DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 3, Issue 01, 2025; p08-14,
E-ISSN 2583-7761

Original Article – Dental Cancer-Genetics

Identification and Functional Analysis of miR-204-3p in Oral Squamous Cell Carcinoma: Insights into Target Gene and Expression Patterns in Indian Population- A Pilot Study

Ishwarya S 1, Ashikha Shirin Usman P P2, Ameya K P2, Dhanraj M Ganapathy1, Durairaj Sekar2*
1- Department of prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India
2- RNA Biology Lab, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India

Corresponding author- Dr. Durairaj Sekar,Professor (Research),RNA Biology Lab,Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai-77,Email id: duraimku@gmail.com, durairajsekar.sdc@saveetha.com,Phone Number: +91 9361216583

Article received on: 03-09-2024 **Article accepted on:** 17-12-2024 **Article published on** :03-05-2025

ABSTRACT

Introduction-Oral Squamous Cell Carcinoma (OSCC) is a significant global health issue, originating from squamous cells in the oral cavity. Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine, plays a crucial role in promoting cell proliferation, metastasis, and invasion in OSCC. This study investigates the role of miR-204-3p in regulating TNF-α, exploring its potential as a therapeutic target and its impact on cancer progression.
Aim-The primary aim of this study is to explore the regulatory relationship between miR-204-3p and TNF-α in OSCC, focusing on miR-204-3p's potential role as a tumor suppressor and its implications for targeted therapies in OSCC.
Methods-Human genome sequences were obtained from NCBI, and OSCC-specific sequences were filtered to create a nucleotide database. miRNA reference sequences were sourced from miRBase, and miRNA targets were predicted using TargetScan based on conserved binding sites. The structural stability of precursor-miRNAs was validated using RNAfold. OSCC tissue samples were ethically collected, and total RNA was isolated using TRIzol. Gene expression levels of TNF-α and miR-204-3p were analyzed using qRT-PCR, with statistical analysis performed using SPSS.
Results-The study identified hsa-miR-204-3p as a regulator of TNF-α, characterized by a stable stem-loop structure. qRT-PCR analysis revealed significant downregulation of miR-204-3p and upregulation of TNF-α in OSCC tissues compared to normal tissues.
Conclusion-hsa-miR-204-3p may act as a tumor suppressor in OSCC by targeting TNF-α, suggesting its potential as a therapeutic target for OSCC treatment.
Key words- OSCC,cancer, RNA,Oral cavity.

1. INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC), is a cancer that arises from squamous cells in the oral cavity or oropharynx. Mainly, this cancer develops in the lining of the buccal cavity, which is made up of squamous cells. It also affects the lips, tongue, and other inner parts.(1) Globally, it is a major public health problem whose incidence has gone up greatly. According to the International Agency for Research on Cancer's (IARC's) Global Cancer Observatory (GLOBOCAN), the yearly incidence of OSCC was 377,713 cases globally, with Asia recording the greatest number of cases.(2) Typically resulting from precancerous lesions, OSCC is associated with risk factors such as excessive alcohol intake, tobacco use, and betel use.(3)For the treatment of OSCC, a multidisciplinary approach is used, including surgery, radiotherapy, and chemotherapy.(4) But treatment success is significantly higher when cancer is detected early in its progression, and there are many methods available for detection. But it's very challenging to identify cancer and provide suitable treatment at an early stage with available tests. This dismal outlook is largely attributed to late-stage diagnosis, high recurrence rates, and metastasis. Thus, early

DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 1, Issue 03, 2025; p08-14,
E-ISSN 2583-7761

diagnosis is crucial for better survival of patients. Understanding the molecular mechanisms underlying OSCC is crucial for developing early diagnostic markers and novel therapeutic strategies. Numerous research concentrates on different genes in OSCC. Nonetheless, research is still being done to determine whether a gene has the potential to be a therapeutic target and how it contributes to the development of cancer. In this case, we are concentrating on tumor necrosis factor (TNF-α), an inflammatory cytokinin that contributes to the development of cancer. Gene-regulating RNA, also known as micro-RNA (mi-RNA), targets mRNA and regulates gene expression. It is a novel biomarker for early cancer detection and prognosis, and its field of study is expanding.

