Journal of Craniomaxillofacial Research

Vol. 11, Issue. 1 Winter 2024

Mir-148 and Mir-375 Expression in Oral Squamous Cell Carcinoma Patients with Oral Infection in Comparison with Oscc Patients without Oral Infection

Naghmeh Bahrami [1,2], Farzaneh Hosseini [3*], Mahsa Hajmali [4], Abdolreza Mohamadnia [5,6], Mona Mohajeri Tehrani [2], Masoume Farhangiyan [2]**

1. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.

2. Craniomaxillofacial Research Center, Tehran University of Medical Sciences, Tehran, Iran.

3. Department of Microbiology, School of Microbiology Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.

4. Department of Biotechnology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.

5. Chronic Respiratory Diseases Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

6. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

ARTICLE INFO

Article Type:
Original Article

Received: 10 September 2023
Revised: 7 October 2023
Accepted: 18 December 2023

**Corresponding author:*
Farzaneh Hosseini, Abdolreza Mohammadnia

Department of Microbiology, School of Microbiology Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran; Chronic Respiratory Diseases Research Center, NRITLD, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Tel: +98-912-5146410
Fax: +98-21-84902473
Email: mohamadnia.ar@gmail.com

ABSTRACT

Introduction: Oral squamous cell carcinoma is a multifactorial disease that is the sixth most common cancer worldwide. MicroRNAs have been confirmed to play a role in oral squamous cell carcinoma, acting as either oncogenes or tumor suppressor genes. This study examined the expression level and role of microR-148 and microR-375 in oral cancer.

Materials and Methods: In this study, we used 30 cancer samples with infection and 30 cancer samples without infection. To analyze the expression of microRNA 375 and microRNA 148, we used real-time PCR. First, we extracted total RNA from the samples. Then, we generated cDNA from it. Finally, the obtained cDNA was used in the real-time PCR technique.

Results: In cancer patients with oral infection, there was an increase in microRNA-148 expression and a decrease in microRNA-375 compared to cancer patients without oral infection.

Conclusion: The downregulation of microRNA-375 and upregulation of microRNA-148 can be utilized as diagnostic biomarkers and prognostic factors in oral cancer.

Keywords: Oral cancer; OSCC; Realtime pcr; MiR-148; MiR-375.

Please cite this Article as:
Bahrami N, Hosseini F, Hajmali M, Mohamadnia A, Mohajeri Tehrani M, Farhangiyan M. Mir-148 and Mir-375 Expression in Oral Squamous Cell Carcinoma Patients with Oral Infection in Comparison with Oscc Patients without Oral Infection. J Craniomaxillofac Res 2024; 11(1): 20-25. DOI: 10.18502/jcr.v11i1.16477

Introduction

Cancer is a set of complex genetic diseases, marked by unrestricted cell growth and the invasion of cells to other parts of the body [1]. Changes and damages in oral cancer make the DNA unstable [2]. MicroRNAs have a crucial role in oral cancer prognosis and function as oncogenes or tumor suppressor genes in this disease. Their expressions exhibit distinct variations between normal and tumor samples, which is utilized as a diagnostic biomarker in oral cancer [3]. Oral cancers are often detected at stages leading to an unfavorable prognosis in many regions around the world [4]. Certain microRNAs have target molecules. Play a significant role, in controlling approximately two thirds of gene expression. These molecules impact expression through mechanisms and their regulation is influenced by various factors [5]. Given the function of microRNAs, in the molecular pathways of eukaryotic cells altering their configurations can lead to disease development, in humans. MicroRNAs play a role in conditions, including cancers [6,7]. miRNA-148 plays a role as a tumor suppressor and innate immune system's regulator so it can serve as a diagnostic biomarker and miRNA-375 functions as both an oncogene and a tumor suppressor; its dual feature depends on the target mRNA [8-12]. The main focus of this study is to analyze and compare the levels of miR-148 and miR-375 in patients with infections who have cell carcinoma and to compare them with OSCC patients without oral infections and healthy individuals.

