



Assessing VEGF mRNA and miR-494 expression in peripheral blood collected from patients suffering from oral squamous cell carcinoma

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ABSTRACT

Introduction: Oral squamous cell carcinoma accounts for 2% of all cancers and also is one of the top 10 causes of death in humans. The goal of this article is to assess changes of VEGF mRNA and miR-494 expression.

Materials and Methods: We chose 30 patients with OSCC and 30 healthy people (as a controlled group) and assess the rate of VEGF mRNA and miR-494 expression in peripheral blood with real-time PCR technique.

Findings: VEGF mRNA marker was positive in 24 out of 30 patients. Also, miR-494 was positive in 20 out of 30 patients.

Results: Overall based on this article's findings, results could count as a diagnostic screening test for OSCC detection in early steps of cancer.

Keywords: Oral squamous cell carcinoma; Early diagnosis; VEGF mRNA; miR-494.

Introduction

OSCC is a fairly rare cancer which has a high rate of mortality [1]. Etiology of OSCC is multifactorial that occur within different steps. Changes and damages have effects on DNA so the instability of

DNA begins. OSCC is the most common Epithelial related cancer detected in oral cavity which accounts about 90% of oral cavity malignancies [2]. It is more frequent in men. (5% in men, 2% in women).

OSCC has a high rate of local invasion and metastasis that result in high mortality. In spite of treatment progress such as chemotherapy, radiotherapy and surgery in recent decades, there is no evidence of decreasing in number of deaths caused by this cancer [3]. miRNAs act completely exclusive in regulating gene expression and their regulating mechanisms function in different ways like; destruction of coding mRNA with targeted gene, inhibition of RNA translation, inducing chromatin changes in target gene which all lead into the end of the transcription [4,5,6]. Increased amount of miR-494 is found in some cancers to suppress the process. Indeed, miR-494 can be a tumor suppressor which could lead to prevent cell cycle and induction of apoptosis [7]. VEGF Family (Vascular endothelial growth factor) consists of some growth factors which effects on endothelial vascular directly and can stimulate proliferation and migration of endothelial cells [8].

Materials and Methods

30 patients which diagnosed with OSCC by a specialist before any treatment selected. 30 people from healthy subjects were selected as control group and participated in this study by filling out consent form. 2ml of peripheral blood sample collected in glass test tube and extraction of RNA began immediately. (Peo-

ple were between 22 and 77 years old and divided into groups with same age). RNA extracted with RNA blood mini kit (qiagen cat no.52304). We used (Cat no.RTPL12) Viva 2-steps RT-PCR Kit For producing cDNA and for investigating VEGF gene CinnaGreen qPCR Mix, 2X (Cat No.MM2041) used by Real-Time RT-PCR technique. For VEGF mRNA we used the reference gene (18s rRNA) (Table 1).

Also, for mi-RNA we used Rotor-Gene-QIAGEN machine with ZIST ROYESH kit. In order to normalizing miRNA in each sample, we need a house keeping calibrator so we used U6 specifically in this study. Temperature and time set up as the kit instruction and each result were analyzed by Amplification and Melting peak.

Gene		Sequence	Length (bp)	Tm (°C)
VEGF	F	AAGGAGGAGGGCAGAATCAT	20	60/2
	R	ATCTGCATGGTATGTTGGA	20	
18s	F	GTAACCCGTTGAACCCATT	20	53/4
	R	CCATCCAATCGGTAGTAGCG	20	

Table 1. Characteristics of primers.

Cycles	Duration of cycles	Temperature
1	15 min	95°C
35-40	15-30 seconds	95°C
	60 seconds	55-60°C
1	Melting Analysis	55-95°C

Table 2. Real-time -PCR reaction temperature conditions.

Findings

Study population consisted of 30 healthy people and 30 patients diagnosed with OSCC which was adapted completely and there was no significant difference between groups therefore age variable didn't affect on study (P value=0.442).

Analysis of Studied Biomarkers

VEGF biomarker was positive in 24 out of 30 in patients (80%) and 3 out of 30 in controlled group (10%). Comparison between two groups by «two sample binomial» technique demonstrated a significant difference between them. (P value<0.001). Also, miR-494 biomarker was positive in 20 out of 30 in patients (67%) and 5 out of 30 in controlled group (17%). Comparison between two groups by «two sample binomial» tech-

nique demonstrated a significant difference between them. (P value<0.001).

Evaluating difference between biomarkers of two groups

The $\Delta\Delta$ Ct method was used. The value of $\Delta\Delta$ Ct for the miR-494 biomarker was calculated to be 1.81 and was found to be 1.40 for the VEGF-mRNA biomarker. Then the formula $2^{-\Delta\Delta Ct}$ was used. Thus, for miR-494, the number of primary biomarker copies in patients is by average 3.48 times more than healthy subjects. And for VEGF mRNA, the number of primary biomarker copies in patients is by average 2.64% more than healthy subjects.

