Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/awkdqg04

EXPRESSION OF mRNA-31 AS A POTENTIAL DIAGNOSTIC BIOMARKER IN ORAL SUBMUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA

Dr. Saba Khan[1], Dr. Eid Muhammad[2], Dr. Uzma Zareef[3], Dr. Dinaz Ghandhi[4], Dr. Muhammad Saqib[5], Dr. Humera Akhlaq[6]

[1]Assistant Professor, Department of Oral Pathology, Hamdard University Dental Hospital, Karachi Pakistan, drsabakhan@outlook.com
[2]Senior Lecturer, Oral Pathology, Quetta College of Dentistry, Quetta, Pakistan eidmohd.raisani@gmail.com
[3*]Professor, Oral Pathology, Liaquat College of Medicine and Dentistry, Karachi Pakistan uzmaz_3@hotmail.com
[4]Associate Professor, Head of Oral & Maxillofacial Department, Altamash Institute of Dental Medicine, Karachi Pakistan, dinaz.ghandhi@altamash.pk
[5]Assistant Professor, Department of Operative Dentistry and Endodontics, Altamash Institute of Dental Medicine, Karachi Pakistan, hafiz_m_saqib@hotmail.com
[6]Associate Professor Oral Pathology, Sindh institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi Pakistan, humeraakhlaq@gmail.com

***Corresponding Author:** Uzma Zareef
*Professor, Oral Pathology, Liaquat College of Medicine and Dentistry, Karachi Pakistan
uzmaz_3@hotmail.com

ABSTRACT

Background:
The death toll attributed to oral cancer ranks high in underdeveloped nations. Oral submucous fibrosis evolves into oral carcinoma in most cases. The oral cavity remains accessible for direct examination yet many cancers are detected only in late stages thus decreasing treatment opportunities during surgery. The investigation began by examining miR-31 levels in saliva samples. The study evaluated how miR-31 acts as an oncogene while analyzing its diagnostic potential.

Methods: A case-control study was conducted at Ziauddin University's department of oral diagnosis and the Altamash Institute of Dental Medicine. We used qRT-PCR on 100 subjects ranging in age from 18 to 70 years to assess salivary miRNA-31 expression between controls (50) and patients. Unstimulated whole saliva samples were collected using the passive drooling technique. All statistical analyses were carried out using SPSS-24.

Results: The results of our study have revealed high expression levels of miRNA-31 in OSCC and OSMF as compared to the control group. The median fold change of miRNA-31 was 7.94 in OSCC, 3.24 in OSMF as compared to controls. In relation to the tumor grading and site the median fold of microRNA31 is higher (median=36.39) in well differentiated and in lower lip (median=39.39)

Conclusion: Results showing elevated miRNA-31 expression in specimens from OSMF and OSCC patients suggest its potential as a diagnostic tool to detect these cancers at their initial stages through salivary analysis.

Keywords: Oral squamous cell carcinoma, Submucous fibrosis, MicroRNA 31, qRT-PCR, Salivary biomarkers

INTRODUCTION

Cancer remains the leading global cause of death resulting in more than 1 million deaths annually which corresponds to one-sixth of total fatalities worldwide during 2020. Oral cancer holds the position of top cancer death cause in developing nations. The most common type of oral cancer is Oral Squamous Cell Carcinoma (OSCC) which represents 90% of oral cancers and 91% of head and neck cancers2. Despite recent advances in treatment methods the survival chances for OSCC patients remain stagnant. Most oral cancer diagnoses happen too late in the healthcare system of Pakistan. Patients remain undiagnosed because they lack understanding about disease symptoms while physicians often hold up treatment and referral processes3. OSMF operates as Pakistan's principal precancerous condition and displays a 7.6% risk of transforming into malignancy during 10 years of standard patient monitoring. The harmful agents in addictive areca nut alkaloids and alcohol-based chewing products accelerate the formation of fatal cancer cells.5 Through molecular pathway analysis the detection of developing cancers and predictions about tumor growth become achievable. Clinical oral examinations along with biopsied tissue analysis remain the standard approach for OSCC diagnosis by assessing suspicious lesions. Scientists study cancer to create less invasive diagnostic techniques using cost-effective approaches that provide detailed cancer information along with enhanced treatment progress tracking for precise therapeutic strategy development6.

