DOI: https://doi.org/10.54393/pjhs.v6i8.3241

PAKISTAN JOURNAL OF HEALTH SCIENCES
(LAHORE)
https://thejas.com.pk/index.php/pjhs
ISSN (E): 2790-9352, (P): 2790-9344
Volume 6, Issue 08 (August 2025)

Original Article

OPEN ACCESS

CLEC3B Expression in Saliva and Serum: A Promising Biomarker Approach for Oral Squamous Cell Carcinoma

Syeda Zehra Ahmed[1*], Shumaila Usman[2], Fouzia Shaikh[1], Atif Iqbal Butt[3], Uzma Zareef[4] and Aliya Irshad Sani[5]

[1]Department of Pathology, Ziauddin Medical University, Karachi, Pakistan
[2]Department of Molecular Medicine, Ziauddin Medical University, Karachi, Pakistan
[3]Department of Oral and Maxillofacial Surgery, Ziauddin Medical University, Karachi, Pakistan
[4]Department of Oral Pathology, Liaquat College of Medicine and Dentistry, Karachi, Pakistan
[5]Department of Biochemistry, Ziauddin Medical University, Karachi, Pakistan

ARTICLE INFO

Keywords:
Squamous Cell Carcinoma of Head and Neck, Gene Expression, Biomarkers, Saliva, Blood

How to Cite:
Ahmed, S. Z., Usman, S., Shaikh, F., Butt, A. I., Zareef, U., & Sani, A. I. (2025). CLEC3B Expression in Saliva and Serum: A Promising Biomarker Approach for Oral Squamous Cell Carcinoma: CLEC3B Expression in Saliva and Serum: Oral Squamous Cell Carcinoma. Pakistan Journal of Health Sciences, 6(8), 84-89. https://doi.org/10.54393/pjhs.v6i8.3241

***Corresponding Author:**
Syeda Zehra Ahmed
Department of Pathology, Ziauddin Medical University, Karachi, Pakistan
zehra.ahmed@zu.edu.pk

Received Date: 11th June, 2025
Revised Date: 29th July, 2025
Acceptance Date: 5th August, 2025
Published Date: 31st August, 2025

ABSTRACT

Metastasis, considered the most important factor for determining the survival rate of oral squamous cell carcinoma (OSCC). Therefore, a useful biomarker is needed to classify patients with OSCC in order to ensure effective treatment. Although CLEC3B has been mentioned in the literature, its relationship to OSCC has not yet been studied. **Objectives:** To evaluate the potential of CLEC3B expression in saliva and blood as a diagnostic biomarker for oral squamous cell carcinoma. **Methods:** A case-control study comprised of 80 samples (40 blood and 40 saliva) from 40 participants was conducted. Both Ziauddin University Hospital and Abbasi Shaheed Hospital served as the recruitment sites for all samples. RT qPCR was used to determine the expression levels of CLEC3B in OSCC and healthy individuals after obtaining written informed consent. Ziauddin University Ethical Review Committee approved this project. SPSS version 20.0 was used to analyze the data. **Results:** When compared to healthy people, OSCC patients' saliva had considerably lower levels of CLEC3B (p-value 0.001). Moreover, the expression was comparatively lower in the saliva of OSCC patients as compared to blood, suggesting that this marker can be better evaluated in saliva. **Conclusions:** Low expression of CLEC3B with the progression of OSCC depicts its tumor suppressive role in the tumor microenvironment. Its high yield in saliva can make it a suitable and easily assessable biomarker for determining OSCC progression.

