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Evaluation of the current microRNAs expression levels as potential biomarkers in Oral Squamous Cell Carcinoma

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ABSTRACT

As the most common malignancy, oral squamous cell carcinoma (OSCC) is typically fatal. The survival of patients with oral cancer has not improved, and tumor recurrence remains high. During tumorigenesis, microRNAs (miRNAs) regulate gene expression. Patients' life expectancy can be determined by prognostic survival biomarkers, which can focus therapy on specific targets. This study evaluated five miRNAs associated with OSCC for their prognostic impact. It was determined through microarray analysis and quantitative reverse transcription polymerase chain reaction that there was a significant difference in the expression of miRNAs between OSCC patients and control patients in plasma. We used the unpaired t-tests and the Mann-Whitney test to conduct the statistical analysis. Based on the study's results, five miRNAs have been found to have significantly different expression levels in the plasma of patients with OSCC; in particular, miR-31 was found to have a significantly higher expression level in OSCC patients' plasma as compared with healthy controls. Aside from that, there was a significant reduction in the expression of miR-100, miR-199a, miR-203, and mir345 in the plasma of OSCC patients ($P < 0.05$). To better understand the importance of miRNAs in OSCC, various OSCC cases were analyzed. Detecting miRNAs in plasma may be a useful diagnostic tool for oral squamous cell carcinoma.

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Introduction

It is generally accepted that head and neck squamous cell carcinomas (HNSCCs) are the most common malignancies of the head and neck (1, 2). One in five neoplasms is cancerous, while the other one in ten is benign. There are an estimated 500,000 cases of this disease every year, with more than 300,000 deaths caused by it every year (3-5). When all stages and anatomic subsites of OSCC are considered, the 5-year overall survival rate is still only about 50%, despite recent improvements in OSCC prognoses. Hematology can detect occult lymph node metastases in up to 40% of OSCC patients with early-stage tumors (clinical T1-T2N0M0 on the pathology report) when the tumor is in an early stage (6-10). Early detection of OSCC could improve the chances of patients surviving the disease. The current approach, which involves multiple solid biopsies, is perceived as uncomfortable by patients. In a liquid biopsy, tumor cells and nucleic acids can be detected in body fluids, and this method is able to detect minimal residual disease, predict treatment response, and provide early diagnosis (11). The membrane-coated particles found in extracellular vesicles (EVs) contain protein chains, lipid chains, DNA strands, mRNA strands, and microRNA strands (9, 10, 12-14). The miRNA family of RNA molecules consists of about 22 nucleotide-long molecules that interact with target sequences to block translation. They are also documented as oncogenes or tumor suppressors and play roles in all cancer hallmarks (15, 16). The microRNAs have a significant role in OSCC's etiology due to the epigenetic effects of regulating gene expression as oncogenic and tumor suppressors. Also, by investigating critical signaling pathways in carcinogenesis that contribute to proliferation, invasion, apoptosis, migration Etc., microRNAs can affect genes that control these pathways. We identified the microRNAs in this research with high targets on genes in these pathways by referring to databases such as miRWalk and miRBase databases (6, 17). Research has shown that miR-31 is associated with oncogenic tumors. Lung, colorectal, esophageal, and OSCC cells express miR-31 more frequently after KRAS mutations (18); it has been reported that a tumor suppressor miRNA in head and neck squamous cell carcinoma can be detected via tissue or oral swirl analysis (19, 20). OSCC resistance to cisplatin is also influenced by miR-203 (21), The prognostic role of these markers in OSCC plasma remains unclear. miR-345 has been studied controversially in the past (22, 23). This study assessed the potential of plasma miRs to be a non-invasive marker for OSCC and found that miR-31, miR-100, miR-199a, miR-203, and miR-345 were differentially

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expressed in OSCC plasmas.