TNF-α is produced by the body's macrophages and monocytes in response to tissue inflammation. It can control several signaling pathways within cells and trigger either apoptosis or necrosis. (5,6) TNF-α is known to be linked to a wide range of diseases, including cancer, according to scientists. The effectiveness of TNF-α in cancer rests on its local application, concentrated on aggressive local soft tissue sarcomas and metastatic melanomas.(7)During acute inflammation, this pro-inflammatory cytokine is produced and participates in signaling processes. It will promote the survival of tumor cells by activating certain signaling pathways and stimulating tumor cell invasion (6). In addition, this tumor factor has role as anti-tumor growth sometimes. Meanwhile, it will provide a link between cancer development and inflammation progression. Thus, it shows the role of potential therapeutic target.(8,9) In certain neoplasms, TNF-α functions as a potent biomarker and prognostic factor.(10)

MicroRNAs (miRNAs) are small noncoding RNA molecules produced from genes in the nucleus. They modulate target mRNA by primarily binding to the 3'-untranslated regions (3'UTR), resulting in either the inhibition of translation or the degradation of the mRNA ,(11)miRNAs with abnormal expression levels have been implicated in the development and spread of human tumours by targeting oncogenes or tumour suppressor genes. These miRNAs are involved in numerous physiological and pathological processes, such as cancer. They play significant roles in tumour development, cell growth, migration, programmed cell death, and metastasis. (12) Human miR-204, part of the miR-183 family cluster, is often downregulated in various malignancies. Notably, miR-204-3p frequently exhibits reduced expression in cancers, acting as a tumor suppressor in tumorigenesis.(13) This downregulation has been observed in gastrointestinal stromal tumors,(14) breast cancer,(15) and hepatocellular carcinoma (HCC),(16) where miR-204 expression in HCC is significantly lower than in adjacent normal hepatic tissues. Several studies suggest that miR-204-5p may act as an inhibitory RNA molecule in OSCC by targeting CXCR4, even if the exact function of miR-204-3p in OSCC is still unknown. (17)Studies have highlighted that miR-204-3p is known to target multiple genes involved in cancer-related pathways. Therefore, understanding the regulatory mechanisms of TNF-α in OSCC is of paramount importance.

The purpose of this work is to look at how miR-204-3p controls TNF-α in OSCC. By analyzing the expression levels of miR-204-3p and TNF-α in OSCC tissues within Indian population and examining their potential interactions, we seek to elucidate the molecular mechanisms underlying OSCC progression. In addition, we hope to investigate the therapeutic potential of miR-204-3p, which may result in the creation of fresh approaches to treating OSCC.

2. MATERIALS AND METHODS

2.1. Data Retrieval

Human genome sequences were sourced from the NCBI portal, part of the International Nucleotide Sequence Database Consortium. To locate OSCC sequences, a search was conducted using the term “Oral squamous cell carcinoma”. After filtering out repetitive and low-quality sequences, an OSCC-specific nucleotide database was created. Reference sequences for miRNAs were obtained from miRbase (http://www.mirbase.org/). This OSCC database was then used to find homologs in the miRNA dataset.(18)

2.2. Identifying miRNAs and target genes

To discover miRNAs that regulate our target gene, we used Target Scan, a bioinformatics tool that predicts miRNA targets by identifying conserved

DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 1, Issue 03, 2025; p08-14,
E-ISSN 2583-7761

binding sites (8mer, 7mer, and 6mer) that match the seed region of each miRNA. This tool provided a list of potential miRNAs for further analysis of their regulatory roles.(18)

2.3. Finding precursor-miRNAs

Mature miRNA sequences were used as queries to search for homologs in the OSCC database. Non-protein coding characteristics were confirmed by validating sequences containing up to three mismatches. Next, it was suggested that the matched sequences may be precursor miRNAs.

