Journal of Craniomaxillofacial Research

Vol. 10, No. 1 Winter 2023

Assessment of miR-182, miR-221 and CEA expression in the peripheral blood of oral squamous cell carcinoma (OSCC) patients

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ARTICLE INFO

Article Type:
Original Article

Received: 8 Apr. 2022
Revised: 5 Jun. 2022
Accepted: 19 Oct. 2022

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ABSTRACT

Introduction: Head and neck cancer is the sixth common cancer in the world, of which the oral cavity is the most frequent type. It was diagnosed on more than 377,700 cases worldwide in 2020. Access to high-quality care leading to a more specific and earlier diagnosis of this cancer is crucial. Therefore, researchers attempt to investigate and detect efficient biological tumor marker. The present study aimed to investigate the changes in the expressions of miR-182, miR-221, and carcinoembryonic antigen (CEA) in the peripheral blood of patients with oral squamous cell carcinoma (OSCC) and compare them with healthy individuals to detect early OSCC.

Materials and Methods: 30 peripheral blood samples from patients with OSCC (19 male and 11 female), aged 25-70, were obtained from the cancer institute of Tehran University of Medical Sciences, and 30 peripheral blood samples from healthy individuals (20 male and 10 female) aged 26-70 were collected. Real-time PCR was carried out to investigate the differences in the expressions of miR-182, miR-221, and ELISA was used to measure CEA protein expression.

Results: Among the subjects with OSCC 83% showed miR-182 expression, 93% revealed miR-221 expression and 96% demonstrated CEA expression. Whereas, these expressions were 26%, 20% and 13%, respectively, for the healthy group. The simultaneous detection of miR-182 and miR-221 was 73% in the individuals with OSCC. The simultaneous observation of miR-182, miR-221, and CEA in the group with OSCC was 60%. The expression level of miR-221 in the group with OSCC was 2.63 times that of the healthy subjects, and the expression level of miR-182 in the individuals with OSCC was 2.29 times that of the healthy group.

Conclusion: The results of this study nominated miR-182, miR-221, and CEA as biomarkers for early diagnosis and consequently improvement of survival rate of patients suffering from OSCC.

Keywords: OSCC; MiR-182; MiR-221; CEA; Biomarker.

Introduction

Recent WHO statistics have reported 1139 new incidences of lip and oral cavity cancers in 2020 in Iran, ranking oral cancers as the 20th most prevalent cancer [1]. In the global map of prevalence of oral cancers Iran stands apace with India, Pakistan and Bangladesh, with affliction rate of 20 to 36.3 in each 100000 persons [2]. Oral and oropharyngeal cancers comprise cancer of the lip, tongue, floor of the mouth, gingiva, palate and buccal mucosa, alveolar mucosa, oropharynx, as well as the pharyngeal tonsils and salivary glands [3].

Oral squamous cell carcinoma (OSCC) constitutes over 90% of oral cancers [4]. Diverse risk factors have been notably related to oral cancer such as age, genetic factor (family history), environmental factors (viral, fungal and bacterial infections, poor oral hygiene and occupational risk) and epigenetic factors (tobacco smoking, alcohol drinking, betel quid chewing, mouthwash, diet and nutrition) [5].

Despite recent progress in diagnosis and therapy strategies, in approximately 53% of patients detection occurred in an advanced stage of OSCC [6], less than half patients with advanced oral cancer had 5-year survival rates [7], and therapy expenses for advanced stages were around $10,532, which imposed economic burden on society. Designing an early diagnosis and screening program for oral cancer would probably decrease the related mortality and health care costs [6]. In this regard, novel molecular techniques merit investigation as they can evaluate valid molecular biomarkers with clinical worth. Tumor biomarkers can ne assorted into nucleic acid-based and protein-based markers [8,9].

MicroRNAs (miRNAs) are non-coding and small single-strand RNA (18-25 nucleotides), which play a role in vital biological processes such as development, proliferation and apoptosis. Changes in the expression of MicroRNAs are critical for clinical prognosis of cancer [10,11]. MiRNAs from primary biopsy lesions, serum or saliva represent auspicious candidates for screening and early detection of oral cancers [12]. The miRNA182 encoding locus is located on chromosome 7q32.2 of the human genome, in a cluster with MIR183 and MIR96 [13]. A Growing body of evidence has revealed the overexpression of miRNA182 in certain human malignancies, such as glioblastoma, melanoma, liver, breast, gastric, colorectal cancers, myeloid leukemia and Hodgkin's lymphoma [14-17].