These small noncoding RNA molecules known as microRNAs (miRNAs) range from 19 to 24 nucleotides in length and naturally occur within endogenous systems. These molecules perform their biological assignments by binding to target genes' 3′ untranslated region (UTR) therefore initiating mRNA degradation or inhibiting translation to control gene expression at post-transcriptional levels. Many studies have identified different miRNAs that play a role in the development of various cancers including OSCC7. Scientific research focuses on understanding the misregulation patterns of miRNAs in both Oral Squamous Cell Carcinoma (OSCC) and Tongue Squamous Cell Carcinoma (TSCC). The expression level of miR-21, miR-24, miR-31, miR-184, miR-211, miR-221, and miR-222 increases in oral cancer but miR-203, miR-100, miR-200, miR-133a, miR-133b, miR-138, and miR-375 show decreased levels in the disease. The modifications in these molecular mechanisms result in substantial changes during oral carcinogenesis8. The miR-31-5p microRNA has a gene located within the p21.39 chromosomal region. The molecular function of miR-31-5p helps cancer cells to migrate beyond their original position as it also drives tumor invasiveness. The upregulation of miR-31-5p in different types of cancer including OSCC has been documented while researchers establish its functional role as an oncogenic miRNA. Research shows that higher expression levels of miR-31-5p correlate with shorter patient survival times9. Tissue biopsy serves as the recognized and most effective diagnostic tool for confirming cases of OSCC. This methodology remains invasive while it also proves costly and time-consuming and shows technical sensitivity. The testing and analysis of bodily fluids such as blood and serum and urine and saliva adds new capabilities to diagnostic techniques10. The continuous interaction between saliva and lesions makes it an ideal diagnostic tool for both OSCC and OSMF because saliva collection allows for non-invasive testing11. Recent research indicates that oral squamous cell carcinoma (OSCC) development involves miRNA deregulation that leads to significant changes in six key miRNAs including miRNA-21, miRNA-184, miRNA-133a, miRNA-221, miRNA-375, and let-7b12. Different aspects of RNA biomarkers consist of dual specificity phosphatase 1 along with H3 histone family members and numerous miRNA genes.

The study investigates the potential of salivary miRNA-31 level evaluation as a diagnostic tool to detect early OSCC cases and monitor OSMF to OSCC malignant progression. Future clinicians and researchers would leverage this discovery to enhance OSMF and OSCC patient management through better diagnostic methods and classification systems.

MATERIALS AND METHODS

A case-control study was conducted following approval from the Ziauddin University Ethical Review Board (ERC no. 3011220SKOM). The estimated duration to complete this research was about eight months (Feb 2021-Oct 2021) Samples were collected from the Department of Oral Diagnosis at Ziauddin University and the Altamash Institute of Dental Medicine. The sample size was calculated with open Epi version 3.01. The power of the test was taken as 90% and the confidence of interval was considered as 95%, the sample size came out to 6 per group which was increased up to 50 per group. A non- probability consecutive sampling technique was used. Total of 100 individuals from Karachi were selected for the study, divided as follows:

GROUP 1 (n=50): 50 clinically healthy individuals of both genders (control group).

GROUP 2 (n=50): 25 patients diagnosed with oral squamous cell carcinoma (OSCC) and 25 patients diagnosed with oral submucous fibrosis (OSMF).

Participants aged 18 to 70 years, with clinically diagnosed cases of OSMF and clinically and histologically confirmed cases of OSCC. Healthy individuals with no history of oral mucosal lesions, hypertension, diabetes, cancers other than those of the oral cavity, pregnant females, and individuals with psoriasis, systemic lupus erythematosus, or rheumatoid arthritis were excluded from the study. Saliva sample analysis was conducted using qRT-PCR at the North Campus of Ziauddin University Hospital, Karachi.

Collection of Saliva:
A total volume of 5 ml whole saliva from unstimulated areas was obtained through passive drooling techniques. Each participant donated saliva into sterile collector tubes while ice maintained sample quality. The centrifugation process at 4˚C included setting the speed to 2500 rpm for 15-20 minutes. Researchers stored the collected supernatant aliquots at -80˚C for subsequent analysis.

RNA extraction and Reverse transcription quantitative polymerase chain reaction (RT-qPCR)
Primer Designing:
All primers were commercially prepared by (Synbio, USA). All primers were received in lyophilized form. Synthesized primers were first re-constituted in nuclease-free water to make 100 micromolar (uM) concentrations. The sequence of used primers were retrived from online data base NCBI, are listed below in Table 3.