Materials and Methods

Subjects and sample collection

The research was conducted on 76 patients; 38 patients were diagnosed with primary OSCC, and 38 were healthy subjects in the control group. This study was approved by the Research Center for Prevention of Oral and Dental Diseases. The age and gender distribution of subjects are presented in Table 1. Blood samples were collected in clot activator tubes. All participants have signed the written informed consent, and no participant underwent chemoradiotherapy before surgery.

Inclusion criteria

All the newly diagnosed cases of primary OSCC and healthy controls who consented to participate in the study have been included.

Exclusion criteria

Cases of SCC that either has or had in the past, any other malignancy. Patients who have AIDS or any other known Immunodeficiency disorder. Patients with active oral infections, hyperglycemic diabetes, Sjogren's metabolic syndrome, inflammation and tendon injuries, CAD (coronary artery disease), and CHD (congenital heart defects). Any patient in a terminal stage of the disease is therefore not operable.

Collection and storage of serum samples and RNA isolation

cDNA with high quality requires pure RNA. For this reason, this part is the most crucial stage. Trizol (Trizol-LS) was used for RNA extraction at this stage. Due to its single-stranded nature, the RNA molecule is more chemically active than DNA and is quickly broken by RNase (RNase-H) enzyme. Working with RNA is much more sensitive than working with DNA, and all the materials and tools used to extract RNA should be free of RNase and DNase (RNase/DNase Free). Therefore, the containers and tubes used to work with RNA were RNase/DNase free and autoclaved for 15 minutes at 15 psi pressure before starting work. 5 ml of venous blood was collected from each patient. After about 60 minutes, blood samples were centrifuged for 15 min at 2300 to 2500 rpm. Until further analysis, plasma was stored at −80°C. RiboEx-LS solution (GeneAll, South Korea) was used to extract RNA from plasma. In extraction steps using RiboEx solution, 200 to 250 microliters of plasma were placed in a microtube free of RNase enzyme, and three times (600 to 750 microliters) of triazole were added to it. The contents of the microtube were mixed with the help of a micropipette and incubated at room temperature (15-30°C) for 5 minutes. Add 200 microliters of chloroform for every 750 microliters of initial triazole. Homogenize the mixture by shaking vigorously for 15 seconds. Incubate the mixture at 15-30°C for 2-3 minutes, and centrifuge the microtube at 2-8°C for 25 minutes at 12,000 rpm. As a result of centrifugation, three phases were created: RNA was present in the colorless upper phase, and protein and DNA were present in the middle and lower phases, respectively. For this reason, the upper phase was slowly transferred to a new tube free of RNase enzyme to prevent protein and DNA contamination. Isopropanol to the volume of the transferred liquid (about 500 microliters), mixing and incubating for 20 minutes at 15-30 degrees Celsius. The sample was centrifuged at 12000 rpm at a temperature of 2-8 degrees Celsius for 20 minutes. Drain the upper phase, add 1 ml of 75% ethanol (prepared with water treated with DEPC), and shake for 20 seconds. The sample was centrifuged at 7500 rpm at a temperature of 2-8 degrees Celsius for 15 minutes. Draining the supernatant solution, inverting the tube on a paper towel, and partially drying the sediment at room temperature for a few minutes. (It should be remembered that completely drying the sediment reduces its solubility.) It dissolves the sediment in 40 microliters of water treated with DEPC and is placed for 10 minutes at a temperature of 56 °C. The RNA sample was transported and stored in a freezer at -80 °C. miRs were quantified using the NanoDrop 1000 (NanoDrop, Wilmington, DE).

