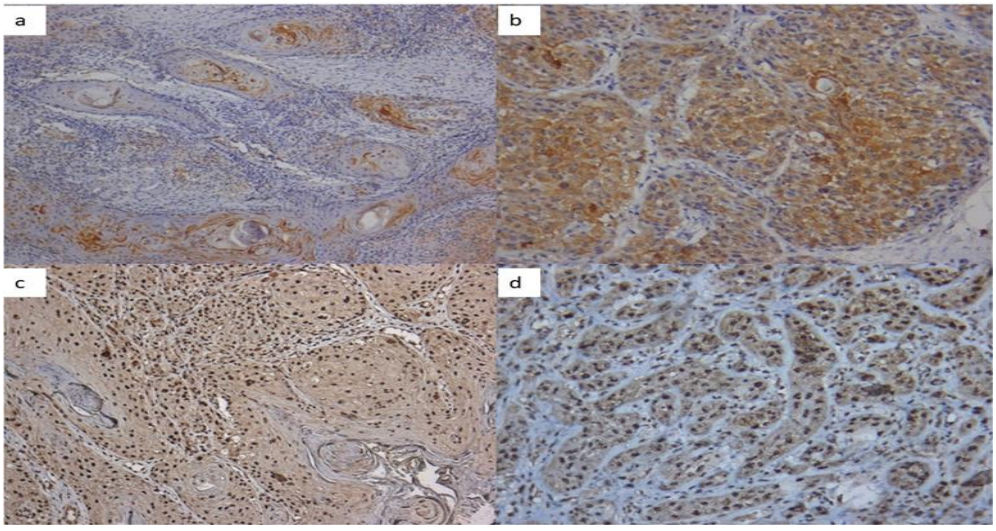

Figura 2. (a) Photomicrograph of well-differentiated SCC showing patchy cytoplasmic expression of TLR4 (x200). (b) Photomicrograph of moderately differentiated SCC showing cytoplasmic immunoexpression in the solid epithelial nests. (x200). (c) Photomicrograph of moderately differentiated SCC revealing diffuse cytoplasmic and nuclear TLR4 immunoexpression. Note the negative expression in the keratin pearl (x 100). (d) Photomicrograph of poorly differentiated SCC showing diffuse cytoplasmic and nuclear TLR4 immunoreaction in the epithelial cords and nests (x200).

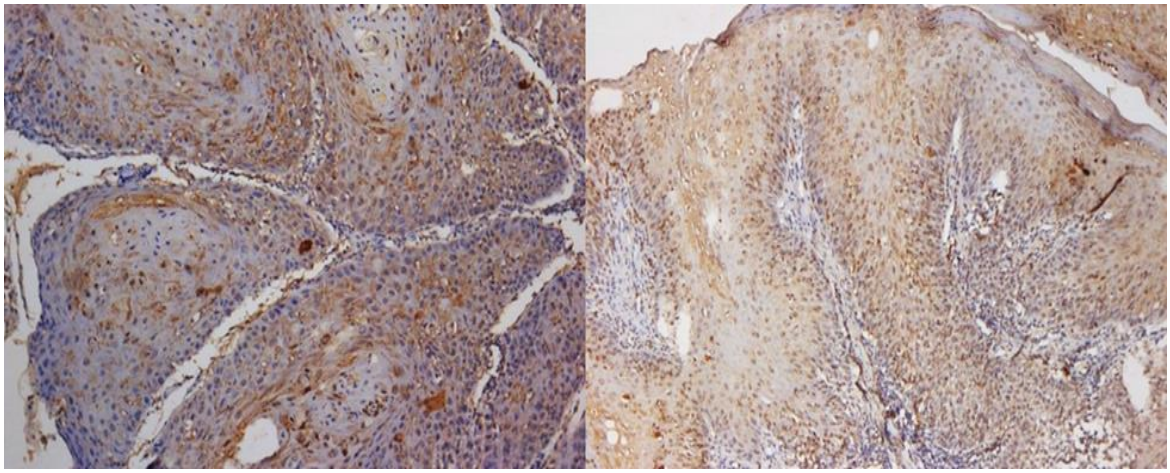

Figure 3. (a) Photomicrograph of papillary SCC showing cytoplasmic and scattered nuclear immunoexpression of TLR4 in the epithelial cells. (x100). (b) A photomicrograph of papillary SCC showing diffuse cytoplasmic and nuclear TLR4 immunoreaction in epithelial cells and some inflammatory cells. Note the negative expression in the keratin layer. (x200)