MiR-221, an onco-miR or oncosuppressor-miR, is located on the human chromosome X. MiR-221 has been indicated to be upregulated in various cancers [18]. The upregulation of MiR-221 has been associated with lesser survival chances in liver, laryngeal and non-small cell lung cancers [19-21]. Also, MiR-221 has an essential role in the prognosis of ovarian cancer and renal cell carcinoma [22-23]. Carcinoembryonic antigen (CEA) is a fetal liver glycoprotein and is not typically generated in significant quantities after birth [24]. The elevated expression of this fetal glycoprotein has been observed in lung and colorectal cancers and it is a key prognostication biomarker for treatment planning and monitoring of patients suffering from these cancers [25, 26]. In the present survey the variations in the expressions of miR-182, miR-221 and CEA in the peripheral blood of patients suffering from OSCC are studied and compared with healthy individuals.

Materials and Methods

The present article is a case-control study comprised of 30 patients of the cancer institute of Tehran Medical sciences university 19 male and 11 female, aged 25-70, diagnosed with initial stage OSCC cancer-as the afflicted group and 30 volunteers 20 male and 10 female, aged 26-70, without any history of cancer or other malignances, as the control group. After it was ensured that the subjects qualified for the study and they had signed a letter of consent, 5ml of peripheral blood was taken from the afflicted and control groups. Subsequently, the red blood cells and hemoglobin were removed by hemolysis, and the resulting sediments were used for RNA extraction.

The RNA extraction was carried out using a RNeasy Midi kit (Qiagen cat no. 75144). The quality and quantity of the extracted RNA was evaluated using NanoDrop spectrophotometery and agarose gel electrophoresis. Subsequently cDNA was synthesized using a Viva 2- steps RT-PCR kit (Cat no.RTPL12). The primers were designed using the oligo7 software, for 6 miR-182 and miR-221 (RNU6 as control) based on the sequences stored in Gen Bank. The specificity of the primers was evaluated using the BLAST algorithm and the NCBI data banks. The primers were synthesized by CinnaGen Co. The primer sequences are shown in table 1. Real Time PCR was carried out using Rotor-Gene OIPGEN according to the reaction temperatures and reaction times stated in table 2. The mean CT for each sample was used to compute expressions via the $2^{-\Delta\Delta CT}$ method.

The serum CEA was measured using a Can Ag CEA EIA kit (which is based on solid-phase-Non-competitive Immunoassay and the direct sandwich method). Concentrations lower than 5µg/L were considered as negative and concentrations higher than 5µg/L were considered as positive. The t-test was used to compare the mean results of the afflicted and control groups and the two-sample binomial test, as implemented in the SPSS 16 software, was used to compare the percentage of positive markers for the two groups. A P value of less than 0.05 was taken as the minimum significance level.

Table 1. Genes and nucleotide sequences of the primers used for Real-time PCR.

Gene	*Sequences of the Primers*	
miR-182	*Forward*	*5'-TGCAGGGTCCGAGGT-3'*
	Reverse	*5'- AATGGTTCTAGACTTGCCAACT-3'*
miR-221	*Forward*	*5'- CGCAGCTACATTGTCTGCTGG-3'*
	Reverse	*5'- GTGCAGGGTCCGAGGT-3'*
RNU6	*Forward*	*5'- TCGCTTCGGCAGCACA -3'*
	Reverse	*5' AACGCTTCACGAATTTGCG -3'*

Table 2. Cycling conditions of real-time PCR for miR-182, miR-221 and RNU6.

Temperature	*Time*	*Cycles*
95 C°	*15min*	*1*
95 C°	*30s*	*35-40*
55-60 C°	*60s*	
55-95 C°	*Melting Analysis*	*1*

Results

In this study the control and afflicted groups consisted of 30 subjects each. Table 3 and figure 1 compare the two groups in terms of the gender and age of the subjects. Clearly the two groups do not show any significant difference in this regard. MiR-182 was observed in the blood of 25 individuals of the OSCC group and 8 members of the control group. The presence of miR-221 was confirmed in 28 members of the afflicted group and only 6 of the control group (Figure 2). As noted in figure 2, the discrepancies between the two groups are clearly significant. The Real-time PCR results in figure 3 exhibit a 2.29 and 2.36 fold increases of the mir-182 and mir-221 expressions, respectively, for the OSCC group compared to the control group. The serum CEA results are positive for 29 subjects in the OSCC group and only 4 members of the control groups (Figure 4).