Table 3: Sequences of reported primers (Al-Malkey et al., 2015)

Primers	**Sequence**	**Primer Length**
MICRO RNA-31 Forward	**5'** GTTTAGGCAAGATGCTGGC**3'**	19
MICRO RNA-31 Reverse	**5'** GTGCAGGGTCCGAGGT**3'**	16
GAPDH Forward	**5'** TCAGCCGCATCTTCTTTTGC**3'**	20
GAPDH Reverse	**3'** TTAAAAGCAGCCCTGGTGAC**5'**	20

RNA isolation
TRizol was used for the extraction of RNA from all samples' specimens. The whole procedure was started with the washing of samples two times with chilled Phosphate Buffer Saline (PBS) followed by lysis of cells by adding 1 ml of TRizol solution in each tube.

cDNA Synthesis
Complementary DNA (cDNA) was synthesized from extracted RNA by taking a calculated amount of DEPC treated nuclease-free water in 0.2 ml tube followed by the addition of 1 ug of extracted RNA and 1 ul of 50uM oligo primers. Adding a calculated amount of DEPC-treated nuclease-free water to a 0.2 ml tube.The cDNA synthesis program was as follows:

Time	**Temperature**	**Storage Condition**
10 minutes	25°C	Initial incubation
60 minutes	42°C	Incubation
10 minutes	70°C	Final incubation
Indefinitely	4°C	Storage condition

Real-Time Polymerase Reaction (qRT-PCR)
After the reaction was completed, the synthesized cDNA was stored at -20°C for further use. The relative gene expression of the miRNA-31 gene in OSMF and OSCC was evaluated using real-time PCR. The reaction was conducted in a thermocycler (Bio-Rad CFX96) with the following conditions:

Step	**Temperature**	**Duration**
Initial Denaturation	95°C	10 minutes
Denaturation	95°C	30 seconds
Annealing	53°C	1 minute (for 40 cycles)
Elongation	72°C	1 minute
Storage	4°C	Indefinitely

STATISTICAL ANALYSES: The researchers conducted statistical tests through SPSS- 24 software. Shapiro-Wilk's test confirmed the data distribution deviated from normality since the P value remained below 0.05. Binary logistics regression calculated the odds ratio. Analysis of mean miRNA-31 differences between groups employed non parametric median test statistics. The researchers used a P-value of < 0.05 to determine statistical significance.

RESULTS

Men comprised 56% of participants in the control group with women making up 44%. Participants in the OSF group included 72% males and 28% females compared to the OSCC group where 88% were male and 12% female. The research involved participants aged 32.6 years for controls, participants aged 36.0 years for OSF and participants aged 47.0 years for OSCC.

Table 1: Demographic information

	Healthy (50)		OSF (25)		OSCC (25)	
Gender	**N**	**%**	N	%	n	%
Male	28	56.0	18	72.0	22	88.0
Female	**22**	44.0	7	28.0	3	12.0
Marital Status						
Married	4	23.5	14	70	6	50
Unmarried	13	76.5	6	30	6	50
Ethnicity						
Baloch	1	2.0	1	4.0	1	4.0
Pathan	1	2.0	2	8.0	3	12.0
Punjabi	14	28.0	8	32.0	4	16.0
Sindhi	3	6.0	0	0.0	4	16.0
Saraiki	7	14.0	5	20.0	3	12.0
Urdu Speaking	**24**	**48.0**	9	36.0	10	40.0
Qualification						
Below 10 grade	2	4.0	5	20.0	2	8.0
Below12th grade	6	12.0	4	16.0	6	24.0
Graduate	**36**	**72.0**	15	60.0	14	56.0
Housewife	0	0.0	0	0.0	1	4.0
no educated	1	2.0	0	0.0	2	8.0
postgraduate	5	10.0	1	4.0	0	0.0
Age						
meanest	**32.6±8.2**		36±7.9		47±8.8	
minimax	20.0	58.0	25.0	54.0	30.0	63.0

Betel nut consumption was highest in the OSF group (100%), followed by the OSCC group (32%). Additionally, 68% of participants in the OSF group and 72% in the OSCC group were smokers,