INTRODUCTION

Oral squamous cell carcinoma gained eighth place among the major cancers that have spread worldwide. The incidence of OSCC is estimated to be highest in the developing countries, particularly in the Southeastern region [1, 2]. The prevalence was reported to be 18.6% in Pakistan, with the majority of cases being caused by the concomitant use of alcohol and cigarettes [3]. Prolonged exposure to tobacco, alcohol, addictive substances, and oncogenic infections can induce genetic alterations, including chromosomal damage, which contribute to the development of premalignant lesions that may progress to invasive carcinoma [4]. Certain cytological and tissue architectural features of squamous cell differentiation are displayed by the uncontrolled proliferation of epithelial cells. This condition can proceed from mild to severe dysplasia, depending on the quantity and severity of these cytological changes. Although science and technology have advanced, the prognosis for OSCC has remained same for more than three decades with a 50% 5-year survival rate [5]. Recent advancements in the application of molecular

DOI: https://doi.org/10.54393/pjhs.v6i8.3241

and biochemical methods have made it simpler to identify biomarkers for the diagnosis and prognosis of OSCC. The Hanahan and Weinberg's proposed Hallmarks of cancer development include invasion and metastasis as the main mechanisms for cancer progression [6]. This crucial feature is governed by the expression of certain biomarkers. Research has identified C-type lectin domain family 3-member B (CLEC3B) as a promising biomarker with potential diagnostic and prognostic significance in oral squamous cell carcinoma (OSCC) [7]. The literature had shown that CLEC3B serves many different functions as It played an oncogenic part in some tumors, but other research indicated that it had a tumor suppressing impact in cases such as hepatocellular carcinoma and pancreatic cancer [8]. Additionally, through the signals of AMP-activated protein kinase and vascular endothelial growth factor, CLEC3B participates in the invasion and metastasis of tumor cells [9]. Given the complexity of OSCC pathogenesis and the urgent need for reliable, non-invasive biomarkers, investigating CLEC3B expression in blood and saliva offers significant approach. This study aims to clarify its diagnostic and prognostic potential in OSCC, helping to resolve its ambiguous role by focusing on a single, well-defined cancer type. Tissue biopsy has continued to be the gold standard method for diagnosing OSCC. However, it is an intrusive approach, some patients might be reluctant to it [10]. For the diagnosis and prognosis of OSCC, saliva is a noninvasive and easily accessible bodily fluid that offers potential indicators [11]. As a result, tissue biopsy may be able to be replaced. However, various saliva collecting methods, including draining, spitting, and suction processes, have an impact on the recovery of particular biomarkers. Additionally, a number of researchers have found that saliva has a higher output of biomarkers than blood [12]. There are significant challenges in identifying prospective blood and salivary biomarkers in OSCC despite continued research on the clinical application of diagnostic biomarkers. Even though saliva is a non-invasive medium, it is not typically employed for diagnostic purposes [13]. There is currently a dearth of information showing how accurately saliva represents the serum levels of particular biomarkers. In the context of OSCC, limited but emerging evidence suggests a potential tumor-suppressive role, particularly through its involvement in extracellular matrix remodeling and immune regulation.

Although CLEC3B has been investigated in several malignancies, its diagnostic relevance in oral squamous cell carcinoma (OSCC), particularly using non-invasive samples such as saliva, remains insufficiently explored. Most previous studies have focused on tissue-based or serum biomarkers, with limited comparative evaluation of salivary and blood gene expression in OSCC patients. Furthermore, there is a lack of region-specific data from Pakistan examining CLEC3B expression in clinically confirmed OSCC cases. Therefore, investigating CLEC3B expression in both saliva and serum is necessary to determine its potential as a reliable and minimally invasive biomarker. This study aims to evaluate the importance of saliva and blood in identifying CLEC3B expression in OSCC patients and control.