2.4. Validating Candidate pre-miRNAs and finding target genes

Candidate pre-miRNAs were assessed using RNA fold to derive their secondary structures. Validation criteria included: 1) Presence of a stem-loop hairpin structure, 2) Location of the mature miRNA on one side of the hairpin, 3) Fewer than seven mismatches with the complementary miRNA in the opposite arm, and 4) Secondary structure with negative energy and an A+U content between 40% and 70%. Additionally, target prediction was performed using TargetScan to in order to help find possible targets (https://www.targetscan.org/vert_80/). One gene identified with more target score and validated by NCBI data set.(11,18)

Sample collection

The institutional ethics committee gave its approval and the samples were obtained in compliance with the Helsinki Declaration for this study. With informed agreement, a total of 20 tissue samples, comprising OSCC and surrounding normal tissues, were taken from patients at hospitals, Saveetha Dental College, and the Oral and Maxillofacial Surgery Department. The dental pathology section at Saveetha Dental College and hospitals confirmed the diagnosis of OSCC. The samples were kept cold for further examination.

Inclusion and exclusion criteria

Individuals with squamous cell carcinoma of the buccal mucosa who were above the age of eighteen and able to give informed permission were considered participants. Exclusion criteria were the presence of cancer at the tongue's border or other active cancers. After applying these criteria, 15 participants were selected for the study.

RNA isolation

Operating within the guidelines provided by the manufacturer, total RNA was extracted from OSCC cells using the TRIzol reagent (Invitrogen). With a Nanodrop 200 Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA), the concentration and purity of the extracted RNA were assessed.

Reverse transcription and gene expression analysis – qRT-PCR

Reverse transcription has been done using qRT-PCR with previously validated primers for TNF-α gene and miR-204 in forward and in reverse. For one-stranded cDNA synthesis, two units of total RNA dT primer and SII RT were used. qRT-PCR was performed on an iCycler using validated primers, and SYBR Premix Ex Taq II. We quantitated the transcripts of specific genes of interest by setting a cycle-number threshold. The transcripts were normalized during the same incubations using GAPDH and U6 as a reference gene respectively. Table 1 illustrate the forward and reverse sequence of each.

Statistical analysis

The statistical analysis was done using SPSS software. The data was presented using the mean ± SD format. The gene expression level in cancerous tissues was compared with normal tissues (adjacent) using an automated t-test program. $P \leq 0.05$ was discovered to be statistically significant.

RESULT

Identification of pre-miRNA and its secondary structure

Using techniques for computation, we identified the miRNA for the target gene TNF-α. Among the list, hsa-miR-204-3p was found. The secondary structure of this miRNA was examined in detail, revealing that its mature sequence has a free energy in negative form of -39.20 kcal/mol. Table 2 provides details of the mature and stem-loop sequences of miR-204-3p, while Figure 1 illustrates its secondary structure with mature sequences. Table 3 summarizes the characteristics of hsa-miR-204-3p, including pre-miRNA length, A+U concentration, mature sequence, degree of match, and minimal free energy.

Identifying the targets

Target scan analysis was performed to determine the targets of miR-204-3p. The analysis identified several significant transcripts targeted by miR-204-3p, including TNF-α, fibroblast growth factor-11

DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 1, Issue 03, 2025; p08-14,
E-ISSN 2583-7761

etc. Table 4 summarizes some target proteins for hsa-miR-204-3p and their associated biological processes and molecular functions. We selected TNF-α as target which is having better score, high impact in cancer progression, and less studied in OSCC of Indian population.

Expression Analysis of miR-204-3p and TNF-alpha Genes

qRT-PCR analysis revealed altered expression levels of miR-204-3p and TNF-α in OSCC patient samples compared to normal samples. miR-204-3p was significantly downregulated, whereas TNF-α was significantly upregulated in OSCC patients. These results imply potential roles for miR-204-3p and TNF-α in OSCC. Refer to Figures 2 and 3 for graphical representations of miR-204-3p and TNF-α expression profiles.