cDNA synthesis

In this study, the Pars Genome kit made in Iran is used to make cDNA. For each sample, the following materials were added to the nuclease-free microtube according to the protocol: First, to normalize all the extracted miRNA221 after reading the miRNA concentration value by the Nanodrop device, we bring the concentration to 1000 ng/uL. Then we pour 1 µL of random hexamer along with 1000 nanograms of miRNAs into a sterile 0.2 microtube; then, we incubate the microtube at 65°C for 5 minutes to unwind the nucleotide strands, then it is immediately placed on ice. Let it cool down. After cooling (preventing premature action of the enzyme), we add 10 µL of RT Master mix enzyme and, according to the temperature program of the kit, in the PCR machine at a temperature of 25 °C for 10 minutes (primer connection), 42 °C for 60 minutes (cDNA synthesis) and 70°C for 10 minutes (enzyme inactivation). After the cDNA synthesis from the extracted RNA samples, RT-PCR was performed to ensure the accuracy of the cDNA synthesis kit and to estimate the quality of miRNAs extracted by 5S control primers. To perform several reactions in parallel and reduce the possible pipetting error, we first prepared a general Master Mix that includes water and cDNA and Master Mix PCR Amplicon for the number of reactions and an additional sample (as a negative control). The negative control consists of water, primers, and Master Mix PCR Amplicon, which is placed as a sample next to other samples inside the PCR to ensure that the Master Mix is not contaminated. After finishing the reaction, we took the samples according to the desired band size on 1.5% agarose gel.

Real-time PCR

The real-time polymerase chain reaction was performed using the SYBR Green PCR Master Mix (Pars Genome, Iran) according to the manufacturer's instructions. Performing Real-time PCR using the Applied biosystem-step one machine to reduce the possibility of errors in pouring the materials, a master mix was prepared, including all the required materials except cDNA. After mixing the contents of the tubes containing the original mixture, 2.9 microliters of the original mixture were poured into each real-time PCR tube. Then in each group, 8 microliters of the desired cDNA were added, and after mixing, it was placed in the Real-time PCR machine. At this stage, due to performing several parallel reactions and to prevent errors

during work, there is a need to prepare a master mix including water, primer, and SYBR Green (Pars Genome, Iran) for the number of reactions and a sample as a negative control according to a specific pattern. After combining these items and gently rotating them, the contents were transferred in 0.2 tubes to the amount of 120 µ, and after preparing the samples. The samples were run in Real-Time PCR. In each PCR reaction, 20 µL of cDNA, 2 µL of universal primer, 10 pmol of each primer, and a QuantiTect SYBR Green PCR Master Mix were used, along with 50 ng of cDNA. Denaturation at 95 °C for 5 minutes, 40 cycles included, and processing at 95 °C for 5 seconds, followed by 62 °C for 20 seconds at which the primers were connected, 72 °C at which they were extended for 30 seconds, forming the melting curve by setting the temperature between 60°C and 95°C. A threshold cycle (Ct) for each sample is obtained after the reaction is completed. A triplicate of each reaction was performed.

The statistical analysis

Statistical analysis was conducted using Graphpad-Prism (version 9.4, GraphPad Software). Categorical variables were counted and categorized according to their mean and standard deviation. The Mann-Whitney and t-Test tests were used to compare the serum expression levels of five miRs 0.05 was set as the statistical significance level.

Results

Characteristics of the study population

The demographic information of the studied people is given in the table. As seen, A total of 38 samples were examined in each group. In the healthy group, 19 samples were female, and 19 samples were male, and in the OSCC group, 18 were female, and 20 were male. It was typical for age and gender to be matched between the control group and the patients. The average age of the studied subjects in the healthy and the case groups was 55.97 ± 5.32 and 55.40 ± 10.87, respectively (Table 1).

miRs expression level in OSCC plasma patients and case-controlled individuals

For each individual, three cDNA vials constructed from Evolution reference genes and markers were analyzed, and the results were interpreted using a Melting peak curve as mentioned earlier. A quantitative real-time PCR assay was used to determine miR-31 expression. Compared with healthy controls, OSCC patients had significantly higher levels of miR-31 expression (upregulation) ($p = 0.0003$), as shown in Figure 1(a). A significant difference (downregulation) occurs in the expression of miR-100 ($p = 0.0059$) Figure 1(b), miR-199a ($p = 0.0499$) Figure 1(c), miR-203 ($p = 0.0007$) Figure 1(d) and miR-345 ($p = 0.0055$) Figure 1(e) in patients compared to case controls (Table 2). The median CT values of miR-31 were 35.37 and 36.45 in the OSCC patients and control group; for miR-100, the values were 37.71 and 36.40; for miR-199a, the values were 38.10 and 37.54; for miR-203, the values were 32.89 and 29.68; for miR-345, the values were 36.46 and 33.50, respectively. According to the Mann-Whitney test, A significant association of miR-31, miR-100, miR-199a, miR-203, and miR-345 expression was found between OSCC plasma patients and the healthy group ($p < 0.05$).