METHODS

This was a case control study comprised of a total of 80 samples, 40 samples were taken from OSCC patients and 40 samples from healthy individuals as controls Using the Open Epi platform, the preliminary calculation indicated that a minimum of 18 participants per group was required, based on a 95% confidence level and a 5% margin of error. Additionally, a prior power analysis was performed to assess the adequacy of the sample size for detecting potential differences in gene expression between groups. This analysis, set at a two-tailed significance level of 0.05 and 80% statistical power, assumed a moderate-to-large effect size (Cohen's d ≈ 0.75). To further enhance the reliability of the results, account for variability in saliva and blood transcript levels, and minimize the risk of data loss due to sample degradation or low-quality RNA, the sample size was increased to 40 subjects per group (total n = 80). Blood and saliva samples from each case and control subject were collected after obtaining written informed consent from June 2023 till January 2024. All the samples were recruited from the Dental Outpatient Departments of Ziauddin University Hospital and Abassi Shaheed Hospital. OSCC patients were diagnosed on the basis of histopathological report. This study was conducted in accordance with the Code of Ethics of the World Medical Association and the Ethics Review Committee (ERC) of Ziauddin (Ref code: 2941220ZAPAT) and data gave its approval. Data collection was given by Abassi Shaheed Hospital (DIRS/ASH/ESTT/3145/2020). Sample size for comparing two means was calculated (Table 1).

Table 1: Sample Size for Comparing Two Means

Variables	Input Data		
Confidence Level (2-Sided)	95%		
Power	80%		
Ratio of Sample Size (Group 2/Group 1)	1%		
Variables	**Group 1**	**Group 2**	**Differences***
Mean	10.8	7.33	3.47
SD	2.8	2.23	–
Variance	7.84	4.9729	–
Sample Size	9	9	–
Total Sample Size	18		

*Difference between the means. Results from Open Epi, version 3,

DOI: https://doi.org/10.54393/pjhs.v6i8.3241

open source calculator—SS Mean. Print from the browser with Ctrl-P, or select text to copy and paste to other programs

OSCC patients were confirmed by histology. Healthy controls were verified through a detailed medical history and a clinical oral examination to confirm the absence of any oral lesions, systemic illnesses, or recent infections. Individuals with autoimmune diseases or other malignancies. Each individual had 5 ml of entire, unstimulated saliva taken from them, and 5 ml of blood was collected at the same time in morning. To minimize pre-analytical variability, saliva samples were collected under standardized conditions: participants were asked to refrain from eating, drinking, or brushing teeth for at least 30 minutes prior to sample collection. Unstimulated whole saliva was collected in sterile tubes, immediately placed on ice, and processed within 2 hours of collection. All samples underwent identical RNA extraction protocols. However, inherent variability in saliva composition remains a potential limitation. All of the obtained samples underwent a 15-minute, 2600 x g centrifugation at 4°C. Then, these samples were kept at -80 degrees Celsius. Blood and saliva samples were utilized to conduct qPCR tests on CLEC3B expression. For gene expression analysis, Trizol technique was used to extract the RNA. For phase separation, 200 µL of chloroform was added. RNA precipitation was carried out by adding 2 mL of isopropanol, followed by centrifugation. The resulting supernatant was discarded, and the RNA pellets were air-dried and subsequently resuspended in 20 µL of nuclease-free water. Samples were stored at -80°C until further use. The concentration and purity of the extracted RNA were assessed using the MultiScan Sky Spectrophotometer. cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit, following the manufacturer's protocol. Primer 3 software was used to design the primer sequence. GAPDH was taken as an internal control. To ensure the specificity and efficiency of the primers designed for CLEC3B and GAPDH, a pilot study was conducted prior to the main experiment. This involved testing the primers on representative saliva and blood samples. Primer specificity was confirmed through melt curve analysis and gel electrophoresis. The following primers were used: Forward 5′-CCAGAACATCATCCCTGCCT-3′ for GAPDH, Reverse: 3′- 5′- CCTGCTTCACCTTCTTG, Forward 5′-TGGTGTAACCTCAGAAGTG- 3′; CLEC3B; Backwards: 5′-GTCAACTCCAGGCTTGTA- 3′. RT-qPCR was used to analyze the expression of CLEC3B. To make a 20 µL volume, 10 µL of SYBR green master mix and 10 µL of cDNA and primer combination were combined. 40 cycles of denaturation (92C),annealing, and extension (72C) were performed The relative fold change was estimated for the study of expression using the CT values. The 2^-ΔΔCt was used to quantify relative expression. The data were analyzed using SPSS version 20.0. Kruskal Wallis and pairwise comparison was performed and median and interquartile ranges were calculated for nonparametric data at a 95% confidence interval. The p-value less than 0.050 was considered statistically significant.

