Journal of Craniomaxillofacial Research

Vol. 10, Issue. 4 Autumn 2023

Comparing Salivary Mir-125 And Mir-30 Expression In Oral Squamous Cell Carcinoma To Healthy Individuals

Naghmeh Bahrami [1,2], Marziyeh Poorahmad [3], Farzaneh Hosseini [4], Farnoush Mohammadi [2,5], Amir Rasouli [6], Mona Mohajeri Tehrani [2], Masoume Farhangiyan [2], Abdolreza Mohamadnia [7,8*]

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 Genetic, School of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.

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

5. Department of Oral and Maxillofacial Surgery, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.

6. Department of Biology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

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

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

ARTICLE INFO

Article Type:
Original Article

Received: 1 August 2023
Revised: 12 September 2023
Accepted: 19 November 2023

**Corresponding author:*
Abdolreza Mohamadnia

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

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

ABSTRACT

Introduction: OSCC (Oral squamous cell carcinoma) accounts for approximately 90% of all oral malignancies and is usually diagnosed at advanced stages. This study investigates changes in miR-125 and miR-30 expression in relation to the clinical findings of oral cavity cancer and their possible use as an early diagnostic tool.

Materials and Methods: A population of 30 individuals with oral squamous cell carcinoma and 30 healthy individuals was studied, and the mean age of two groups were compared using a t-test, with no significant difference found in terms of age so age will not be an interfering factor in this study. The levels of these two biomarkers (miR-125 and miR-30) were measured and evaluated using real-time PCR technique.

Results: After evaluating the results of real-time PCR technique, it was found that miR-125 was positive in 25 out of 30 patients, while it was positive in 5 out of 30 healthy individuals (p-value≤0.001). miR-30 was a positive biomarker in 10 out of 30 patients. The amount of this biomarker in the group of healthy individuals was 26 out of 30 (p-value<0.001).

Conclusion: The miR-125 profile is upregulated in the saliva of OSCC cases, whereas the miR-30 profile is downregulated in the aforementioned patients compared with the healthy group. Therefore, measurement of miR-125 and miR-30 may be a protentional diagnostic test to identify OSCC. We suggest more extensive studies with a larger sample size to support this claim.

Keywords: Oral squamous cell carcinoma; MiR-125; MiR-30; Biomarker.

Please cite this Article as:
Bahrami N, Poorahmad M, Hosseini F, Mohammadi F, Rasouli A, Mohajeri Tehrani M, et al. Comparing Salivary Mir-125 And Mir-30 Expression In Oral Squamous Cell Carcinoma To Healthy Individuals. J Craniomaxillofac Res 2023; 10(4): 162-166. DOI: 10.18502/jcr.v10i4.15307

Introduction

Squamous cell carcinoma of the oral cavity accounts for 2% of cancers of the body and is one of the 10 leading causes of death in humans. 95% of cases occur in people over 40 years of age, and most are elderly. Men are three times more likely to develop this cancer than women; in general, this cancer accounts for 5% of cancers in men and 2% of cancers in women [1]. The poor prognosis for oral cavity cancer in most parts of the world is due to the fact that most oral cavity cancers are identified in advanced stages, as a result of clinical manifestations [2]. Cancers in men and 2% of cancers in women [1]. The poor prognosis for oral cavity cancer in most parts of the world is due to the fact that most oral cavity cancers are identified in advanced stages, as a result of clinical manifestations [2]. Cancer in the oral cavity refers to the development of cancerous cells in either the mouth or tongue. It can manifest as an ulcer or as a growth in the submucosal region, which is not visible externally but can be detected by touch [3]. The length of miRNAs is usually between 18 to 25 nucleotides. Molecules regulate gene expression in a very specific way [7-9]. The specific expression of miRNAs in various tissues and diseases has led scientists to consider them as a diagnostic marker [6]. Many cancer types such as nasopharyngeal carcinoma (NPC), retinoblastoma (RB), glioblastoma (GBM), non-small cell lung cancer (NSCLC), and gastric cancer have reported overexpression of miR-125 as an oncogene [7]. miR-125 can act as a strong promoter or inhibitor in different tumors depending on the molecular context.