Materials and Methods

For our analysis, we first selected 30 patients referred to the Cancer Institute of Tehran University of Medical Sciences. These patients were suspected of having OSCC with oral infection based on physical examination and diagnosis by an expert. People with chronic or acute inflammatory diseases were excluded from this study. Additionally, we excluded those for whom pathological findings were not available for any reason. In our study, we also included 30 samples from patients who were diagnosed with OSCC but did not have oral infection after being thoroughly examined by a physician and giving consent by filling out the required forms. According to the code of ethics (IR.TUMS.AMIRALAM.REC.1400.033) both groups consisted of individuals aged between 22 and 77 years. Once the participants were chosen a conventional blood collection syringe was used to collect 2mm of blood which was then immediately processed for RNA extraction. The RNA extraction process was conducted using the RNeasy Mini Kit, from Qiagen (Cat no.75144). To assess the quality of the extracted RNA we utilized a Nanodrop device. In the next step, the ZIST ROYESH kit was used to make cDNA. This kit also contains the necessary materials to perform Real-time PCR, including forward and reverse primers and SYBR Master Mix Green. U6 was used as housekeeping. Real-time PCR was performed in a final volume of 25mL containing: 10mL Amplicon master mix, 0.5mL of each of F and R primers, 5μL DNA and distilled water. The test was done in 35 cycles using rotor-gene cycler. To ensure results follow these temperature and time conditions; start by denaturing at 95°C for 5 minutes then continue denaturation at 95°C for 20 seconds. Connect the primers at 56°C for 40 seconds. Amplify the samples, at 72°C, for 30 seconds (repeated for a total of 35 cycles). Finally, perform an amplification step at 72°C for 5 minutes. We analyzed the results using software called SPSS Version 22. We calculated the standard deviation. To determine the difference or relationship, between gene expression level and clinicopathological characteristics we used a paired t-test. The difference was considered significant if the p-value was less than or equal, to 0.05.

Results

As previously mentioned, the study consisted of 30 samples of patients diagnosed with cell carcinoma (OSCC) who had oral infections. The average age of these patients was 45 years old, with a deviation of 10.12. Additionally, there were 30 samples of OSCC patients without infections with an age of 46 years old and a standard deviation of 12.24. Both groups were carefully matched in terms of age variables. To compare the two groups a t-test was conducted to assess the age difference between them. The results indicated that there was no difference in terms of age suggesting that age does not play a role in causing issues, within the studied groups. We conducted a Real-Time RT PCR reaction. Analyzed the results based on the melting curve. the biomarker miR-148 was positive in 26 of 30 patients with oral infection. The positive rate of this biomarker in the group of patients without oral infection was 20 of 30 individuals. The amount of this biomarker in the group of healthy subjects was 6 out of 30. Statistical comparison of the positivity of this biomarker in two groups was performed using the two-sample binomial test, which showed a statistically significant difference between the two groups studied (P value<0.001). MiR-375 biomarker was positive in 19 out of 30 patients with oral infection. The positive rate

infection was 24 out of 30. The amount of this biomarker in the group of healthy people was 26 out of 30 people. The statistical comparison of the positivity of this biomarker in two groups was done using the two-sample binomial test, which showed a statistically significant difference between the two studied groups (P-value<0.001). To determine the folding change, we first need to calculate the Ct value for each sample. Then we can use the formula $\Delta\Delta$ Ct 2 to calculate the difference, in marker expression between the two groups. In patients with infection, the expression level of miR 148 is 1.39 times higher than in patients without infection. Additionally, the expression level of miR 375 in infected patients is 1.28 times lower than, in infected patients. Also, the difference in expression of miR-148 and miR-375 genes in cancer patients and healthy people is shown in Figure 4.

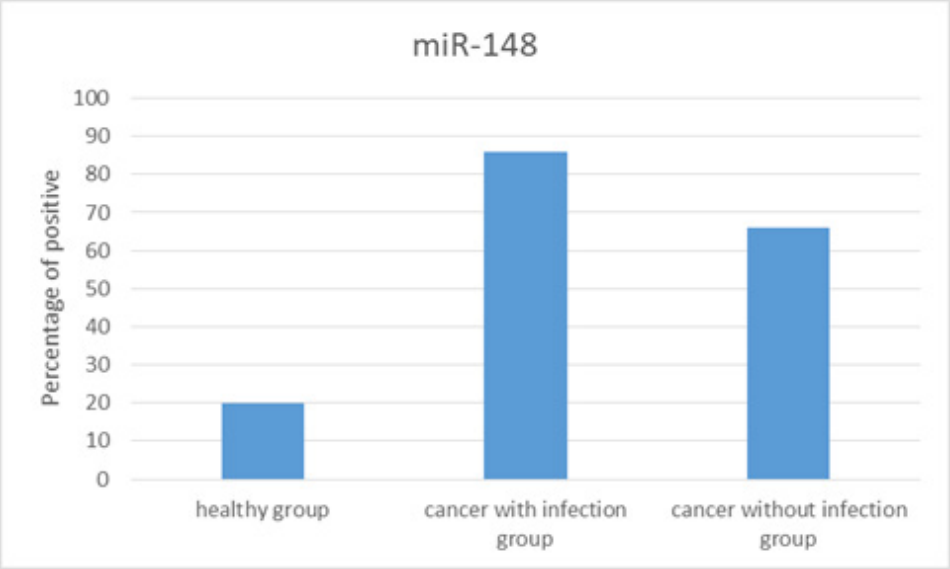

Figure 1. The rate of miR-148 positivity in the peripheral blood of patients with and without oral infection and healthy individuals.