DISCUSSION

The study aimed to investigate the role of miR-204-3p in OSCC by exploring its regulatory effects on TNF-α gene. The result provides significant insight into the molecular mechanisms underlying OSCC and the potential therapeutic targets that could be explored further. Our work is an addition to the increasing amount of research that shows how miR-204-3p functions in OSCCThe dual involvement of miR-204 in cancer is due to its induction of apoptosis, increase in chemotherapy resistance, inhibition of cancer stem cell self-renewal, and epithelial to mesenchymal transition (EMT).(19) Moreover, TNF-α also plays dual role in prostate and endometrial cancers, its expression is repressed by XRN1 and TRKB, forming oncogenic feedback loops that promote cancer development. Adenomatous polyposis coli 2 (APC2) is a functional target of miR-204-3p, and previous research has shown that circ-PKD2 acts as a sponge for miR-204-3p. As such, the circ-PKD2/miR-204-3p/APC2 axis is a novel pathway involved in the pathogenesis of OSCC and a potential therapeutic target.(20) Using chromogen-based in situ hybridisation (ISH) and immunohistochemistry (IHC), Rajthala et al. also sought to develop a technique for the dual visualisation of miR and protein (pan-cytokeratin). stromal expression of miR-204 as a predictive biomarker in OSCC was investigated using this methodology. Their findings further support the significance of miR-204 in OSCC, reinforcing its potential utility in prognosis and targeted therapy.(21) Furthermore, Brierly et al. review the role of TNF-α in OSCC, highlighting its impact on tumor progression through promotion of inflammation and cell proliferation. The study emphasizes TNF-α's potential as a biomarker for OSCC and explores the therapeutic promise of TNF-α-targeting biologics.(22) Another study by Ridha Azimudin et al. investigated salivary TNF-α levels in OSCC patients with and without diabetes mellitus, revealing significant differences that suggest TNF-α's involvement in OSCC and its potential interactions with diabetes. Further research is needed to clarify TNF-α's prognostic value in OSCC and the influence of diabetes mellitus.(23) Further this research is warranted to explore these findings in clinical settings and refine therapeutic strategies targeting the miR-204-3p and TNF-α pathways.

In conclusion, our study underscores the pivotal role of miR-204-3p in the pathogenesis of OSCC, revealing its function as a tumor suppressor through its regulatory effects on TNF-α. The dual role of TNF-α in cancer progression, as highlighted by previous studies, further emphasizes the complexity of its involvement in OSCC. This research enriches our understanding of the molecular mechanisms underpinning OSCC and highlights the potential of miR-204-3p and TNF-α as therapeutic targets. The dual role of miR-204-3p and TNF-α in cancer emphasizes the complexity of their interactions and suggests that targeting these pathways could provide novel approaches for treatment.

Conclusion

In conclusion, our study identifies hsa-miR-204-3p as a critical regulator in OSCC, with its downregulation leading to the upregulation of TNF-α, an important player in cancer inflammation. The comprehensive analysis of the precursor miRNA, secondary structure, and expression profiles underscores the relevance of miR-204-3p in OSCC pathogenesis. These findings pave the way for further investigation into miR-204-3p as a potential biomarker and therapeutic target. Upcoming research endeavours ought to concentrate on verifying these findings in more extensive groups

DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 1, Issue 03, 2025; p08-14,
E-ISSN 2583-7761

and investigating the remedial possibilities of adjusting miR-204-3p levels in the management of OSCC.

Abbreviations

TNF-α- Tumor necrosis factor -alpha
OSCC- Oral squamous cell carcinoma
HCC- Hepatocellular carcinoma

Conflict of Interest-None

Acknowledgement-Not applicable

CRedit of Authorship Statement

Ishwarya S: writing original draft. Ashikha shirin Usman PP: Methodology, Investigation. Ameya KP . Visualization, Validation. Dhanraj M Ganapathy: Review and Editing. Durairaj Sekar: Conceptualization, Formal analysis.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval

Ethical approval for the sample collection were obtained in compliance with the Helsinki Declaration for this study, from the Department of oral maxillofacial surgery, Saveetha Dental College

TABLES

Table 1 represents the list of primers used in the reverse transcription.

PRIMERS	FORWARD SEQUENCES	REVERSE SEQUENCES
GAPDH	5'-GTCTCCTCTGACTTCAACAGCG-3'	5' ACCACCCTGTTGCTGTAGCCAA-3'
TNF-α	5'- AGTGAGGAACAAGCCAGAGC -3'	5' GTCAGGGGTGGTTATTGCAT 3'
miR-204	5'-GGACTTCCTGATCGCGTA-3'	5'-TTTCACTCCTTCCTAATTCCAGA-3'
U6	5'- CTCGCTTCGGCAGCACA-3'	5'- ACGCTTCACGAATTTGCGT-3'

Table 2: Represents the stem loop and mature sequences of miR-204-3p.