More importantly, miR-125 can serve as a diagnostic and prognostic marker for multiple tumors [8]. miR-30 has been studied as a biomarker in various cancers, including lung, pancreatic, colorectal, and melanoma, and the available data are relatively controversial. In any case, miR-30 plays a role in cancer prognosis and metastasis and as a tumor suppressor marker [9-11]. The aim of this study is to investigate the expression of miR-125 and miR-30 in saliva samples from patients with oral squamous cell carcinoma compared with saliva samples from healthy individuals.

Materials and Methods

Initially, 30 patients referred to the Cancer Institute of Tehran University of Medical Sciences and suspected of oral squamous cell carcinoma based on physical examination and diagnosis by an expert were selected before treatment was performed on them (Code of Ethics: IR.TUMS.AMIRALAM.REC.1401.014). Individuals who had chronic or acute inflammatory diseases were also excluded from this study. Individuals whose pathologic findings were not available for any reason were excluded from this study. thirty healthy subjects as a control group voluntarily participated in this study after being examined by a physician and completing an informed consent form. The subjects in the two groups were in the same age group with a minimum age of 24 years and a maximum age of 68 years. After the subjects were selected, saliva samples were taken. and immediately entered the RNA extraction stage. RNA extraction steps were performed using (qiagen Cat no.75144) RNeasy Mini Kit. Nanodrop device was used to evaluate the quality of extracted RNA. 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 house keeping. Real-time PCR was performed in a final volume of 25ml containing: 10ml Ampliqon master mix, 0.5ml each of F and R primers, 5µl DNA, and distilled water. The assay was performed in 35 cycles using a rotor gene cycler. Temperature and time conditions: initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 20 seconds, primer connection at 56°C for 40 seconds and amplification at 72°C for 30 seconds (for 35 cycles) and the final amplification at 72°C for 5 minutes. The results were analyzed using statistical software: SPSS Version 22 and the mean and standard deviation were calculated. Paired t-test was used to analyze the difference or relationship between gene expression level and clinicopathological characteristics. The difference was considered significant at the $P \leq 0.05$ level.

Results

The study group is composed of 30 healthy individuals and 30 people diagnosed with oral squamous cell cancer, as previously noted. These 2 groups were matched based on age variables. The groups were compared using t-test in terms of average age and did not show any significant difference in terms of average age (Table 1). Real-time RT-PCR reaction was performed. The results were interpreted based on the melting curve. miR-125 was positive in 25 of 30 patients. The amount of this biomarker in the healthy group was 5 out of 30 subjects. Statistical comparison of the positivity rate of this biomarker showed a statistically significant difference between these two groups (P value<0.001) (Figure 1). The biomarker miR-30 was positive in 10 of 30 patients. The amount of this biomarker in the group

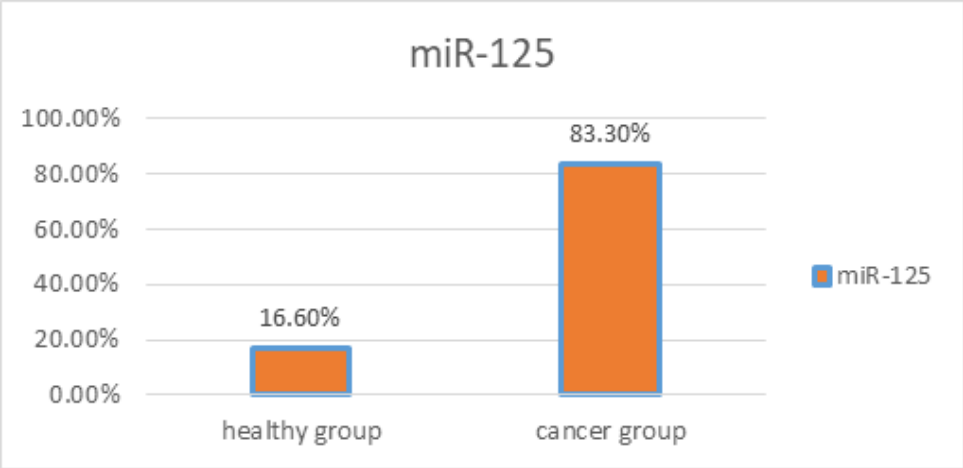

Figure 1. The rate of miR-125 positivity in the saliva of OSCC patients and healthy individuals.