Table 2: Consumption of Tobaco

	Healthy (50)		**OSF (25)**		**OSCC (25)**	
Batal Nuts	**N**	**%**	**n**	**%**	**N**	**%**
Yes	-	-	**25**	100.0	8	32.0
No	-	-	0	0.0	3	12.0
total	**-**		**25**		**11**	
Smoking						
Smoker	1	2.0	**17**	**68.0**	**18**	**72.0**
non-smokers	49	98.0	8	32.0	7	28.0
Total	**50**		**25**		**25**	
Betel Quid/Pan & Tobacco						
Yesa	7	14	**24**	96	**21**	84
No	43	86	1	4	4	16
Total	50		25		25	

Regarding the clinical staging of OSMF, 16 cases (64%) were in stage 2, 7 cases (28%) were in stage 3, and only 2 cases (8%) were in stage 1. Based on the histological grading of OSCC, 16 cases (64%) were poorly differentiated, 8 cases (32%) were moderately differentiated, and just 1 case (4%) was well-differentiated, as outlined in Table 3.

Table 3: Staging and grading of OSCC and OSF

OSF Group	**n**	**%**
Stage 1	2	8
Stage 2	16	64
Stage 3	7	28
Total	25	100
Historical grading of OSCC (n=25)		
Moderately differentiated	8	32
Poorly differentiated	16	64
well differentiated	1	4
Total	25	100
Site of OSCC		
Buccal mucosa	14	56%
Lower lip	1	4%
Floor of mouth	8	32%
Tongue	2	8%

The results revealed that the median fold change of miRNA-31 was 7.94 in OSCC, 3.24 in OSMF as compared to controls. Any patient who had a value equal to or more than7.94 will be considered as having a malignant tumor as shown in Table 4.

Table 4: Median Fold change of MicroRNA-31 among case and control Groups

S.No	**Group**	**Median Fold Change**	**P-Value**
1.	Controls	1	**0.001**
2.	OSMF	3.24	
3.	OSCC	**7.94**	

In relation to the tumor site and grading the median fold of microRNA31 is higher (median=36.39) in well differentiated and in lower lip (median=39.39). In relation to clinical staging of OSMF the median fold of microRNA31 is higher (median=35.45) in Stage II, as shown in Table 5.

Table.5: Median Fold change of MicroRNA-31 associated with different variable in OSCC group

OSCC Group			
Histological grading of OSCC	**N**	**median fold**	**p-value**
Moderately differentiated	8	32.97	0.494*
Poorly differentiated	16	33.09	
Well differetiated	**1**	**36.39**	
Total	25		
Site of OSCC			
Buccal mucosa	14	33.05	0.250*
Lower lip	**1**	**39.39**	
Floor of mouth	8	32.24	
Tongue	2	36.14	
OSMF Group			
Staging of OSMF	**Median Fold Change**	**P-value**	
Stage-I	32.77	**0.484***	
Stage-II	35.45		
Stage-III	32.87		
***P-values derived from Non-Parametric Median Test**			

DISCUSSION

Tobacco exposure combined with alcohol consumption and infection by human papillomavirus cause significant risk for developing OSCC. Various health factors simultaneously harm tumor suppressor genes while affecting proto-oncogenes and oncogenes and also disturb normal cellular operations that include chromosomal instability with copy number variations and loss of heterozygosity and telomere stability and DNA damage repair mechanisms and cell cycle regulation and Notch signaling pathways. The timing of a patient's disease progression at treatment onset influences their expected OSCC results. Medical reports indicate that many Pakistanian patients receive oral cancer diagnoses at an advanced stage.

The urgent requirement demands innovative biomarkers for prompt OPMD diagnosis alongside early OSCC detection and post-treatment patient monitoring. Saliva-liquid biopsy offers promise because it interacts directly with oral tissues to provide information about the disease status. MicroRNAs (miRNAs) function as small non-coding RNA molecules to contribute to tumorigenesis by regulating multiple mRNAs which results in controlling cell proliferation alongside differentiation and apoptosis while enabling migration and signal transduction. Healthcare professionals can use miRNAs as potential tools for early cancer detection and postoperative disease monitoring.

The demographic data revealed higher quantities of male subjects within both OSF and OSCC disease groups than females.A research conducted by Marcela Gomes Reis et al16 and Khan O et al17 demonstrated OSCC occurred more frequently in males than females. not only impact tumor suppressor genes, proto-oncogenes, and oncogenes but also cause disruptions in normal cellular processes such as chromosomal instability, copy number variations, loss of heterozygosity, telomere stability, DNA damage repair mechanisms, cell cycle regulation, and Notch signaling pathways. Patients with OSCC experience different treatment outcomes based on their disease stage at initial diagnosis. Research reveals that numerous patients in Pakistan receive their first cancer diagnosis when their condition has progressed to an advanced stage.