PCR Results:

According to the RT-PCR results, PDSCC tumor cells had the highest mRNA levels for TLR4, followed by MDSCC. In contrast, WDSCC showed lower expression than the previous grades. OPSCC biopsies displayed variation in TLR4 expression from low to high, with higher levels observed in cases showing more pronounced dysplastic features. All OPSCC cases coincided with TLR4 expression in well and moderately differentiated SCC. Furthermore, TLR4 mRNA expression was higher in tumor samples than in normal controls after normalization to GAPDH expression.

Statistical Analysis

Area Percent

The comparison of the area percent of TLR4 in tumor cells among the studied groups was statistically significant. The highest mean area percentage was recorded in the PDSCC group, while the lowest was recorded in the control group (Table 2, Figure 4a).

Table 2. Showing area percent of TLR4 in all groups and significance of the difference using (ANOVA) test

P.O.C.	Control	WDSCC	MDSCC	PDSCC	OPSCC
Mean	6.52^{a}	9.23^{b}	$30.77^{c,d}$	33.76^{c}	22.94^{d}
SD	1.01	2.31	5.31	2.56	7.78
Min	5.48	5.9	24.69	29.22	12.31
Max	7.66	12.74	37.96	36.44	30.03
F-value	31.876				
P-value	$< .00001^{*}$				

*Significant at p<0.05

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.

Nuclear Count

The highest mean nuclear count was recorded in the PDSCC group, whereas the lowest was found in the WDSCC group. The comparison of nuclear count among the groups showed statistically significant differences (P<0.001). Tukey's post hoc revealed no significant difference between MDSCC and OPSCC. (Table 3, Figure 4b)

Table 3. TLR4 nuclear count in all groups and significance of the difference using (ANOVA) test.

P.O.C	WDSCC	MDSCC	PDSCC	OPSCC
Mean	20.4[a]	57.4[b]	234.8[c]	55.8[b]
SD	11.9	66.7	14.9	53.6
Min	6	5	221	8
Max	39	140	254	135
F-value	24.311			
P-value	< .00001			

*Significant at p<0.05

Tukey's post hoc test: means sharing the same superscript letter are not significantly different.

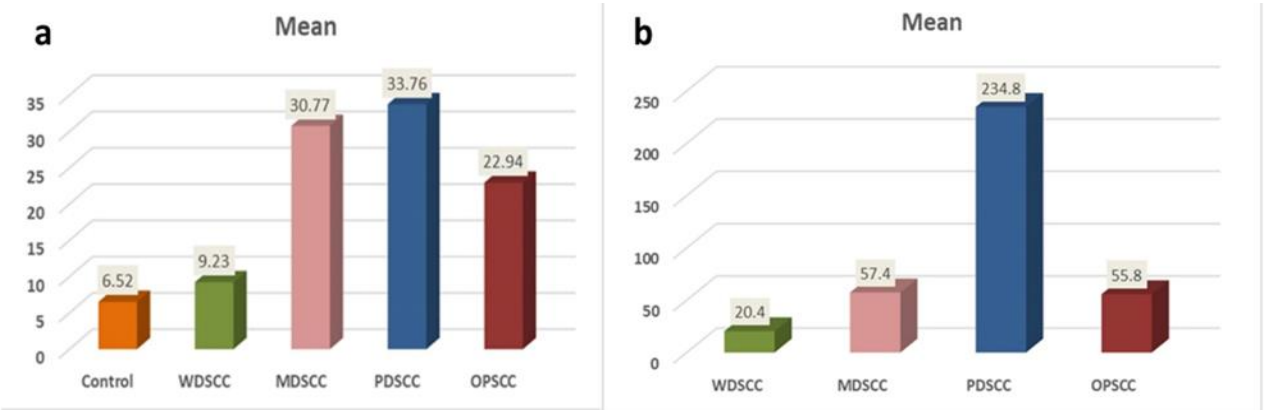

Figura 4. (a) Column chart showing mean area percent of TLR4 in all groups. (b) Column chart showing mean TLR4 nuclear count in all groups.