RESULTS

The study analyzed the expression of CLEC3B in 40 blood and saliva samples by determining the relative gene expression. The study observed the highest median (IQR) values in cases of saliva, 5.1 (2.06%), compared with blood samples, 2.7 (0.4%), at a confidence interval of 95% (Figure 1).

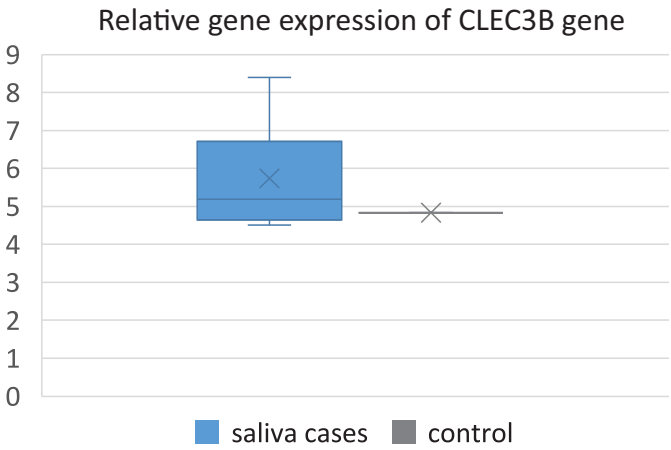

Figure 1: Relative Gene Expression of CLEC3B in Saliva OSCC Cases Compared with Controls

The Kruskal-Wallis test was applied to compare CLEC3B expression between OSCC cases and controls. These Boxplots represent the median and Interquartile ranges. The p-value less than 0.05 considered significant.

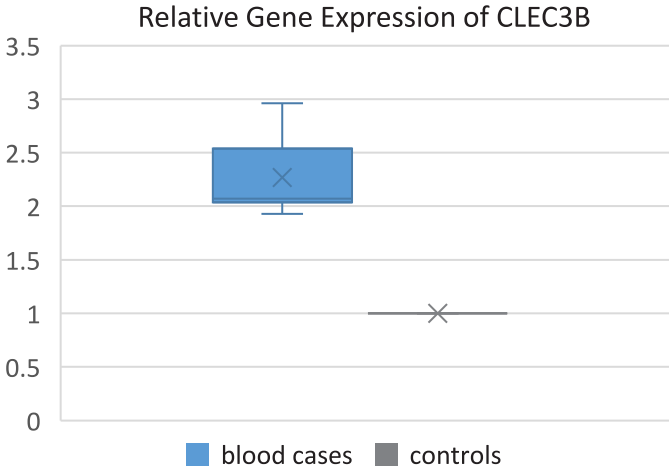

Figure 2: Relative Gene Expression of OSCC Cases and Controls in Blood

In both saliva and blood samples, significantly increased relative gene expression was observed in OSCC cases compared with controls(p-value=0.001).

Test result variable (s) for saliva and blood was applied (Table 2).

DOI: https://doi.org/10.54393/pjhs.v6i8.3241

Table 2: Test Result Variable(s): Saliva and Blood

Area	Std. Errora	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.953	0.035	0.000	0.885	1.
0.90	0.085	0.03	0.812	0.988

To evaluate the significance of CLEC3B in blood and saliva, ROC curve was generated and area under the curve was calculated. The study found significantly increased area under the curve in blood samples (AUC 0.9) and in saliva samples as (AUC 0.95). If study allow the cutoff point to be 3, the sensitivity would be 100% and 1 - specificity would be 94% for saliva samples and for blood cut off point is 0.4 and 1 - specificity would be 100%. The study shows ROC analysis in AUC (0.953), at point 3 we observed 100% sensitivity and 1-specificity was 94% in saliva (a). Analysis shows ROC analysis in AUC (0.90), at point 0.4 we observed 95% sensitivity and 1-specificity was 100% in blood samples (b) (Figure 3).