NO.	Structure	Sequence
1.	Stem loop	GGCUACAGUCUUUCUUCAUGUGACUCGUGGACUUCCCUUU GUCAUCCUAUGCCUGAGAAUAUAUGAAGGAGGCUGGGAAG GCAAAGGGACGUUCAAUUGUCAUCACUGGC
2.	Mature miRNA	GCUGGGAAGGCAAAGGGACGU

DOI-10.5455/AJMRHS.20250503
Asian Journal of Medical Research & Health Sciences, Vol 1, Issue 03, 2025; p08-14,
E-ISSN 2583-7761

Table 3: Represents the pre-miRNA length, minimum free energy, mature sequence, match extent, and A+U% content of has-miR-204-3p.

Source miRNA	Source Organism	Pre-miRNA Length	Minimum Free Energy	Mature Sequence	Match Extent	Strand	A+U%
miR-204-3p	*Homo sapiens*	110	-39.20 Kcal per mole	GCUGGGAAGGCAAAGGGACGU	21/21	3p	51.8%

Table 4 represents other target genes of the same miRNA with its molecular functions.

Sl no	Target protein	Uniprot acc. no	Molecular function	Biological process
1.	**Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase**	NX_P26572	Transferase activity, Catalytic activity, Acting on a protein, Other molecular functions	Protein glycolysation, Anatomical structure development, Carbohydrate derivative metabolic process.
2.	MICAL-like protein 1	NX_Q8N3F8	Lipid binding, Other molecular functions	Cytoskeleton organization, Vesicle-mediated transport, Cell differentiation.
3.	**Serine/threonine-protein kinase greatwall**	NX_Q96GX5	Transferase activity, Hydrolase activity, Catalytic activity.	Mitotic cell cycle, meiotic nuclear division, Cell differentiation.

4.	**DNA damage-inducible transcript 3 protein**	NX_P35638	DNA binding, Molecular adaptor activity, Transcription regulator activity.	Autophagy, Programmed cell death, Cell signalling, differentiation.
5.	**Fibroblast growth factor 11**	NX_Q92914	Cytoskeletal protein binding, molecular transducer activity,	Signalling, Anatomical structure development, Transmembrane transport.

REFERENCE

1. Guimarães OS, Cangussu LMB, dos Reis Pereira Queiroz L, Santos EMS, de Souza MG, de Paula AMB, et al. Inhibition of GATA3, ABCB1, and TYR by gallic acid: A real-world approach to uncovering a preventive agent against oral squamous cell carcinoma development. Gene Rep. 2023;33:101840. DOI: 10.1016/j.genrep.2023.101840
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. DOI: 10.3322/caac.21660
3. Kumari P, Debta P, Dixit A. Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. Front Pharmacol. 2022;13. DOI: 10.3389/fphar.2022.825266
4. Arboleda LPA, de Carvalho GB, Santos-Silva AR, Fernandes GA, Vartanian JG, Conway DI, et al. Squamous Cell Carcinoma of the Oral Cavity, Oropharynx, and Larynx: A Scoping Review of Treatment Guidelines Worldwide. Cancers (Basel). 2023;15(17):4405. DOI: 10.3390/cancers15174405
5. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr. 2010;20(2):87–103. DOI: 10.1615/critreveukargeneexpr.v20.i2.10
6. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech. 2000;50(3):184–95. DOI: 10.1002/1097-0029(20000801)50:3<184::AID-JEMT2>3.0.CO;2-H
7. van Horssen R, ten Hagen TLM, Eggermont AMM. TNF-α in Cancer Treatment: Molecular Insights, Antitumor Effects, and Clinical Utility. Oncologist. 2006;11(4):397–408. DOI: 10.1634/theoncologist.11-4-397
8. ANDERSON G. Tumor necrosis factor-? in the pathogenesis and treatment of cancer. Curr Opin Pharmacol. 2004;4(4):314–20. DOI: 10.1016/j.coph.2004.04.004
9. Sethi G. TNF: A master switch for inflammation to cancer. Frontiers in Bioscience. 2008;Volume(13):5094. DOI: 10.2741/3066
10. Mahdavi Sharif P, Jabbari P, Razi S, Keshavarz-Fathi M, Rezaei N. Importance of TNF-alpha and its alterations in the development of cancers. Cytokine. 2020;130:155066. DOI: 10.1016/j.cyto.2020.155066
11. Shreya Reddy CS, Usman P.P AS, Ganapathy DM, K.P. A, Sekar D. MicroRNA-21 as a biomarker in terminal stage oral squamous cell carcinoma (OSCC) in the South Indian population. Oral Oncology Reports. 2024;9:100139. DOI: 10.1016/j.oor.2023.100139
12. Sekar D, Mani P, Biruntha M, Sivagurunathan P, Karthigeyan M. Dissecting the functional role of microRNA 21 in osteosarcoma. Cancer Gene Ther. 2019;26(7–8):179–82. DOI: 10.1038/s41417-019-0092-z
13. Sümbül AT, Göğebakan B, Ergün S, Yengil E, Batmacı CY, Tonyalı Ö, et al. miR-204-5p expression in colorectal cancer: an autophagy-associated gene. Tumor Biology. 2014;35(12):12713–9. DOI: 10.1007/s13277-014-2596-3
14. Liang Y, Zhang C-D, Zhang C, Dai D-Q. DLX6-AS1/miR-204-5p/OCT1 positive feedback loop promotes tumor progression and epithelial–mesenchymal transition in gastric cancer. Gastric Cancer. 2020;23(2):212–27. DOI: 10.1007/s10120-019-01002-1
15. Fan X, Fang X, Liu G, Xiong Q, Li Z, Zhou W. MicroRNA-204 inhibits the proliferation and metastasis of breast cancer cells by targeting PI3K/AKT pathway. J BUON. 2019;24(3):1054–9.