RT-qPCR of TLR4

The highest mean value was recorded in the PDSCC group, while the lowest was in the control group. One-way ANOVA indicated that the differences among all groups were statistically significant (P<0.001). (Table 4, Figure 5)

The results demonstrated that TLR4 expression was significantly elevated in all tumor tissues, both at the protein and gene levels, compared to normal oral mucosa. Among the OSCC subtypes, PDSCC exhibited the highest TLR4 expression, suggesting a correlation between TLR4 overexpression and increased tumor aggressiveness. Therefore, therapies aimed at modulating TLR4 activity could complement existing treatments, potentially improving response rates and reducing the risk of metastasis in patients with OSCC.

Table 4. RT-qPCR values of TLR4 in all groups and significance of the difference using (ANOVA) test

P.O.C.	Control	WDSCC	MDSCC	PDSCC	OPSCC
Mean	0.24[a]	0.34[a]	0.6[c]	1.02[d]	0.42[a]
SD	0.06	0.13	0.05	0.4	0.1
Min	0.16	0.21	0.54	0.67	0.27
Max	0.32	0.48	0.68	1.54	0.52
F-value	12.729				
P-value	< .00001*				

*Significant at p<0.05

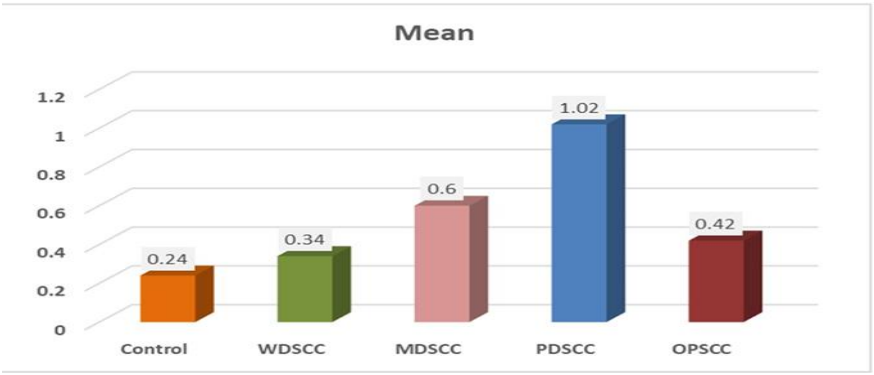

Figura 5. Column chart showing mean RT-qPCR values of TLR4 in all groups

DISCUSSION

In the current study, OPSCC was more prevalent in older male patients. The most frequently affected sites were alveolar mucosa followed by the buccal mucosa, lip, and tongue. The palate and floor of the mouth were not involved. These results align with those of Matsuo et al., who reported a higher prevalence of OPSCC in elderly males, and those of Bao et al., who stated that OPSCC affects the alveolar mucosa, followed by the buccal mucosa, tongue, palate, lower lip, and floor of the mouth. [21-22]

This male predominance may be attributed to the differences in lifestyle behaviors as men engage more in high-risk habits such as tobacco use and alcohol consumption. [23] Moreover, sex hormones, especially estrogen, may significantly protect women against the formation and spread of head and neck tumors. [24]

It is widely recognized that the innate immune system and chronic inflammation play important roles in the development of cancer. TLR4 is crucial regulating chronic inflammation and triggering innate immunity. Moreover, it is expressed in human OSCC cell lines, with higher expression levels correlating with tumor differentiation .[25] Furthermore, TLR4 stimulation can facilitate epithelial-mesenchymal transition, invasion, and metastasis by increasing the expression of matrix metalloproteinases and integrins,allowing tumor cells to evade the immune response.[19]

Several studies have shown that TLR4 can serve as an indicator of metastatic potential and a predictor of survival rates in OSCC. [26] Therefore, in this study, TLR4 was chosen to compare its expression in conventional OSCC and OPSCC. TLR4 is normally located on the surface of epithelial cells. Upon activation, it can be internalized, triggering downstream signaling pathways, including the activation of the transcription factor nuclear factor (NF)-κB. Consequently, the production of pro-inflammatory cytokines increases. This mechanism explains the cytoplasmic expression of TLR4, which represents its typical localization following activation in tumor samples. [27-28]