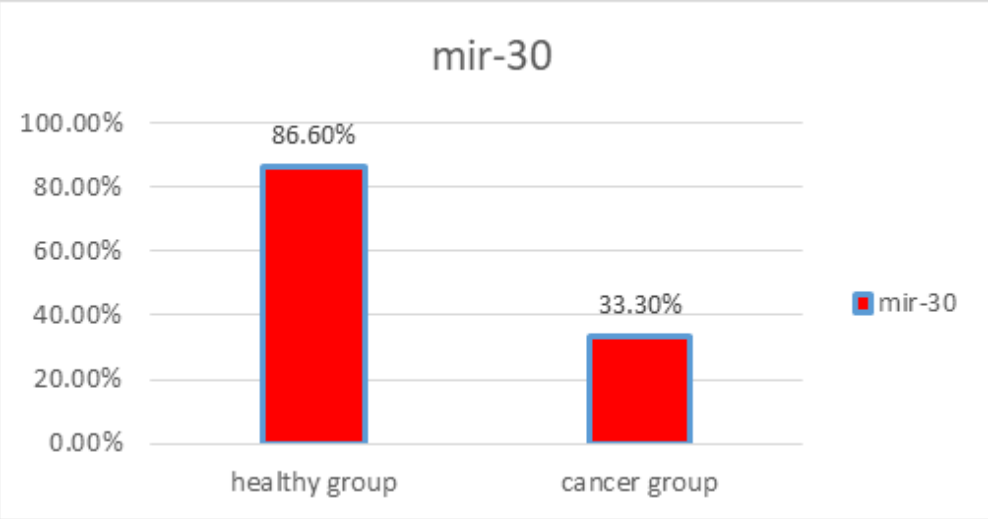

Figure 2. The rate of miR-30 positivity in the peripheral blood of OSCC patients and healthy individuals.

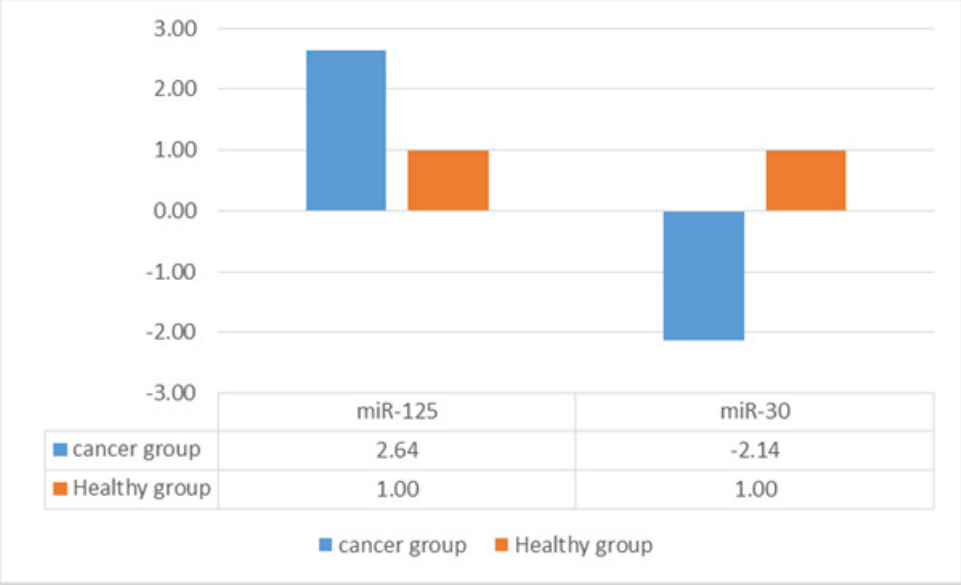

Figure 3. Differences in expression of miR-125 and miR-30 genes in cancer group and Healthy group.