16. Luo Y-H, Tang W, Zhang X, Tan Z, Guo W-L, Zhao N, et al. Promising significance of the association of miR-204-5p expression with clinicopathological features of hepatocellular carcinoma. Medicine. 2017;96(30):e7545. DOI: 10.1097/MD.0000000000007545
17. Wang X, Li F, Zhou X. miR-204-5p regulates cell proliferation and metastasis through inhibiting CXCR4 expression in OSCC. Biomedicine & Pharmacotherapy. 2016;82:202–7. DOI: 10.1016/j.biopha.2016.04.060
18. Suthagar P, Purayil ASUP, Parambath AK, Sekar D. In silico identification and gene expression of miR-148a-5p and IL-6 in hypothyroid patient blood samples. Biomedical Research and Therapy. 2024;11(5):6379–86. DOI: 10.15419/bmrat.v11i5.884
19. Li T, Pan H, Li R. The dual regulatory role of miR-204 in cancer. Tumor Biology. 2016;37(9):11667–77. DOI: 10.1007/s13277-016-5144-5
20. Gao L, Zhao C, Li S, Dou Z, Wang Q, Liu J, et al. circ-PKD2 inhibits carcinogenesis via the miR-204-3p/APC2 axis in oral squamous cell carcinoma. Mol Carcinog. 2019;58(10):1783–94. DOI: 10.1002/mc.23065
21. Rajthala S, Dongre H, Parajuli H, Min A, Nginamau ES, Kvalheim A, et al. Combined In Situ Hybridization and Immunohistochemistry on Archival Tissues Reveals Stromal microRNA-204 as Prognostic Biomarker for Oral Squamous Cell Carcinoma. Cancers (Basel). 2021;13(6):1307. DOI: 10.3390/cancers13061307
22. Brierly G, Celentano A, Breik O, Moslemivayeghan E, Patini R, McCullough M, et al. Tumour Necrosis Factor Alpha (TNF-α) and Oral Squamous Cell Carcinoma. Cancers (Basel). 2023;15(6). DOI: 10.3390/cancers15061841
23. Azimudin R, Palati S, R P. Evaluating Tumor Necrosis Factor (TNF)-Alpha Pro-inflammatory Cytokines in Healthy and Oral Squamous Cell Carcinoma Patients With and Without Diabetes. Cureus. 2024;16(1):e52890. DOI: 10.7759/cureus.52890

Asian Journal of Medical Research & Health Sciences, vol 2, issue 03, 2024; p50-55,
doi: 10.5455/AJMRHS.1107202300028, E-ISSN 2583-7761