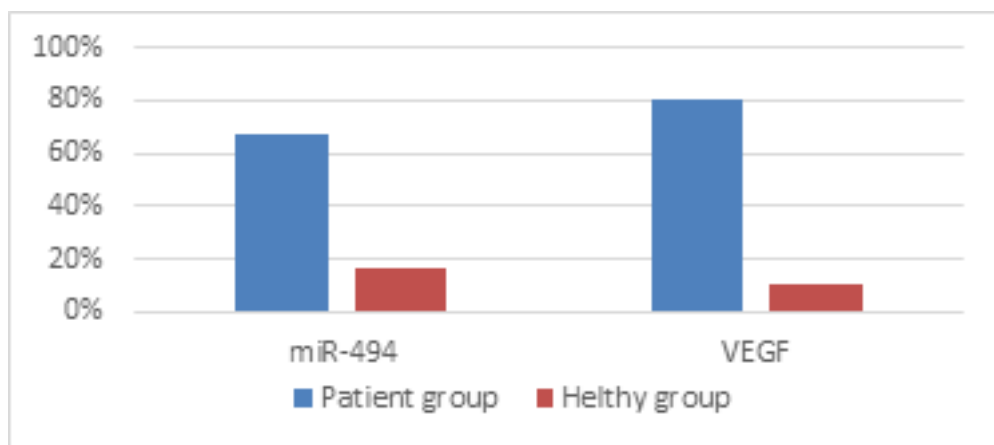


Figure 1. The rate of Positive VEGF-mRNA and miR-494 in the peripheral blood of OSCC patients and healthy subjects.

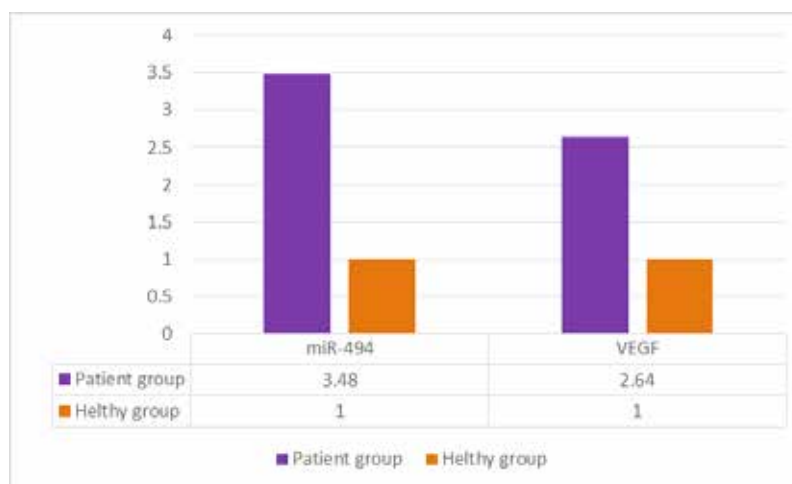


Figure 2. Difference in expression of miR-494 and VEGF genes in case and control

Discussion

Oral cancer is 8th most common cancer in men and 15th most common cancer in women [9]. Because of staging in diagnosis can seriously affect on survival rate, not only the prevention and intervention treatments can decrease the illness but also can lead into put a stop to progression of OSCC [10,11]. In this study with the aim of diagnosing the biomarkers which can demonstrate the difference between expression of RNA in normal and injured tissues, we tried to state the differences between VEGF mRNA and miR-494 in peripheral blood samples from patients and controlled group with Real-time PCR method.

VEGF biomarker was positive in 24 out of 30 in patients (80%) and 3 out of 30 in controlled group (10%). Also, miR-494 biomarker was positive in 20 out of 30 in patients (67%) and 5 out of 30 in controlled group (17%). Comparison between these two biomarkers stated a significant difference between groups. Nowadays understanding the accurate relationship between biomarkers and clinical symptoms and presenting a non-surgical diagnostic method in early steps of illness is a big challenge [12,13]. There are various kind of biomarkers such as protein biomarkers, DNA biomarkers and RNA biomarkers [14].

mi-RNA effectively found in body fluids like blood, saliva, urine and respiratory droplets. Wong et al specified the presence of plasma miR-184 in 80% of patients suffering from OSCC in comparison with presence of the same marker in 13% of controlled group [15]. In a research (2012) on 10 pairs of tissue samples from OSCC and non-cancerous revealed that expression of miR-21 is higher in cancerous tissues [16]. Same to the result of previous study stated the higher rate of miR-155. In another study 60 samples of OSCC and 20 samples of normal tissue evaluated and expression of VEGF-A mRNA specified more than 50% increase. [17] Also, the increase of expressing CEA was announced. Overall the results of current studies suggest that biomarkers like VEGF mRNA and miR-494 can play a major role in diagnosing OSCC. In addition, further studies recommended.

Conflict of Interest

There is no conflict of interest to declare.

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