DOI: 10.18502/jcr.v10i4.15307

of healthy subjects was 26 out of 30. Statistical comparison of the rate of positivity of this biomarker in the sick group and the healthy group showed a statistically significant difference between these two groups (P value<0.001) (Figure 2). To calculate folding change, Ct of each sample was determined first. The relative difference in the expression of markers in two groups was calculated using the formula 2-ΔΔ Ct. Therefore (Figure 3). The expression level of miR-125 in sick people is 2.64 times higher than in healthy people. The expression level of miR-30 in sick people is 2.14 times lower than in healthy people.

Discussion

Oral cancer is one of the most common malignancies worldwide. Although oral cancer tends to occur in middle-aged and elderly people, recent studies have shown that 4% of oral cancers occur in people younger than 40 years of age, and surprisingly, they can even be detected in children as young as 10 years of age [12] therefore, early diagnosis of this disease is very important. In this study, the expression of miR-125 was increased in patients compared with healthy individuals, and the expression of miR-30 was decreased in patients compared with healthy individuals.

Usually, the diagnosis of OSCC depends on a detailed clinical examination of the oral cavity, followed by biopsy for histological analysis. However, despite the ease of access to visual examination, OSCC is often diagnosed at advanced stages, which reduces patient survival [13,14] miR-125 plays an important role in cell proliferation and can affect genes involved in MAPK metabolism [15]. In some cases, miR-125 can regulate the target oncogene as a tumor suppressor, reducing tumor proliferation and metastasis. On the other hand, the tumor-stimulating function shows that miR-125 is considered as a tumor promoter [16].

We should definitely consider miR-125 as a strong predictor for early detection of malignant and other diseases [17]. MiRNAs are actually present in human body fluids such as blood, saliva, urine, and breath, so they can be used as a noninvasive diagnostic tool. Wong et al. demonstrated the presence of miR-184 in the plasma of 80% of patients with squamous cell carcinoma of the tongue [18]. In addition, Liu and colleagues demonstrated a high concentration of miR-31 in the saliva of patients with oral cancer, whereas the concentration of miR-31 in plasma increased [19,20]. Similarly, in the study conducted, there was an increase in miR-125 expression in patients with OSCC compared with healthy individuals, and there was a statistically significant difference between them. miR-30 has been studied as a biomarker in various cancers, including lung, pancreatic, colorectal, and melanoma, and the available data are relatively controversial. In any case, miR-30 plays a role in cancer prognosis and metastasis and as a tumor suppressor marker [9-11]. In a recent study, a decrease in the expression of miR-30 was observed in patients with OSCC compared with healthy individuals, with a statistically significant difference between them.

Conclusion

The miR-125 and miR-30 genes are produced in oral squamous cell carcinoma tumors, and the expression of these genes in the saliva of the OSSC group is significantly higher than that of the healthy group. Measurement of the expression of these genes in saliva can be used as a non-invasive method for the early diagnosis of patients with oral squamous cell carcinoma.

Conflict of Interest

There is no conflict of interest to declare.

References

[1] Nevile B, Douglas D, CARL M, Jerry E. Oral & maxillofacial pathology. 2nd ed. W B South Carolina Saunders Co; 2002. P. 356.

[2] Yu-Jen J, Chia-Der L, Chin-Ho L, Chih-Hsin T, Su-Hua H, Ming-Hsui T, et al. Salivary zinc finger protein 510 as a novel biomarker for detection of oral squamous cell carcinoma early stages. Clin Chim Acta. 2011 Jul 15; 412(15-16):1357-65.

[3] Jou YJ, Lin CD, Lai CH, Chen CH, Kao JY, Chen SY, et al. Proteomic identification of salivary transferin as a biomarker for early detection of oral cancer. Anal Chim Acta. 2010 Nov 29; 681(1-2):41-8.