The urgent need exists to identify new biomarkers that will support OPMD and OSCC early detection and postoperative patient monitoring. Direct interactions between saliva-liquid biopsy and oral tissues suggest this approach could offer valuable diagnostic information about oral mucosal status. Small non-coding RNA molecules known as microRNAs (miRNAs) promote tumorigenesis through their ability to target multiple mRNAs and control key cellular processes such as cell proliferation, differentiation, migration, apoptosis and signaltransduction. MiRNAs show promise for both early tumor screening and monitoring patients after cancer removal treatments15.

Demographic date concluding that there was high percentage of males in diseased group (OSF and OSCC) as compare to the females. A study by Marcela Gomes Reis et al16 and Khan O et al17 reported the greater ratio for male as compared to females. Research indicates male patients experience oral cancer occurrences at rates two to four times higher than females in the population according to 18 and 19. Males display higher prevalence

for this disease possibly because of several factors such as geographical differences along with their heavy tobacco and alcohol consumption and limited monitoring of oral health prevention.

The research data revealed patient average ages of 32.6 for controls, 36.0 for OSF cases and 47.0 for OSCC patients. Studies demonstrate a strong relationship between middle age populations (41-50 years) and higher occurrence of OSCC. Not many studies in this geographical area found that older cases of OSCC occurred between 40 to 65 years of age. This study produced different findings than all research examined here. The fusion of genetic predispositions with persistent environmental elements throughout life spans can elevate the tendency to develop oral submucous fibrosis (OSF) in patients.

The receiver operator characteristic (ROC) curve analysis identified seven-point-ninety-four as the optimal cutoff point for miRNA-31 detection. Patients with miRNA-31 values exceeding 7.94 are identified as having malignant tumors. Data analysis using Spearman rank test revealed no significant correlation between patient age and miRNA31 expression change but indicated a negative trend ($r=-0.236$, $P<0.05$).

Research analyzing oral keratinocytes and tobacco exposure found a positive correlation between increased tobacco exposure and enhanced miRNA-31 levels which increased by three-fold22.The current study revealed that patients had markedly increased expression of miRNA-31 with median fold changes being 7.94 in patients compared to the control group value of 3.24; this finding had a highly significant statistical difference. the world have reported that majority of the OSCC patients were in the age group of 40 to 65 years20. All these studies are inconsistent with results from our study. The risk of developing oral submucous fibrosis (OSF) may be heightened by genetic variability, coupled with prolonged exposure to certain environmental factors over a person's lifetime21.

ROC curve analysis established a cutoff level of 7.94 for miRNA-31 detection. A value greater than or equal to 7.94 indicates a malignant tumor according to this measurement. Analysis through Spearman rank test illustrated a non-significant negative correlation between age of patients and miRNA31 fold change values ($r=-0.236$, $P<0.05$).

Research tracking miRNA expression alterations in oral keratinocytes revealed tobacco exposure through smoking and chewing results in the expression of miRNA-31 increasing 3-fold22.The data shows marked differences between patient and control groups with respect to median fold change in miRNA-31 expression, 7.94 in patients compared to the control's 3.24, leading to statistically significant results and demonstrating miRNA-31's relationship with OSCC development.patients group than in control group, 7.94 versus3.24 and control group 1.; this difference was statistically highly significant indicating its strong correlation with OSCC. The research results confirmed observed correlations between tobacco exposure times and miRNA-31 expression changes according to published literature with a statistical significance level of $P=0.00323$. Research data validates evidence-based health education approaches aimed at supporting people who sustain their lifestyle choices during prolonged periods. periods of time.

CONCLUSION

The present study demonstrates elevated miRNA 31 expression during oral submucous fibrosis and oral carcinoma when compared to control samples indicating this biomarker shows potential for early disease detection through saliva tests. Oral squamous cell carcinomas display clinical behaviors which make early detection difficult thus requiring the development of better biomarkers for patients with oral cancers and precancerous conditions. Saliva provides distinct information about oral mucosa disease status due to its consistent presence within the mouth but differs from other permanent bodily fluids that surround the tissues.