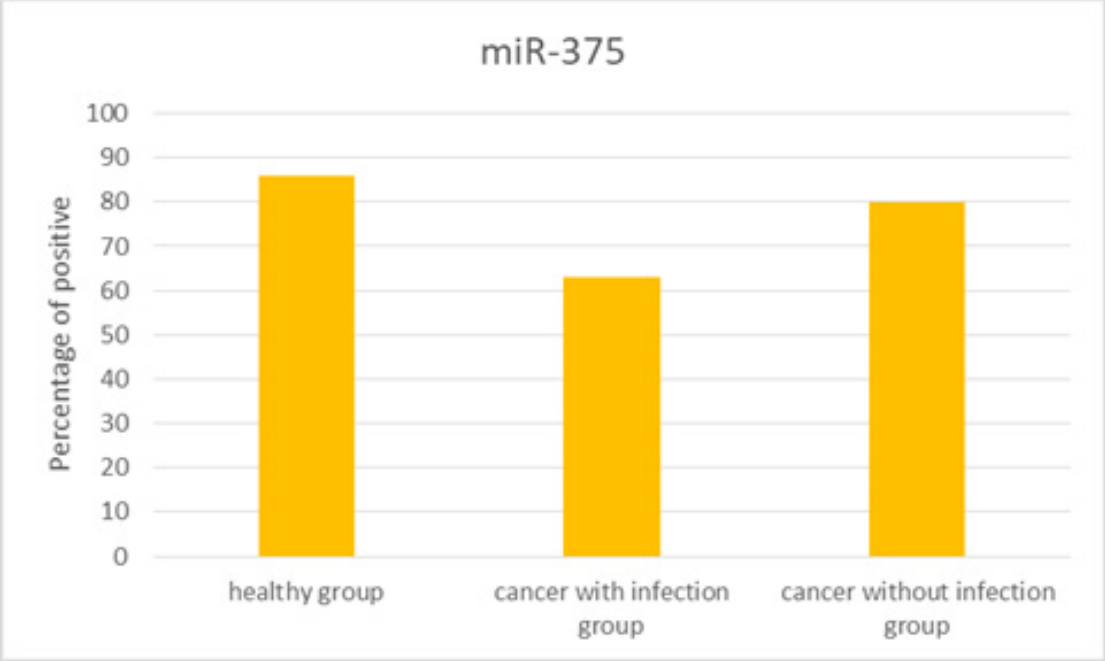

Figure 2. The rate of miR-375 positivity in the peripheral blood of patients with and without oral infection and healthy individuals.

DOI: 10.18502/jcr.v11i1.16477

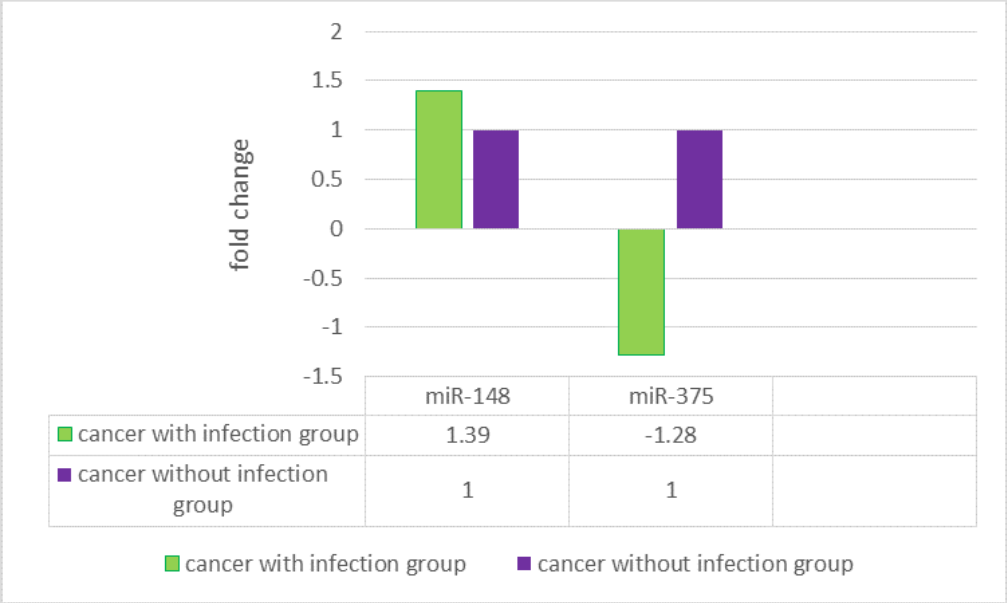

Figure 3. The difference in expression of miR-148 and miR-375 genes in patients with and without oral infection.