Moreover, inhibiting TLR4 with the small molecule inhibitor TAK-242 suppressed NF-κB-related anti-apoptotic genes such as BCL-xL, BCL-2, and survivin in breast and ovarian cancer cells, resulting in enhanced apoptosis. [29]

A statistically significant difference in TLR4 immunoexpression was observed between the control and different grades of OSCC and OPSCC. The highest mean area percentage was found in poorly differentiated SCC. These findings are consistent with those of several studies Li et al., Sharma et al., and Mäkinen et al., that reported upregulation of TLR4 in OSCC samples compared to healthy controls as well as its correlation with tumor differentiation and progression. [14, 25, 30]

In this study, moderately and poorly differentiated SCC and OPSCC exhibited variable nuclear expression. Jouhi et al., explain that the change in TLR4 expression from cytoplasmic to nuclear may reflect the functional change of TLRs in cancer progression.[31] The nuclear presence of TLR4 may lead to its interaction with transcription factors such as NF-κB, further regulating inflammatory cytokines and gene expression in a way that might enhance the microenvironment for tumor cell proliferation and immune suppression.[32] Therefore, nuclear expression may serve as an indicator of poorer prognosis.

In the present study, TLR4 immunoexpression in normal epithelial tissue was observed in 16% of cases. This may be attributed to the protective role of epithelial cells against bacteria. When the epithelial layer comes into contact with bacteria, it activates the expression of various cell surface molecules that play roles in the innate immune response. [33]

Additionally, the highest mean TLR4 by RT-qPCR was recorded in the PDSCC group, while the lowest was observed in the control group. Furthermore, the differences among all groups were statistically significant. These results align with those of Pakdel et al., who concluded that TLR4 expression in the tongue surface epithelium was higher in OSCC lesions than in healthy tissue. [20]

The examination of area percent and nuclear count in the immunohistochemically stained tissues revealed no significant difference between MDSCC and OPSCC. Moreover, the RT-qPCR results showed no significant difference between the WDSCC and OPSCC

groups. These findings suggest that OPSC and conventional OSCC may exhibit comparable biological behavior in terms of TLR4 expression. This is consistent with the results of Takeda et al., who observed no significant difference in Ki67 expression between the two types, supporting the implication that their proliferative and aggressive potentials are similar. [34]

However, our results contrast with those of Mahmoud et al., who suggested that the biological behavior of OPSCC is more favorable than that of various histological grades of conventional OSCC. This conclusion was based on a comparison of α-SMA and TGF-β expression in both lesions. [35] This discrepancy may stem from the lack of molecular analysis in their study, as they relied solely on immunohistochemical analysis; the expression of α-SMA and TGF-β should ideally also be examined using additional methods, including qPCR to detect mRNA levels.

Based on these findings, we suggest that OPSCC demonstrates molecular characteristics comparable to those of conventional OSCC of similar histological grades. Although OPSCC typically exhibits an exophytic architecture with limited invasion into the underlying connective tissue, it appears to retain a comparable biological potential for proliferation and aggressiveness. These results indicate that differences in growth pattern do not necessarily correspond to a less aggressive molecular phenotype. Therefore, clinicians should consider that assessing TLR4 expression may assist in risk stratification and treatment planning.

Limitations of the Study

The primary limitation of this study was the relatively small sample size, which may have affected the generalizability of the findings and limited the statistical power of subgroup comparisons. Larger multicenter studies are recommended to confirm these observations and explore their prognostic implications.

Future Research Direction

Future studies should include follow-up data to assess the correlation between TLR4 expression levels and patient outcomes such as recurrence, metastasis, and overall survival. This could help establish TLR4 as a potential prognostic biomarker in oral squamous cell carcinoma.

CONCLUSION

In conclusion, this study demonstrated that TLR4 expression was significantly higher in OSCC and its papillary variant compared to normal oral mucosa, with the highest expression observed in poorly differentiated OSCC. Based on these findings, we suggest that OPSCC resembles OSCC at comparable histological grades, regardless of invasion into the underlying connective tissue. Therefore, TLR4 expression may serve as a useful indicator of tumor differentiation and biological behavior, contributing to the assessment of OPSCC prognosis.