Quantitative expression data of miR-203, miR-199a, miR-100, miR-31, and miR-345 genes were measured with ΔCT and reported as median and IQR (25-75) (Table 3). The result of the expression of each of these genes in two healthy and OSCC groups is as follows:

The differences in miRs expression in the two groups

The relative differences in expressions were calculated by the ΔΔct method for miRs. After completing the analyses, miR-31 expression was 4.37 times higher in the OSCC plasma patients than in the case-controlled group, and miR-100 expression was 4.67 times, miR-199a was 1.75 times, miR-203 was 7.33 times, and miR-345 was 9.18 times lower in the plasma of OSCC patients than in healthy individuals (Figure 2**)**.

Discussion

The most common childhood cancer is oral cancer,

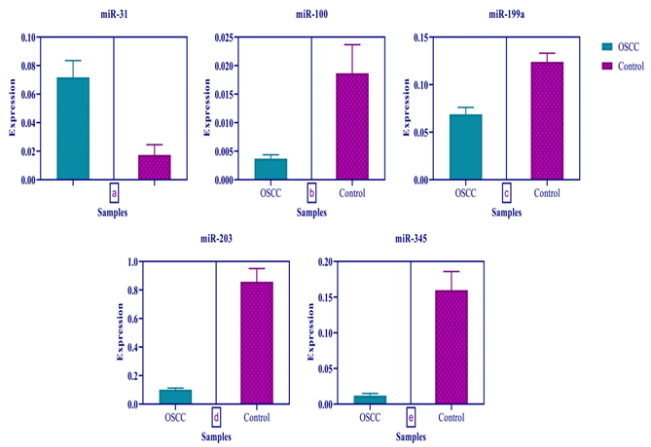

Figure 1. The microRNAs expression level in the OSCC samples and healthy groups;(a): the expression level of miR-31; (b): miR-100; (c): miR-199a; (d): miR-203 and (e): miR-345.

Table 1. The study subjects' demographic and clinical information.

Factor	Group with primary OSCC n=38		Healthy group n=38	
Average Age	55.40±10.87		55.97±5.32	
Gender	F (47.3%) n=18	M (52.6%) n=20	F (50%) n=19	M (50%) n=19

Table 2. Unpaired t-test statistical analysis.

t-Test	miR-31	miR-100	miR-199a	miR-203	miR-345
P value	0.0003	0.0059	0.0499	0.0007	<0.0001
P value summary	***	**	*	***	****

Table 3. Distribution (median and IQR 75 - 25) of the expression values of the studied variables in two OSCC and healthy groups.

miR-expression (ΔCT)	IQR	Sample Group (OSCC) n=38	Control Group n=38
miR-31 expression (ΔCT)	Percentile 25	6.19	3.98
	Median	7.15	3.57
	Percentile 75	7.46	7.67
miR-100 expression (ΔCT)	Percentile 25	0.53	5.89
	Median	8.48	8.37
	Percentile 75	8.82	8.27
miR-199a expression (ΔCT)	Percentile 25	8.78	4.81
	Median	6.45	4.01
	Percentile 75	4.98	1.04
miR-203 expression (ΔCT)	Percentile 25	4.73	0.94
	Median	4.18	2.04
	Percentile 75	4.86	3.67
miR-345 expression (ΔCT)	Percentile 25	8.96	6.79
	Median	8.97	9.51
	Percentile 75	9.54	7.55

which consists of malignant neoplasms inside the mouth. Tonsils, salivary glands, oropharynx, nasopharynx, hypopharynx, lip, tongue, gums, the floor of the mouth, hard palate, tonsils, and soft palate (18).In addition to genetic and environmental factors, epigenetic factors are also involved in cancer etiology (19). According to recent studies, exciting ways to regulate gene expression have been identified, which are mediated by small mRNA. Genes silencing, DNA methylation, gene transcription, and RNA interference pathways can be mentioned (20).