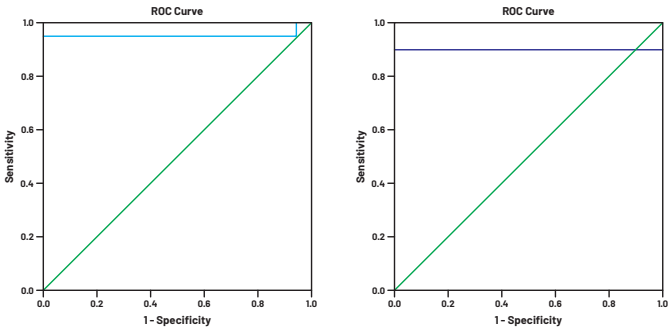

Figure 3: ROC Curve Analysis in Blood and Saliva Samples

DISCUSSION

Genetic mutations and uncontrolled tumor cell proliferation cannot be the only causes of cancer progression. The development of tumors and their spread are greatly influenced by a variety of growth factors and signaling pathways. The delayed diagnosis is the primary cause of the OSCC patient's poor prognosis, though. Numerous studies have explored differences in salivary transcriptome profiles between healthy individuals and those with disease, contributing to disease detection and monitoring of its progression [14, 15]. In the present research, we examined the potential of saliva as a non-invasive diagnostic tool and evaluated the diagnostic relevance of CLEC3B in identifying OSCC through salivary biomarker analysis. Our findings revealed that blood and saliva samples from OSCC cases had considerably lower levels of CLEC3B expression than samples from controls (p-value=0.001). In blood and saliva samples from OSCC patients, Arellano *et al.* demonstrated the expression of CLEC3B and contrasted it with healthy control subjects. When compared to a healthy person, he had a lower level of CLEC3B in OSCC [16]. Another case-control research on saliva samples from OSCC patients found that CLEC3B was downregulated relative to healthy people [17, 18]. These findings might point to OSCC's tumor-suppressive properties. Studies using blood samples from patients with ovarian cancer, lung, breast, and colon carcinoma revealed similar findings, with considerable downregulation of CLEC3B [19-21]. Additionally, hepatocellular carcinoma tissue samples showed comparable outcomes [8]. The earlier investigations were supported by our study. The delayed expression of CLEC3B observed in OSCC samples in this study and in previous studies indicated the fact that decreased expression leads to decreased phosphorylation of AMP activated kinases that inhibits cell growth and proliferation, resulting in the activation of VEGF signaling, leading to invasion and progression of OSCC [9]. This work would suggest that CLEC3B expression reduced in the blood and saliva of OSCC patients compared to healthy people, can be thought of as a potential diagnostic biomarker (p-value=0.001). Compared to blood CLEC3B levels, the differences in salivary CLEC3B levels between OSCC and healthy participants seemed more significant. This may be due to the fact that the salivary environment is flooded with oral cancer cells. Tetranectin, a translational byproduct, seems to be ingested within the tumor microenvironment. The extracellular matrix must be broken down by proteases for metastasis to occur. Tetranectin is therefore present in lower concentrations in OSCC patients compared to healthy persons [22]. Therefore, this study may aid in the classification of OSCC patients. While CLEC3B expression was evaluated in OSCC patients, correlation with clinical staging and histopathological grading was not included in this study. Future investigations incorporating these parameters would be valuable to confirm CLEC3B's utility as a prognostic biomarker and to understand its potential role in tumor progression. However, it suffers some limitation due to its limited sample size , potential variability in saliva collection and composition, which may affect CLEC3B expression levels and the utility of this gene should further be validated and studied with large samples in future.