List of abbreviations:

WHO: World Health Organization
OSCC: Oral squamous cell carcinoma
OPSCC: Oral papillary squamous cell carcinoma
TLR4: Toll-like receptor 4
WDSCC: well-differentiated squamous cell carcinoma
MDSCC: Moderately differentiated squamous cell carcinoma
PDSCC: Poorly differentiated squamous cell carcinoma
IHC: Immunohistochemical.

DECLARATION

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Funding

This research received no external funding.

REFERENCES

1. Ng JH, Iyer NG, Tan MH, Edgren G. Changing epidemiology of oral squamous cell carcinoma of the tongue: a global study. Head Neck. 2017;39(2):297–304.
2. Alotaiby F, Song F, Boyce BJ, Cao D, Zhao Y, Lai J. Unusual papillary squamous cell carcinoma of the tip of tongue presenting in a patient status post heart transplant. Anticancer Res. 2018;38(7):4203–6.
3. Benedetti MD, Marangi A, Bozzetti S, Gobbin F, Turatti M, Pea M, et al. HPV-related papillary squamous cell carcinoma of the tonsil during treatment with fingolimod.*Mult Scler RelatDisord*.2018;23:24–6.
4. Betz SJ. HPV-related papillary lesions of the oral mucosa:a review.*Head Neck Pathol*.2019;13(1):80–90.
5. Wenig BM. Squamous cell carcinoma of the upper aerodigestive tract: dysplasia and select variants. *Mod Pathol*. 2017;30(1):112–8.
6. Karthik KP, Balamurugan R, Pushpa TS. Oral papillary squamous cell carcinoma (OPSCC): a rare presentation in the palate. *J Oral Med Oral Surg*. 2020;26(2):16.
7. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin*. 2017;67(2):93–9.
8. Jo VY, Mills SE, Stoler MH, et al. Papillary squamous cell carcinoma of the head and neck: frequent association with human papillomavirus infection and invasive carcinoma. *Am J Surg Pathol*. 2009;33(11):1720–4.
9. Poeta ML, Manola J, Goldwasser MA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck.*NEnglJMed*.2007;357(25):2552–61.
10. Russell JO, Hoschar AP, Scharpf J. Papillary squamous cell carcinoma of the head and neck: a

clinicopathologic series. *Am J Otolaryngol*. 2011;32(6):557–63.

11. Wester A, Eyler JT, Swan JW. Topical imiquimod for the palliative treatment of recurrent oral squamous cell carcinoma. *JAAD Case Rep*. 2017;3(4):329–31.

12. Jiang Y, He L, Ju C, et al. Isolation and expression of grass carp toll-like receptor 5a (CiTLR5a) and 5b (CiTLR5b) gene involved in the response to flagellin stimulation and grass carp reovirus infection. *Fish Shellfish Immunol*. 2015;44(1):88–99.

13. Kumar AR, Devan AR, Nair B, Vinod BS, Nath LR. Harnessing the immune system against cancer: current immunotherapy approaches and therapeutic targets. *Mol Biol Rep*. 2021;48(8):5821–41.

14. Li L, Zhou Z, Mai K, Li P, Wang Z, Wang Y, et al. Protein overexpression of toll like receptor 4 and myeloid differentiation factor 88 in oral squamous cell carcinoma and clinical significance. Oncol Lett. 2021;22(5):1–11.

15. Visioli F, Nunes JS, Pedicillo MC, Leonardi R, Santoro A, Zannoni GF, et al. TLR4 expression in ex-lichenoid lesions—oral squamous cell carcinomas and its surrounding epithelium: the role of tumor inflammatory microenvironment. *Biomolecules*. 2022;12(3):385.

16. Yang J, Liu D, Khatri KS, Wang J, Zhang G, Meng C, et al. Prognostic value of toll-like receptor 4 and nuclear factor-κBp65 in oral squamous cell carcinoma patients. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2016;122(6):753–64.

17. Papadakos SP, Arvanitakis K, Stergiou IE, Vallilas C, Sougioultzis S, Germanidis G, et al. Interplay of extracellular vesicles and TLR4 signaling in hepatocellular carcinoma pathophysiology and therapeutics. *Pharmaceutics*. 2023;15(10):2460.