In 2021, Kumari et al. investigated the expression level of miR-31 in patients with OSCC. In this research, they found that other parameters such as age, sex, tumor area, tumor size, and stage of the disease did not make a difference in the obtained results. Also, in Ouyang (22) et al.'s research in 2013, no significant difference in the amount of this microRNA was proven in different cancer stages. However, in the article by Siow (23) et al. in 2014, the results were different from the results of two previous studies, and the expression level of this microRNA was different with various conditions, for example, with different stages of cancer. The results of the current research are in line with the results of this research. miR-31 is one of the micro RNAs in which the amount increases in the body, but previous research investigated the expression level of miR-31 in saliva (24). However, in the present research, this amount was checked in plasma. In a study by Jakob and his colleagues in 2019 (25), they examined 36 OSCC tissue samples, most of which were from the tongue area. Their research with the delta c method showed that the expression level of miR-100-5p in the tumoral tissue decreased. Furthermore, the higher the decrease, the decreased survival rate. The result of this research was similar to Yap's (26) and Hou's (27) research. In which miR-100-5p is mentioned as a diagnosis of SCC lesions in the mouth, and also this microRNA is introduced as a predictor of the patient's prognosis; considering that this research also examines miR-100 in plasma patients, the reduction of this microRNA in the patients' plasma can also be related to their survival rate. In Manikandan et al.'s research in 2016, it was also done on punched cancer tissues; it also

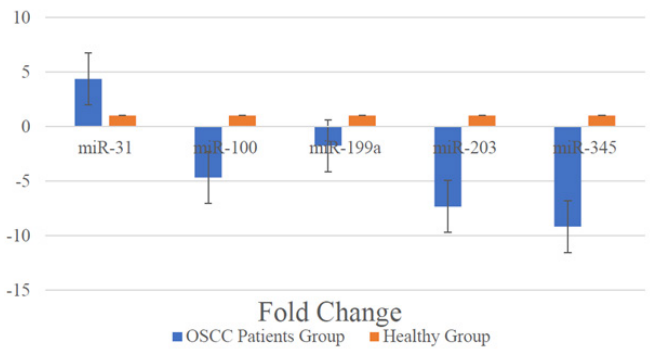

Figure 1. Fold Changes, differences in the expression of miRs (miR-31, miR-100, miR-199a, miR-203, miR-345) in OSCC patients and Control group.

showed a decrease in the expression of miR-203 in cancer tissues, but the research done in this field was all on isolated tissues (28); the present research to identify this biomarker faster, used patients' plasma and showed that the expression level of this microRNA decreases significantly. In a study in 2021, Jin et al. investigated the role of miR199a-3p expression in squamous cell cancer tissues isolated from the lesion. Through the TTN-AS1/RUNX1 pathway, they investigated miR-199a-3p's effect on oral cancer cell proliferation and apoptosis. Based on the results of various tests, it was determined that oral cancer cells expressed more TTN-AS1/RUNX1 when compared with the control group. Increasing expression of TTN-AS1 and RUNX1 decreased cell migration, adhesion, and proliferation but increased apoptosis. By reducing miR-199a-3p expression, cancer cells become more susceptible to TTN-AS1 and RUNX1 expression, increasing the risk of becoming malignant (29).