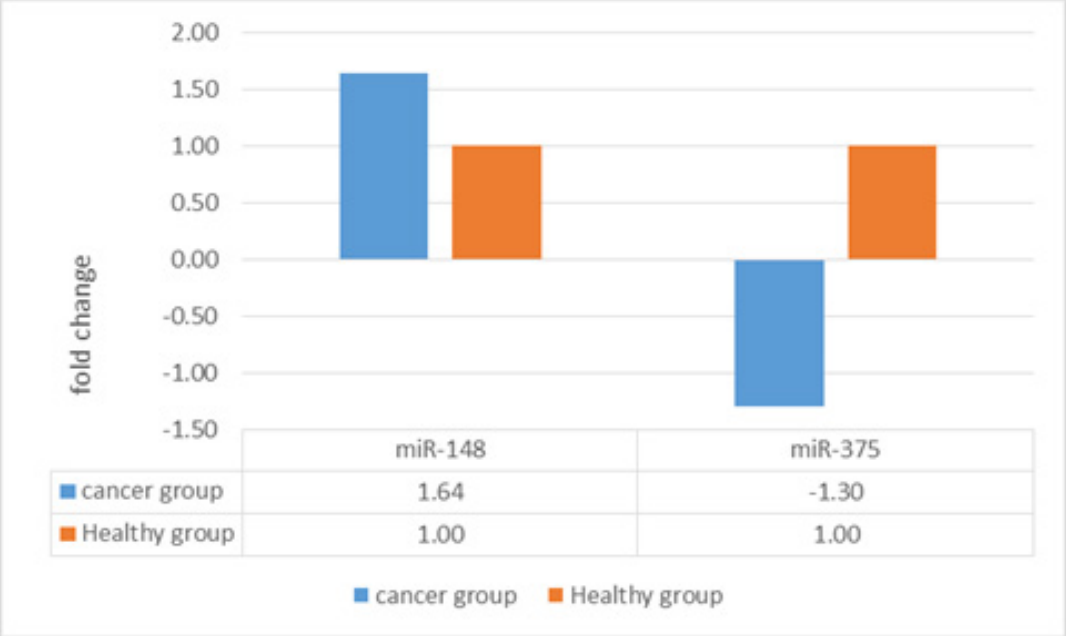

Figure 4. The difference in expression of miR-148 and miR-375 genes in cancer patients and healthy people.

Discussion

Oral cancer occurs due, to alterations in the composition of DNA found in the cells of oral tissue. These changes can arise from exposure to substances like alcohol, smoking, viral infections and other carcinogens. The genetic alterations involve mechanisms such, as deletions, point mutations, promoter methylation, gene and oncogene amplification as well as the inactivation of tumor suppressors. MicroRNAs can serve as a biomarker as numerous studies have demonstrated their expression, in oral squamous cell carcinoma and its derived cells compared to normal conditions. This increased expression is known to play a role, in cancer development and tumor formation [13]. According to the research conducted by Zheng et al., it has been discovered that miRNAs selectively enter the blood circulation through intercellular communication. The presence of miRNAs in peripheral blood is believed to be a factor, in the development of cancer [14]. Wong et al. also observed that miR-184 was found in the plasma of 80% of patients with OSCC of the tongue whereas it was only present in 13% of healthy individuals [15]. Furthermore, In the saliva of patients

with oral squamous cell cancer, miR-125a and miR-200 have significantly increased expression compared to the control sample [16]. In other studies, it has been demonstrated that patients, with squamous cell carcinoma exhibit elevated levels of two biomarkers, namely CEA and miR-155 compared to healthy individuals. These markers have the potential to play a role in the early diagnosis of OSCC [17]. Furthermore, previous studies have examined the levels of miR-4291 and miR-4303 in OSCC. These investigations reported an expression of both microRNAs, in tumor samples compared to healthy ones [13]. It is worth mentioning that other microRNAs, including miR-4652 and miR-1304, have also been studied in oral cancer. Likewise, these investigations have confirmed an increased expression of both microRNAs, in tumor samples compared to healthy ones [19].