18. Ran S. The role of TLR4 in chemotherapy-driven metastasis. Cancer Res. 2015;75(12):2405–10.

19. Mishra V, Pathak C. Human toll-like receptor 4 (hTLR4): structural and functional dynamics in cancer. Int J Biol Macromol. 2019;122:425–51.

20. Pakdel F, Pouralibaba F, Pakdel S, Khiyavi RK, Falsafi P, Eslami H, et al. Expression of toll-like receptors in squamous cell carcinoma of the tongue. Arch Appl Sci Res. 2015;7(2):20–4.

21. Matsuo K, Akiba J, Kusukawa J, Yano H. Squamous cell carcinoma of the tongue: subtypes and morphological features affecting prognosis. Am J Physiol Cell Physiol. 2022;323(6):C1611–23.

22. Bao Z, Yang X, Shi L, Feng J, Liu W, Zhou Z. Clinicopathologic features of oral squamous papilloma and papillary squamous cell carcinoma: a study of 197 patients from eastern China. *Ann Diagn Pathol*. 2012;16(6):454–8.

23. Lipsky MS, Su S, Crespo CJ, Hung M. Men and oral health: a review of sex and gender differences. Am J Mens Health. 2021;15(3):15579883211016361.

24. Kranjčević JK, Čonkaš J, Ozretić P. The role of estrogen and estrogen receptors in head and neck tumors. Cancers (Basel). 2024;16(8):1575.

25. Sharma Y, Bala K. Role of toll like receptor in progression and suppression of oral squamous cell carcinoma. *Oncol Rev*. 2020;14(1):1–8.

26. Visioli F, Nunes JS, Pedicillo MC, Leonardi R, Santoro A, Zannoni GF, et al. TLR4 expression in ex-lichenoid lesions—oral squamous cell carcinomas and its surrounding epithelium: the role of tumor inflammatory microenvironment. *Biomolecules*. 2022;12(3):385.

27. Ruuskanen M, Leivo I, Minn H, Vahlberg T, Haglund C, Hagström J, et al. Expression of toll-like receptors in non-endemic nasopharyngeal carcinoma. *BMC Cancer*. 2019;19(1):12.

28. Rusanen P, Marttila E, Amatya SB, Hagström J, Uittamo J, Reunanen J, et al. Expression of toll-like receptors in oral squamous cell carcinoma. *PLoS One*. 2024;19(4):e0300437.

29. Li Y, Tang T, Sun Y, Chen GE, Yuan X, Cai D. The role of TLR-4 in chemoresistance of cancer. Discover Oncology. 2025 May 22;16(1):865.

30. Mäkinen LK, Ahmed A, Hagström J, Lehtonen S, Mäkitie AA, Salo T, et al. Toll-like receptors 2, 4, and 9 in primary, metastasized, and recurrent oral tongue squamous cell carcinomas. *J Oral Pathol Med*. 2016;45(5):338–45.

31. Jouhi L, Renkonen S, Atula T, Mäkitie A, Haglund C, Hagström J. Different toll-like receptor expression patterns in progression toward cancer. *Front Immunol*. 2014;5:638.

32. Kashani B, Zandi Z, Pourbagheri-Sigaroodi A, Bashash D, Ghaffari SH. The role of toll-like receptor 4 (TLR4) in cancer progression: a possible therapeutic target? J Cell Physiol. 2021;236(6):4121–37.

33. Calcaterra R, Di Girolamo M, Mirisola C, Baggi L. Expression of pattern recognition receptors in epithelial cells around clinically healthy implants and healthy teeth. Implant Dent. 2016;25(3):348–52.

34. Takeda Y, Satoh M, Nakamura SI, Yamamoto H. Papillary squamous cell carcinoma of the oral mucosa: immunohistochemical comparison with other carcinomas of oral mucosal origin. J Oral Sci. 2001;43(3):165–9.

35. Mahmoud EA, Abdellatif MK, Mahmoud SAM. Oral papillary squamous cell carcinoma and oral squamous cell carcinoma: a histopathological and immunohistochemical comparative study. *Head Neck Pathol*. 2024;18(1):47.