Also, in the research that Wei et al. conducted in 2019 regarding the effect of the miR-199a-5p expression on SOX4 in the EMT (Epithelial-to-Mesenchymal Transition) pathway, the results of this research showed that the effects of the expression of this microRNA reduce cell migration and invasion by increasing the expression of SOX4 in can-

cer cells, which leads to increased cell migration and cell invasion so that this microRNA can be mentioned as a predictive factor (30). In general, the research conducted on this microRNA was isolated entirely on tissue cells, and the research was not done on the patient's plasma. In the research of Huang and his colleagues in 2012, the effects of this microRNA were mentioned as tumor oncogenes in some cancers such as cervical cancer (31), while the results of the research conducted on OSCC showed it as a tumor suppressor factor, however. Due to the lack of sufficient research on this miR-199a, the current research investigated this microRNA to cover this information gap. The data obtained from the current research showed that this microRNA has different behavior. However, it can be said that, in general, it has significantly decreased (32). In examining the expression level of miR-345 in patients with OSCC, there are many different results related to this microRNA. For example, in the research that Scholtz and his colleagues conducted in 2022 to examine the expression level of miR-345-3p miR-424- 3p in cancer tissues, their results showed an increase in the expression of miR-345-3p (33), which was contrary to the results of the research conducted by Wu et al., in 2020 on 22 cancer tissues, because, in their research, the expression of miR-3p 345 has been significantly reduced (34), also in the research conducted by Gissi and colleagues in 2018 on 14 OSCC patients by scrubbing and brushing from the lesion, their results showed that the expression level of this microRNA was not significantly different (35). The results of the present research are in line with the research results of Wu and his colleagues and show that the expression level of this microRNA is significantly reduced in the plasma of patients. The difference in the results of this research can be considered as the result of several factors; for example, in the research done by Cervigne and his colleagues in 2009 and they stated the increase in the expression of this microRNA, patients with leukoplakia were also included in their research (36-38), but this was not the case in the present research. Also, the difference in the sampling method can be another factor. For example, in Gissi's study, sampling was done from a tissue block, but the present study used the patient's plasma sample. Moreover finally, perhaps one of the most important reasons is the population from which sampling was done; for example, in Wu's research, he studied the Chinese people, while Gissi studied the Italian people (34, 35). As a result, it can be said that the expression level of this microRNA is different. As a result of changes in the levels of microRNAs present in a patient's plasma, it can be assumed that changes in microRNA expression levels may indicate the presence of early progression of primary OSCC in a patient. Furthermore, there is a great deal of potential in this area for assisting in the early detection of OSCC by using these tests as good screening tests that needs further research with more samples.