This situation is similar to the result we obtained from this research, with the difference that we examined the expression levels of miR-148 and miR-375 in OSCC, which showed an increase in the expression of miRNA-148 and a decrease in the expression of miRNA-375 in tumor samples. We compared OSCC patients with oral infection with the ones without oral infection. Thus, our investigation focused on exploring these microRNAs roles within the context of cancer and oral infection, an area that has not been extensively studied before.

Conclusion

In this study, we examined the correlation, between the levels of miR-148 and miR-375 expression and the clinical findings of patients with oral cancer. The results indicate that there is an increase in miR-148 expression among OSCC patients who also have infections compared to those without such infections. Additionally, a decrease in miRNA 375 expression has been observed in individuals with OSCC and oral infections. Early detection of cancer plays a role in predicting the progression of the disease and its treatment outcomes. As a result, biomarkers can be utilized to identify targets, for treatment and prognosis purposes.

Conflict of Interest

There is no conflict of interest to declare.

References

[1] Collins FS, Barker AD. Mapping the cancer genome. Scientific American. 2007; 296(3):50-7.

[2] Little J, Miller C, Rhodus N. Dental management of the medically compromised patient 6th ed. Missouri: Mosby. 2002:387-9.

[3] Lodish HF. Molecular cell biology: Macmillan; 2008.

[4] Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. Nature reviews Molecular cell biology. 2005; 6(5):376-85.

[5] Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. Genomics, proteomics & bioinformatics. 2009; 7(4):147-54.

[6] Nagy Z, Igaz P. Introduction to microRNAs: Biogenesis, action, relevance of tissue micrornas in disease pathogenesis, diagnosis and therapy—the concept of circulating microRNAs. Circulating microRNAs in Disease Diagnostics and their Potential Biological Relevance. 2015:3-30.

[7] Singh SR, Rameshwar P. MicroRNA in Development and in the Progression of Cancer: Springer; 2014.

[8] Chen Y, Song Y-X, Wang Z-N. The microRNA-148/152 family: multi-faceted players. Molecular cancer. 2013; 12:1-8.

[9] Friedrich M, Pracht K, Mashreghi MF, Jäck HM, Radbruch A, Seliger B. The role of the miR-148/-152 family in physiology and disease. European journal of immunology. 2017; 47(12):2026-38.

[10] Fujita Y, Kojima K, Ohhashi R, Hamada N, Nozawa Y, Kitamoto A, et al. MiR-148a attenuates paclitaxel resistance of hormone-refractory, drug-resistant prostate cancer PC3 cells by regulating MSK1 expression. Journal of biological chemistry. 2010; 285(25):19076-84.

[11] Liu X, Zhan Z, Xu L, Ma F, Li D, Guo Z, et al. MicroRNA-148/152 impair innate response and antigen presentation of TLR-triggered dendritic cells by targeting CaMKIIα. The Journal of Immunology. 2010; 185(12):7244-51.

[12] Patoulias D. Is miRNA-375 a promising biomarker for early detection and monitoring of patients with type 2 diabetes? Archives of Medical Science-Atherosclerotic Diseases. 2018; 3(1):119-22.

[13] Liu CJ, Lin SC, Yang CC, Cheng HW, Chang KW. Exploiting salivary miR-31 as a clinical biomarker of oral squamous cell carcinoma. Head & neck.

 DOI: 10.18502/jcr.v11i1.16477

2012; 34(2):219-24.

[14] Zheng Y, Cui L, Sun W, Zhou H, Yuan X, Huo M, et al. MicroRNA-21 is a new marker of circulating tumor cells in gastric cancer patients. Cancer biomarkers. 2012; 10(2):71-7.

[15] Wong T-S, Liu X-B, Wong BY-H, Ng RW-M, Yuen AP-W, Wei WI. Mature miR-184 as potential oncogenic microRNA of squamous cell carcinoma of tongue. Clinical Cancer Research. 2008; 14(9):2588-92.

[16] Lin S-C, Liu C-J, Lin J-A, Chiang W-F, Hung P-S, Chang K-W. miR-24 up-regulation in oral carcinoma: positive association from clinical and in vitro analysis. Oral oncology. 2010; 46(3):204-8.

[17] Narimani A, Hosseini F, Bahrami N, Mohamadnia A. The Expression of MicroRNA-155 (miR-155) and Carcinoembryonic Antigen Messenger RNA (CEA mRNA) in Peripheral Blood of Patients with Oral Squamous Cell Carcinomas (OSCC). J Isfahan Med Sch 2019; 36(510): 1597-601.