List of abbreviations

1. **OSCC** - Oral Squamous Cell Carcinoma
2. **OSMF** - Oral Submucous Fibrosis
3. **miRNA** - MicroRNA
4. **UTR** - Untranslated Region
5. **TSCC** - Tongue Squamous Cell Carcinoma
6. **qRT-PCR** - Quantitative Reverse Transcription Polymerase Chain Reaction
7. **cDNA** - Complementary DNA
8. **DEPC** - Diethyl Pyrocarbonate
9. **PBS** - Phosphate Buffer Saline
10. **SPSS** - Statistical Package for the Social Sciences
11. **ERC** - Ethical Review Committee

LIMITATIONS

- Expression of miRNA-31 should be evaluated on a larger sample size.
- An array of miRNA could have been included in this evaluation.
- Sample selection should have been done with purposive inclusion of all stages and grades of OSCC.

FUTURE RECOMMENDATION

- To decipher the molecular pathway of miRNA-31 in OSCC and its interactions with tumour suppressor genes and oncogenes and research on therapies targeting the pathway utilized by miRNA-31.
- To compare miRNA-31 expression level in HPV positive Vs HPV negative oropharyngeal carcinoma.

CONFLICT OF INTEREST/COMPETING INTERESTS: The authors declare that there are no conflicts of interest or competing interests associated with this manuscript.

ETHICS APPROVAL/ DISCLOSURE: Ethical approval for the study was obtained from the relevant ethics committee, and all procedures were conducted in accordance with ethical standards. The committee's reference number is granted by the **Ziauddin University Ethical Review Board (ERC no. 3011220SKOM)**.

FUNDING: The study did not receive any funding or financial support from any institution or company.

AUTHORS' CONTRIBUTIONS: All authors contributed significantly to the design, data analysis, and writing of the manuscript.

REFERENCES

1. Krishnan M, Babu S, Jayaraman S, Daniel P, Solomon A, Chinnaiyan M. Microrna-31 as a potential therapeutic biomarker for oral squamous cell carcinoma: current evidence and future prospects. Experimental Oncology. 2022;44(3):263-4. DOI: https://doi.org/10.32471/exp-oncology.2312-8852.vol-44-no-3.18569
2. Tan Y, Wang Z, Xu M, Li B, Huang Z, Qin S, Nice EC, Tang J, Huang C. Oral squamous cell carcinomas: state of the field and emerging directions. International journal of oral science. 2023 Sep 22;15(1):44. https://doi.org/10.1038/s41368-023-00249-w
3. Kumari P, Syed SA, Wahid M, Qureshi MA, Kumar R. Expression of miR-31 in saliva-liquid biopsy in patients with oral squamous cell carcinoma. Journal of Taibah University Medical Sciences. 2021 Oct 1;16(5):733-9. https://doi.org/10.1016/j.jtumed.2021.03.007
4. Yuwanati M, Ramadoss R, Kudo Y, Ramani P, Senthil Murugan M. Prevalence of oral submucous fibrosis among areca nut chewers: A systematic review and meta-analysis. Oral diseases. 2023 Jul;29(5):1920-6. https://doi.org/10.1111/odi.14235
5. Peng QS, Cheng YN, Zhang WB, et al. circRNA_0000140 suppresses oral squamous cell carcinoma growth and metastasis by targeting miR-31 to inhibit Hippo signaling pathway. Cell Death Dis. 2020;11(1):112. https://doi.org/10.1038/s41419-020-2273-y
6. Menini M, De Giovanni E, Bagnasco F, Delucchi F, Pera F, Baldi D, Pesce P. Salivary micro-RNA and oral squamous cell carcinoma: A systematic review. Journal of personalized medicine. 2021 Feb 4;11(2):101. https://doi.org/10.3390/jpm11020101
7. Lin X, Wu W, Ying Y, Luo J, Xu X, Zheng L, Wu W, Yang S, Zhao S. MicroRNA-31: a pivotal oncogenic factor in oral squamous cell carcinoma. Cell Death Discovery. 2022 Mar 29;8(1):140.
8. Osan C, Chira S, Nutu AM, Braicu C, Baciut M, Korban SS, Berindan-Neagoe I. The connection between MicroRNAs and oral cancer pathogenesis: emerging biomarkers in oral cancer management. Genes. 2021 Dec 15;12(12):1989. https://doi.org/10.3390/genes12121989
9. Mohtasham N, Ghorbani Z, Ayatollahi H, et al. Serum mir-31-5p is a reliable biomarker in patients with oral squamous cell carcinoma and oral lichen planus. Egypt J Med Hum Genet. 2024;25:77. https://doi.org/10.1186/s43042-024-00531-4
10. Michela B. Liquid Biopsy: A Family of Possible Diagnostic Tools. Diagnostics. 2021;11(8):1391. https://doi.org/10.3390/diagnostics11081391
11. Dikova V, Jantus-Lewintre E, Bagan J. Potential Non-Invasive Biomarkers for Early Diagnosis of Oral Squamous Cell Carcinoma. J Clin Med. 2021;10(8):1658. https://doi.org/10.3390/jcm10081658
12. Chang YA, Weng SL, Yang SF, Chou CH, Huang WC, Tu SJ, Chang TH, Huang CN, Jong YJ, Huang HD. A three–microRNA signature as a potential biomarker for the early detection of oral cancer. Int J Mol Sci. 2018 Mar 5;19(3):758. https://doi.org/10.3390/ijms19030758