This study has certain limitations, too. Its single-center design and relatively small sample size may limit the generalizability of the findings. Furthermore, the lack of correlation with clinical staging, histopathological grading, and long-term patient outcomes restricts the ability to establish the prognostic significance of CLEC3B in OSCC. Variability in saliva collection and inherent differences in salivary composition may also influence gene expression levels. Future multicenter studies with larger cohorts, longitudinal follow-up, and comprehensive clinicopathological correlation are recommended to further validate CLEC3B as a reliable diagnostic and prognostic biomarker and to clarify its role in tumor progression.

DOI: https://doi.org/10.54393/pjhs.v6i8.3241

CONCLUSIONS

It was concluded that CLEC3B is downregulated in OSCC and may act as a diagnostic biomarker. In addition, low expression of CLEC3B with progression of the disease depicts its tumor suppressive role in tumor microenvironment. Furthermore, its high yield in saliva can make it a suitable and easily assessable biomarker for determining OSCC progression.

Authors' Contribution

Conceptualization: SZA
Methodology: SZA, SU, AAB, AIS
Formal analysis: UZ
Writing and Drafting: SZA, FS, AIS
Review and Editing: SZA, FS, AIS, UZ, SU, AAB

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2019. CA: A Cancer Journal for Clinicians. 2019 Jan; 69(1): 7-34. doi: 10.3322/caac.21551.

[2] Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M *et al.* Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research On Cancer. 2020 Nov; 20182020.

[3] Harwansh RK and Deshmukh R. Breast Cancer: An Insight into Its Inflammatory, Molecular, Pathological and Targeted Facets with Update on Investigational Drugs. Critical Reviews in Oncology/Hematology. 2020 Oct 1; 154: 103070. doi: 10.1016/j.critrevonc.2020.103070.

[4] Karim AA, Inam S, Zaidi AB. Inadequate Checks On Smokeless Tobacco Usage in Pakistan: Cultural Heritage or Policy Neglect. Journal of the Pakistan Medical Association. 2020 Sep; 70(1): 134-8. doi: 10.5455/JPMA.6459.

[5] Bschorer M, Schneider D, Goppold K, Sperling J, Schön G, Bschorer R. Quality of Life and Survival Rate After Primary Surgical Treatment of Oral Squamous Cell Carcinoma: A Retrospective Study with 18 Years of Follow-Up. Journal of Cranio-Maxillofacial Surgery. 2022 Feb; 50(2): 170-7. doi: 10.1016/j.jcms.2021.09.016.

[6] Tan Y, Wang Z, Xu M, Li B, Huang Z, Qin S *et al.* Oral Squamous Cell Carcinomas: State of the Field and Emerging Directions. International Journal of Oral Science. 2023 Sep; 15(1): 44. doi: 10.1038/s41368-023-00249-w.

[7] Shan J, Sun Z, Yang J, Xu J, Shi W, Wu Y *et al.* Discovery and Preclinical Validation of Proteomic Biomarkers in Saliva for Early Detection of Oral Squamous Cell Carcinomas. Oral Diseases. 2019 Jan; 25(1): 97-107. doi: 10.1111/odi.12971.

[8] Xie XW, Jiang SS, Li X. CLEC3B as a Potential Prognostic Biomarker in Hepatocellular Carcinoma. Frontiers in Molecular Biosciences. 2021 Jan; 7: 614034. doi: 10.3389/fmolb.2020.614034.

[9] Dai W, Wang Y, Yang T, Wang J, Wu W, Gu J. Downregulation of Exosomal CLEC3B in Hepatocellular Carcinoma Promotes Metastasis and Angiogenesis Via AMPK and VEGF Signals. Cell Communication and Signaling. 2019 Sep; 17(1): 113. doi: 10.1186/s12964-019-0423-6.

[10] Umapathy VR, Natarajan PM, Swamikannu B. Review Insights on Salivary Proteomics Biomarkers in Oral Cancer Detection and Diagnosis. Molecules. 2023 Jul; 28(13): 5283. doi: 10.3390/molecules28135283.