Figure 5 shows the results for the simultaneous detection of miR-182 and miR-221 as well as miR-182, miR-221 and CEA. It can be noted that mir-182 and miR-182 were simultaneously observed in 22 of the afflicted group, and miR-182, miR-221 and CEA were simultaneously detected in 18 members of the OSCC group.

Table 3. The range of age for OSCC and healthy groups.

Age (year)			Group
SD	Mean	Range of age	
10.22	46.25	26-70	OSCC (30 individual)
12.12	47.84	25-70	Healthy (30 individual)

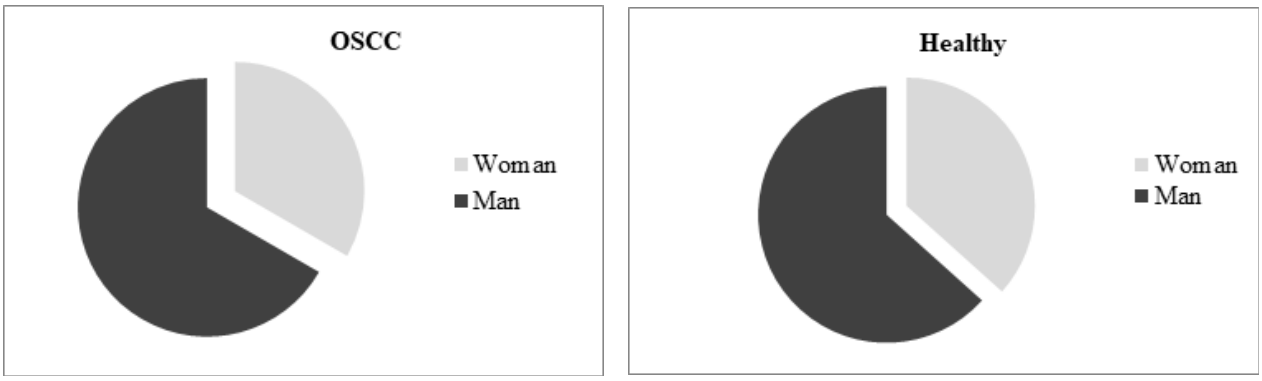

Figure 1. Gender distribution in OSCC and healthy groups.

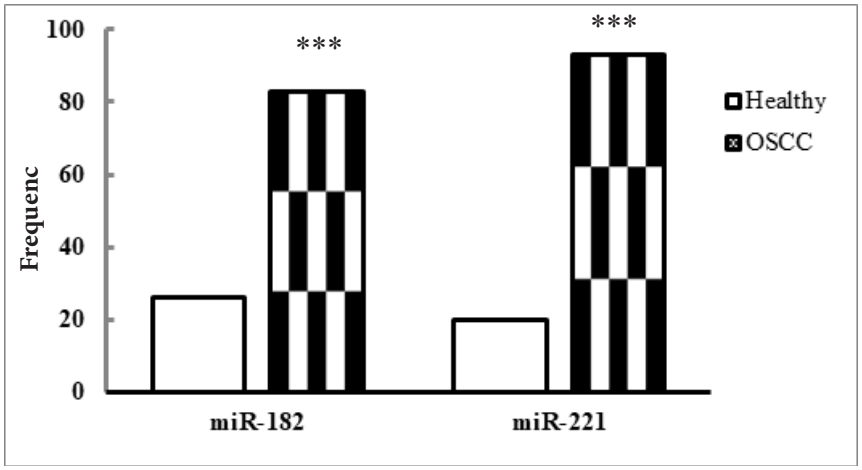

Figure 2. Frequency of presence of miR-182 and miR-221 in OSCC and healthy groups.