According to the analysis, miR-31, miR-100, miR-199a, miR-203, and miR-345 expression in plasma could serve as a diagnostic screening test for early detection of oral squamous cell carcinoma and could also serve as potentially useful therapeutic interventions in the future.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Johnson DE, Burtness B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. Nat Rev Dis Primers. 2020;6(1):1-22.
2. Seifi Kafshgari H, Yazdanian M, Ranjbar R, Tahmasebi E, Mirsaeed S, Tebyanian H, et al. The effect of Citrullus colocynthis extracts on Streptococcus mutans, Candida albicans, normal gingival fibroblast and breast cancer cells. J Biol Res. 2019;92(1):8201.
3. Ramos M, Benavente S, Giralt J. Management of squamous cell carcinoma of the head and neck: updated European treatment recommendations. Expert Rev Anticancer Ther. 2010;10(3):339-44.
4. Zolfaghar M, Amoozegar MA, Khajeh K, Babavalian H, Tebyanian H. Isolation and screening of extracellular anticancer enzymes from halophilic and halotolerant bacteria from different saline environments in Iran. Mol Biol Rep. 2019;46(3):3275-86.
5. Rezaeeyan Z, Safarpour A, Amoozegar MA, Babavalian H, Tebyanian H, Shakeri F. High carotenoid production by a halotolerant bacterium, Kocuria sp. strain QWT-12 and anticancer activity of its carotenoid. EXCLI J. 2017;16:840-51.
6. Pedersen NJ, Jensen DH, Lelkaitis G, Kiss K, Charabi BW, Ullum H, et al. MicroRNA-based classifiers for diagnosis of oral cavity squamous cell carcinoma in tissue and plasma. Oral Oncol. 2018;83:46-52.
7. Mosaddad SA, Beigi K, Doroodizadeh T, Haghnegahdar M, Golfeshan F, Ranjbar R, et al. Therapeutic applications of herbal/synthetic/bio-drug in oral cancer: An update. Eur J Pharmacol. 2021;890:173657.
8. Hajmohammadi E, Molaei T, Mowlaei SH, Alam M, Abbasi K, Khayatan D, et al. Sonodynamic therapy and common head and neck cancers: in vitro and in vivo studies. Eur Rev Med Pharmacol Sci. 2021;25(16):5113-21.
9. Hajmohammadi E, Ghahremanie S, Alam M, Abbasi K, Mohamadian F, Khayatan D, et al. Biomarkers and common oral cancers: Clinical trial studies. JBUON. 2021;26(6):2227-37.
10. Tahmasebi E, Alikhani M, Yazdanian A, Yazdanian M, Tebyanian H, Seifalian A. The current markers of cancer stem cell in oral cancers. Life Sci. 2020;249:117483.
11. He T, Guo X, Li X, Liao C, Wang X, He K. Plasma-derived exosomal microRNA-130a serves as a noninvasive biomarker for diagnosis and prognosis of oral squamous cell carcinoma. J Oncol. 2021;2021.
12. Chang Y-A, Weng S-L, Yang S-F, Chou C-H, Huang W-C, Tu S-J, et al. A three–microRNA signature as a potential biomarker for the early detection of oral cancer. Int J Mol Sci. 2018;19(3):758.
13. Hussain A, Tebyaniyan H, Khayatan D. The Role of Epigenetic in Dental and Oral Regenerative Medicine by Different Types of Dental Stem Cells: A Comprehensive Overview. Stem Cells Int. 2022;2022:5304860.
14. Soudi A, Yazdanian M, Ranjbar R, Tebyanian H, Yazdanian A, Tahmasebi E, et al. Role and application of stem cells in dental regeneration: A comprehensive overview. EXCLI J. 2021;20:454-89.
15. Minhas S, Kashif M, Altaf W, Afzal N, Nagi AH. Concomitant-chemoradiotherapy-associated oral lesions in patients with oral squamous-cell carcinoma. Cancer Biol Med. 2017;14(2):176.
16. Alam M, Abbasi K, Nouri F, Golkar M, Ranjbar R, Yazdanian M,