[4] Nafisa Momeni. What is the treatment of oral Cancer? 1393. Available at: URL: http://www.dandane.ir/. Accessed Jan 10, 2017.

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

[6] Lodish, H., et al. (2008). Molecular cell biology, Macmillan.

[7] Chen J, Fu X, Wan Y, Wang Z, Jiang D, Shi L.

miR-125b inhibitor enhance the chemosensitivity of glioblastoma stem cells to temozolomide by targeting Bak1. Tumour Biol. 2014; 35 (7):6293–6302. doi:10.1007/s13277-014-1821-4.

[8] Liu Z, Smith KR, Khong HT, et al. miR-125b regulates differentiation and metabolic reprogramming of T cell acute lymphoblastic leukemia by directly targeting A20. Oncotarget. 2016; 7(48):78667–78679. doi:10.18632/oncotarget.12018.

[9] Wang J, Paris PL, Chen J, et al. Next generation sequencing of pancreatic cyst fluid microRNAs from low grade-benign and high gradeinvasive lesions. Cancer Lett. 2015; 356(2):404-409.

[10] Kandimalla R, Gao F, Matsuyama T, et al. Genome-wide discovery and identification of a novel miRNA signature for recurrence prediction in stage II and III colorectal cancer. Clin Cancer Res. 2018; 24(16):3867-3877.

[11] Gaziel-Sovran A, Segura MF, Di Micco R, et al. MiR-30b/30d regulation of GalNAc transferases enhances invasion and immunosuppression during metastasis. Cancer Cell. 2011; 20(1):104-118

[12] LI, C. I., URIBE, D. J., DALING, J. R. (2005). Clinical characteristics of different histologic types of breast cancer. British Journal of Cancer. 93(9), 1046-1052.

[13] Pende, M., et al. (2004). "S6K1-/-/S6K2-/-mice exhibit perinatal lethality and rapamycin-sensitive 5′-terminal oligopyrimidine mRNA translation and reveal a mitogenactivated protein kinase-dependent S6 kinase pathway." Molecular and cellular biology 24(8): 3112-3124.

[14] Sebolt-Leopold JS (June 2008). "Advances in the development of cancer therapeutics directed against the RAS-mitogen-activated protein kinase pathway". Clin. Cancer Res. 14 (12): 3651–6. doi:10.1158/1078-0432.CCR-08-0333. PMID 18559577

[15] Radhika T, Jeddy N, Nithya S, Muthumeenakshi RM. Salivary biomarkers in oral squamous cell carcinoma - An insight. J Oral Biol Craniofac Res. 2016 Nov; 6(Suppl 1):S51-S54.

[16] Sun, Yu-Meng; LIN, Kang-Yu; CHEN, Yue-Qin. Diverse functions of miR-125 family in different cell contexts. Journal of hematology & oncology, 2013, 6: 1-8.

[17] Ghosh RD, Pattatheyil A, Roychoudhury S. Functional Landscape of Dysregulated MicroRNAs in Oral Squamous Cell Carcinoma: Clinical Implications. Front Oncol. 2020 May 12; 10:619

[18] Ma, J.; Fu, Y.; Tu, Y.Y.; Liu, Y.; Tan, Y.R.; Ju,W.T.; Pickering, C.R.; Myers, J.N.; Zhang, Z.Y.; Zhong, L.P. Mutation allele frequency threshold does not affect prognostic analysis using next-generation sequencing in oral squamous cell carcinoma. BMC Cancer 2018, 18, 758.

[19] Coutinho-Camillo, C.M.; Lourenço, S.V.; Nishimoto, I.N.; Kowalski, L.P.; Soares, F.A. Caspase expression in oral squamous cell carcinoma. Head Neck 2011, 33, 1191–1198.

[20] Liu, P.F.; Hu, Y.C.; Kang, B.H.; Tseng, Y.K.; Wu, P.C.; Liang, C.C.; Hou, Y.Y.; Fu, T.Y.; Liou, H.H.; Hsieh, I.C.; et al. Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. PLoS ONE 2017, 12, e0180620.

 DOI: 10.18502/jcr.v10i4.15307