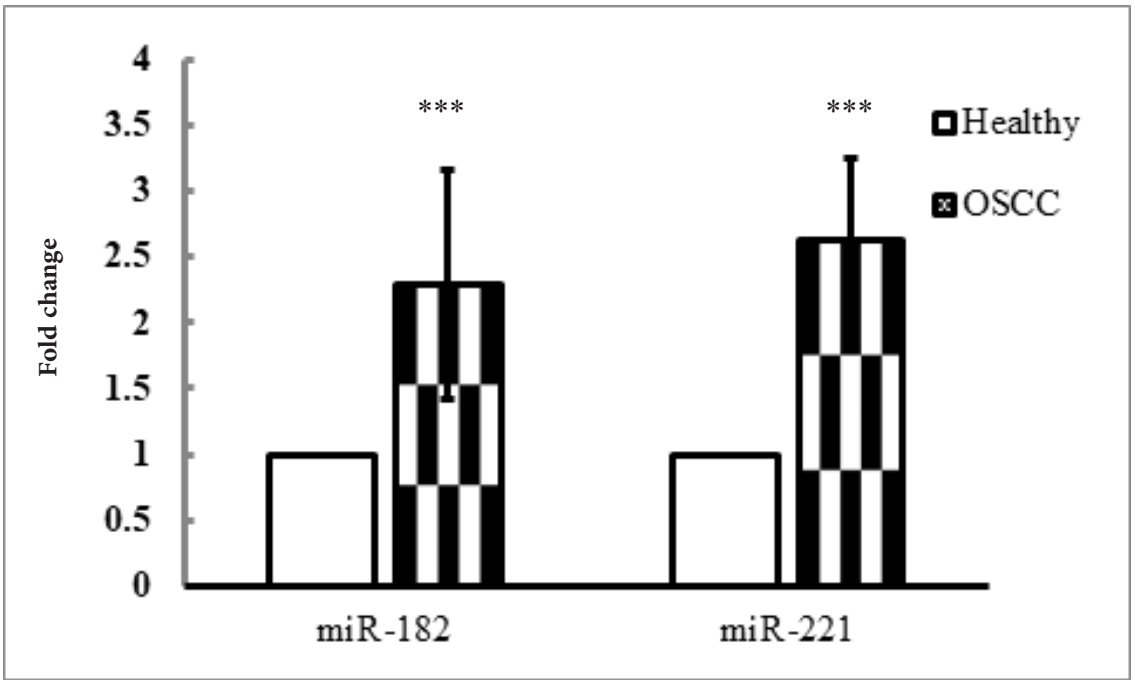

Figure 3. The expression of miR-182 and miR-221 in OSCC and healthy groups.

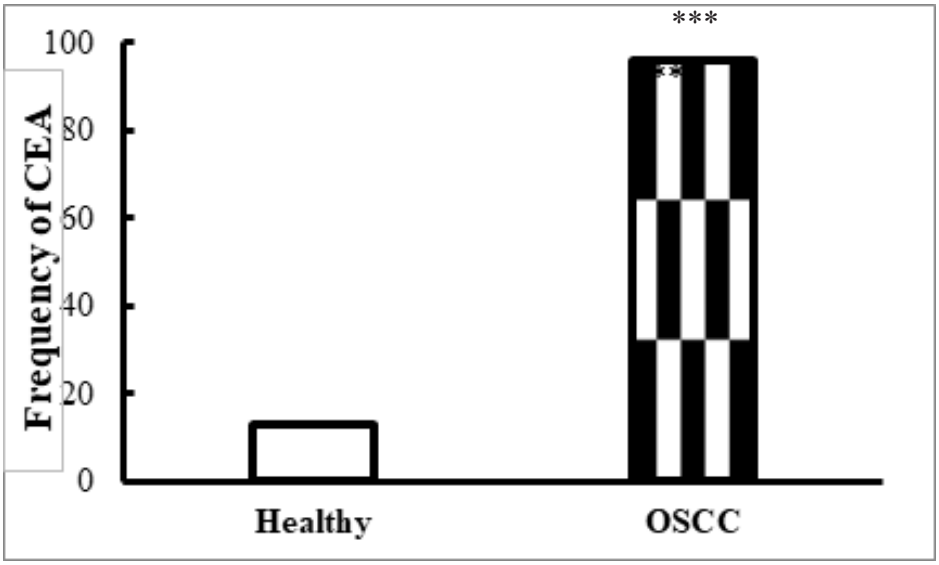

Figure 4. Frequency of presence of CEA in OSCC and healthy groups.

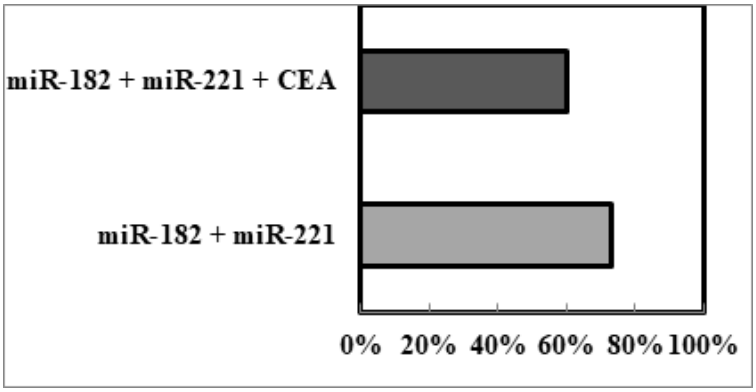

Figure 5. Frequency of simultaneous detection of miR-182 and miR-221, and miR-182, miR-221 and CEA in OSCC group.