13. Bayraktar E, Bayraktar R, Oztatlici H, Lopez-Berestein G, Amero P, Rodriguez-Aguayo C. Targeting miRNAs and other non-coding RNAs as a therapeutic approach: an update. Non-coding RNA. 2023 Apr 13;9(2):27.
14. Basharat S, Shaikh BT, Rashid HU, Rashid M. Correction to: Health seeking behaviour, delayed presentation and its impact among oral cancer patients in Pakistan: a retrospective qualitative study. BMC Health Serv Res. 2019;19(1):835. https://doi.org/10.1186/s12913-019-4716-7
15. Lu Z, He Q, Liang J, Li W, Su Q, Chen Z, et al. miR-31-5p is a potential circulating biomarker and therapeutic target for oral cancer. Mol Ther Nucleic Acids. 2019;16:471-80. https://doi.org/10.1016/j.omtn.2019.03.012
16. Reis MG, Lopes LC, Sanches AB, Guimarães NS, Martins-Chaves RR. Diet and Oral Squamous Cell Carcinoma: A Scoping Review. Int J Environ Res Public Health. 2024 Sep 10;21(9):1199.
17. Khan O, Shaikh AH, Ahmed S, Naz S, Memon R, Baig QA. Evaluating the effects of frequency and duration of tobacco use on oral mucosa in patients visiting Hamdard University Dental Hospital.
18. Warnakulasuriya S, Kerr AR. Oral cancer screening: past, present, and future. J Dent Res. 2021 Nov;100(12):1313-20. https://doi.org/10.1177/002203452110147
19. Lipsky MS, Su S, Crespo CJ, Hung M. Men and Oral Health: A Review of Sex and Gender Differences. Am J Men's Health. 2021;15(3). https://doi.org/10.1177/15579883211016361
20. Salehiniya H, Raei M. Oral cavity and lip cancer in the world: an epidemiological review. Biomed Res Ther. 2020;7(8):3898-905. https://doi.org/10.15419/bmrat.v7i8.619
21. Pradhan D, Mehta T, Srivastava A, Patel D, Dash KC, Hittalamani V, Makkad RS. Evaluation of the Importance of Genetic Polymorphisms in Genes Expressing Cancer-Metabolizing Enzymes (Cyp1a1 and Gstm1) in Oral Submucous Fibrosis. J Pharm Bioallied Sci. 2024;16(Suppl 3):S2785-S2787. https://doi.org/10.4103/jpbs.jpbs_413_24
22. Bhat MY, Advani J, Rajagopalan P, Patel K, Nanjappa V, Solanki HS, et al. Cigarette smoke and chewing tobacco alter expression of different sets of microRNAs in oral keratinocytes. Sci Rep. 2018;8(1):7040. https://doi.org/10.1038/s41598-018-25498-2
23. Uma Maheswari TN, Nivedhitha MS, Ramani P. Expression profile of salivary micro RNA-21 and 31 in oral potentially malignant disorders. Braz Oral Res. 2020 Feb 10;34:e002.
24. Uma Maheswari TN, Nivedhitha MS, Ramani P. Expression profile of salivary micro RNA-21 and 31 in oral potentially malignant disorders. Brazilian oral research. 2020 Feb 10;34:e002. https://doi.org/10.1590/1677-3175.2020.e002