et al. The Cytotoxicity and Anticancer Effects of Propolis against the Oral Squamous Cell Carcinoma: In Vitro Study. Open Access Maced J Med Sci. 2022;10(B):2374-81.
17. Liu C-J, Lin J-S, Cheng H-W, Hsu Y-H, Cheng C-Y, Lin S-C. Plasma miR-187* is a potential biomarker for oral carcinoma. Clin Oral Investig. 2017;21(4):1131-8.
18. Solomon B, Young RJ, Rischin D, editors. Head and neck squamous cell carcinoma: genomics and emerging biomarkers for immunomodulatory cancer treatments. Semin Cancer Biol; 2018: Elsevier.
19. Hema KN, Smitha T, Sheethal HS, Mirnalini SA. Epigenetics in oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2017;21(2):252-9.
20. Zaratiegui M, Irvine DV, Martienssen RA. Noncoding RNAs and gene silencing. Cell. 2007;128(4):763-76.
21. D'Souza W, Kumar A. microRNAs in oral cancer: Moving from bench to bed as next generation medicine. Oral Oncol. 2020;111:104916.
22. Ouyang S, Wang J, Huang Z, Liao L. Expression of microRNA-31 and its clinicopathologic significance in oral squamous cell carcinoma. Zhonghua Kou Qiang Yi Xue Za Zhi. 2013;48(8):481-4.
23. Siow M, Karen Ng L, Vincent Chong V, Jamaludin M, Abraham M, Abdul Rahman Z, et al. Dysregulation of mi R-31 and mi R-375 expression is associated with clinical outcomes in oral carcinoma. Oral Dis. 2014;20(4):345-51.
24. 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. J Taibah Univ Medical Sci. 2021;16(5):733.
25. Jakob M, Mattes LM, Küffer S, Unger K, Hess J, Bertlich M, et al. MicroRNA expression patterns in oral squamous cell carcinoma: hsa-mir-99b-3p and hsa-mir-100-5p as novel prognostic markers for oral cancer. Head Neck. 2019;41(10):3499-515.
26. Yap T, Koo K, Cheng L, Vella LJ, Hill AF, Reynolds E, et al. Predicting the Presence of Oral Squamous Cell Carcinoma Using Commonly Dysregulated MicroRNA in Oral SwirlsOSCC Prediction from MicroRNA in Oral Swirls. J Cancer Prev. 2018;11(8):491-502.
27. Hou B, Ishinaga H, Midorikawa K, Shah SA, Nakamura S, Hiraku Y, et al. Circulating microRNAs as novel prognosis biomarkers for head and neck squamous cell carcinoma. Cancer Biol Ther. 2015;16(7):1042-6.
28. Manikandan M, Deva Magendhra Rao AK, Arunkumar G, Manickavasagam M, Rajkumar KS, Rajaraman R, et al. Oral squamous cell carcinoma: microRNA expression profiling and integrative analyses for elucidation of tumourigenesis mechanism. Mol Cancer. 2016;15(1):1-17.
29. Jin Z, Jiang S. Long non-coding RNA TTN-AS1/microRNA-199a-3p/runt-related transcription factor 1 gene axis regulates the progression of oral squamous cell carcinoma. Bioengineered. 2021;12(1):7724-36.
30. Wei D, Wang W, Shen B, Zhou Y, Yang X, Lu G, et al. MicroRNA-199a-5p suppresses migration and invasion in oral squamous cell carcinoma through inhibiting the EMT-related transcription factor SOX4. Int J Mol Med. 2019;44(1):185-95.
31. Huang L, Lin J-X, Yu Y-H, Zhang M-Y, Wang H-Y, Zheng M. Downregulation of six microRNAs is associated with advanced stage, lymph node metastasis and poor prognosis in small cell carcinoma of the cervix. PLoS One. 2012;7(3):e33762.
32. Chen J, Shin VY, Siu MT, Ho JC, Cheuk I, Kwong A. miR-199a-5p confers tumor-suppressive role in triple-negative breast cancer. BMC Cancer. 2016;16(1):1-12.
33. Scholtz B, Horváth J, Tar I, Kiss C, Márton IJ. Salivary miR-31-5p, miR-345-3p, and miR-424-3p Are Reliable Biomarkers in Patients with Oral Squamous Cell Carcinoma. Pathogens. 2022;11(2):229.
34. Wu J, Cao J, Li X, Wu B, Zhang S. MicroRNA-345 functions as a tumor suppressor via targeting ZEB2 in oral squamous cell carcinoma. Arch Oral Biol. 2020;116:104732.
35. Gissi DB, Morandi L, Gabusi A, Tarsitano A, Marchetti C, Cura F, et al. A noninvasive test for MicroRNA expression in oral squamous cell carcinoma. Int J Mol Sci. 2018;19(6):1789.
36. Mirzaei, A., Heidari, M. (2022). 'Comprehensive analysis of microRNA (miRNA) in cancer cells', Cell Mol Biomed Rep. Accepted. doi: 10.55705/cmbr.2022.364591.1070.
37. Raju K, Sharifa A, Soumya P, Khanam R, Bhargav KKB. Comparative study on the efficacy of statin therapy in lowering cholesterol and assessing the risk of metabolic syndrome in patients under statins therapy. Cell Mol Biomed Rep 2023; 3(1): 1-8. doi: 10.55705/cmbr.2022.361364.1064
38. Cervigne NK, Reis PP, Machado J, Sadikovic B, Bradley G, Galloni NN, et al. Identification of a microRNA signature associated with progression of leukoplakia to oral carcinoma. Hum Mol Genet. 2009;18(24):4818-29.