Discussion

Head and neck cancers are the sixth most prevalent cancers, among which Oral cancer is more common [27,28]. OSCC is the most common type of oral cancer, with an invasive growth pattern. It attacks the neck lymphatic nodes, can disrupt speech, breathing and deglutition, with serious detrimental effects on physical and emotional wellbeing [29-31]. Due to its obscure behavior, detection of OSCC is usually at later stages which greatly reduces patient survival chances [32]. However early detection of OSCC, when the damage is small and not widespread, can greatly improve treatment results and survivability, and reduce treatment period and the associated deformities [33]. Adopting an approach which evaluates many biomarkers can provide early detection and better treatment strategies for OSCC [34,35]. MicroRNAs have been associated with resistance to chemo and radiotherapy, and proliferation, apoptosis, invasion, metastasis, EMT and cell cycle arrest of oral cancer cells. Abnormal microRNA expression can have a significant clinical role in early detection of OSCC [36], therefore in the present research the expression of miR-188 and mir-221 mircoRNAs and CEA protein levels have been measured and compared in healthy and OSCC afflicted individuals. The study involved 19 male and 11 female subjects with OSCC (a male to female ratio of 1.7). This is in agreement with the reports that the risk of OSCC in men is 1.6 times greater than in women. The risk of acquiring head and neck cancers for men over 60 is greater than women, even irrespective of smoking and drinking history [37]. Mir-182 is a conserved microRNA whose elevated expression has been reported in many human malignancies such as breast cancer, gastric cancer, glioblastoma and hepatocellular carcinoma [38]. It is also associated with proliferation of OSCC cells [39]. Our study results showed an increase in mir-182 expression

in the peripheral blood of OSCC patients compared to healthy individuals. This is in agreement with the in vitro findings of Li et al. in 2018, which reported increased miR-182 expression in several OSCC cell lines and tissues excised from OSCC patients. It is worth noting that they describe the mechanism of action of miR-188 in cancer cell proliferation as inhibiting the Ca^{2+}/ calmodulin kinase ii pathway [39].

The function of miR-221 in the progression of bladder, prostate and breast cancers has been established via impeding the modulation of downstream targets such as phosphatase, E-cadherin, PTEN/AKT pathway and cytokine signaling [40-44]. Investigations on various oral squamous cell carcinoma cell lines revealed that downregulation of miR-221 promotes apoptosis via upregulation of PTEN, whereas it's upregulation causes resistance to Doxorubicin, which in turn downregulates the tissue inhibitor metalloproteinase-3 (TIMP3) [45-46]. The present study confirmed the presence of and remarkable elevation in the expression of miR-221 in the peripheral blood of oral cancer patients in comparison with healthy individuals. Carcinoembryonic antigen decreases cell-cell and basal-cell adhesionmolecules, therefore boosts malignancy [47]. A radioimmunoassay study on 29 patients with primary squamous cell carcinoma showed that 34.5% tested positive for serum CEA [48]. Narimani et al., detected mRNACEA in %77 of oral cancer cases and %17 of healthy individuals [49]. Their results are in accord with our findings which exhibited significant presence of CEA in OSCC patients.

Conclusions

The present investigation displayed the presence of miR-182, miR-221 and CEA individually or together in the peripheral blood of patients with oral squamous cell carcinoma. Considering the importance of early diagnosis of OSCC in the efficiency of the treatment and survival rate, the assessment of these three potential tumor biomarkers is highly recommended. However, establishing miR-182, miR-221 and CEA as promising tumor biomarkers for early detection of OSCC requires further clinical investigation.

Acknowledgment

This study was carried out under the research code…… issued by Islamic Azad University Share Qods Branch. The authors would like to thank Islamic Azad University Share Qods Branch and Shahid Beheshty University. We thank Dr. R. Rajaie Khorasani for editing the manuscript.

Conflict of Interest

There is no conflict of interest to declare.

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Please cite this paper as:
Rasouli A, Arzi L, Khademi S, Mohamadnia A. Assessment of miR-182, miR-221 and CEA expression in the peripheral blood of oral squamous cell carcinoma (OSCC) patients. J Craniomax Res 2023; 10(1): 18-26