[11] Jayasinghe RD, Hettiarachchi PV, Amugoda D, Kumaraarachchi M, Liyanage RL, Siriwardena BS *et al.* Validity of Toluidine Blue Test as A Diagnostic Tool for High Risk Oral Potentially Malignant Disorders-A Multicentre Study in Sri Lanka. Journal of Oral Biology and Craniofacial Research. 2020 Oct; 10(4): 547-51. doi: 10.1016/j.jobcr.2020.08.002.

[12] Delrue C, De Bruyne S, Speeckaert MM. Unlocking the Diagnostic Potential of Saliva: A Comprehensive Review of Infrared Spectroscopy and Its Applications in Salivary Analysis. Journal of Personalized Medicine. 2023 May; 13(6): 907. doi: 10.3390/jpm13060907.

[13] Bel'skaya LV and Sarf EA. Biochemical Composition and Characteristics of Salivary FTIR Spectra: Correlation Analysis. Journal of Molecular Liquids. 2021 Nov; 341: 117380. doi: 10.1016/j.molliq.2021.117380.

[14] Roi A, Rusu LC, Roi CI, Luca RE, Boia S, Munteanu RI. A New Approach for the Diagnosis of Systemic and Oral Diseases Based on Salivary Biomolecules. Disease Markers. 2019; 2019(1): 8761860. doi: 10.1155/2019/8761860.

[15] Song M, Bai H, Zhang P, Zhou X, Ying B. Promising Applications of Human-Derived Saliva Biomarker Testing in Clinical Diagnostics. International Journal of Oral Science. 2023 Jan; 15(1): 2. doi: 10.1038/s41368-022-00209-w.

[16] Arellano-Garcia ME, Li R, Liu X, Xie Y, Yan X, Loo JA *et al.* Identification of Tetranectin as A Potential Biomarker for Metastatic Oral Cancer. International Journal of Molecular Sciences. 2010 Sep; 11(9): 3106-21. doi: 10.3390/ijms11093106.

DOI: https://doi.org/10.54393/pjhs.v6i8.3241

[17] Sajan T, Murthy S, Krishnankutty R, Mitra J. A Rapid, Early Detection of Oral Squamous Cell Carcinoma: Real Time PCR Based Detection of Tetranectin. Molecular Biology Research Communications. 2019 Mar; 8(1): 33.

[18] Khidhir KG. Expression Profile of PBRM1, PLAU and CLEC3B Genes in Head and Neck Squamous Cell Carcinoma. Passer Journal of Basic and Applied Sciences. 2023 Jun; 5(1): 128-33.

[19] Chen H, Liao K, Li L, Zhou Y, Wu S. CLEC3B Inhibits Proliferation and Radioresistance and Induces Apoptosis in BT549 Breast Cancer Cells. Journal of Radiation Research and Applied Sciences. 2024 Jun; 17(2): 100895. doi: 10.1016/j.jrras.2024.100895.

[20] Zhu HF, Zhang XH, Gu CS, Zhong Y, Long T, Ma YD *et al.* Cancer-Associated Fibroblasts Promote Colorectal Cancer Progression by Secreting CLEC3B. Cancer Biology and Therapy. 2019 Jul; 20(7): 967-78. doi: 10.1080/15384047.2019.1591122.

[21] Sun J, Xie T, Jamal M, Tu Z, Li X, Wu Y et al. CLEC3B as a Potential Diagnostic and Prognostic Biomarker in Lung Cancer and Association with the Immune Microenvironment. Cancer Cell International. 2020 Apr; 20(1): 106. doi: 10.1186/s12935-020-01183-1.

[22] Madunić J. The Urokinase Plasminogen Activator System in Human Cancers: An Overview of Its Prognostic and Predictive Role. Thrombosis and Hemostasis. 2018 Dec; 118(12): 2020-36. doi: 10.1055/s-0038-1675399.