Gene expression of one important microRNA in blood and tissue samples of oral squamous cell carcinoma

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is one of the most common oral malignancies, which accounts for 80-90% of malignant neoplasms of the oral cavity. MicroRNAs (miRNAs) are small RNA molecules that regulate post-transcriptional gene expression by targeting mRNAs.

Materials and Methods: In this case-control study, 40 patients with oral squamous cell carcinoma and 40 healthy individuals as control were studied. Blood samples were collected from both groups. Also, 30 cancer tissue samples and 30 healthy tissue samples were prepared and evaluated. RNA was extracted from collected peripheral blood and tissue samples and evaluated for the expression level of miR-494 via real-time PCR technique. P. value values<0.05 were considered statistically significant.

Results: The expression level of miR-494 in serum (peripheral blood) of patients with oral squamous cell carcinoma increased by 1.12 fold (P-value<0.001) compared with healthy individuals. Also, the expression level of miR-494 in samples of oral squamous cell carcinoma infected tissue showed a 1.28-fold increase compared to healthy tissue.

Conclusion: The results of this study indicate an increase in the expression level (up-regulation) of miR-494 in oral squamous cell carcinoma. This biomarker can be used in screening and early detection of oral squamous cell carcinoma.

Keywords: Oral squamous cell carcinoma; Microrna; Real-time pcr; MiR-494.

Introduction

Oral Squamous Cell Carcinoma (OSCC) is one of the most common oral malignancies, which accounts for 80-90% of malignant neoplasms of the oral cavity [1]. It is the 6th to 9th most prevalent malignancy depending on patients' geographical location (country) and sex across the world [1]. Southeast Asia has so far been detected as the place where the disease is most prevalent, which could be attributed mostly to smoking, drinking and HPV infections [2]. MiRNAs are a natural class of small 19-25 nucleotide non-coding RNAs, which are one of the most significant gene expression regulators in cells [3]. MiRNAs function through a full sequence with

3'UTR ends in cellular mRNAs and termination translation or degradation of the corresponding mRNA [4]. The functioning of some miRs has been known as oncogenic or tumor-suppressor, which is involved in various kinds of cancer [3,5,6]. miR-494 is located on 14q 32.31 [7]. A change in its expression level has been reported in various types of cancer. Its dysregulation can lead to an increase in cell proliferation, cell migration, and cell invasion and developing drug resistance by invading its target mRNAs [8]. Its deregulation has also been reported in Hepatocellular carcinoma [7], cervical cancer [9], gastric cancer [10], oral cancer [11] and renal cancer [12]. Expression profile of miR-494 is of great paradox about oral squamous cell carcinoma in humans and requires close scrutiny for clinical use.

Circulating miRNAs have been shown to be stable in body fluid such as urine and blood [13] and since there is a difference in miRNA expression level in cancerous patients compared to healthy individuals [14], the present study decided to target the changes in expression levels of miR-494 in the serum and tissues of OSCC patients compared to healthy samples as a diagnostic biomarker.

Materials and Methods

Selecting participants and sampling

In this research, the permission of the ethics committee IR.SBMU.NRITLD.REC.1398.046 was received. In this case-control study, 2 ml of peripheral blood was taken from 40 OSCC-infected individuals and 40 healthy persons. A total of 30 cancer tissue samples and 30 healthy ones were also collected by a specialist. RNA extraction was performed for each sample. RNA extraction and Real-time polymerase chain reaction were performed in accordance with the protocols stated in RNeasy Mini Kit (QIAGEN Cat. No. 75144). The extraction of total and small RNAs from the blood and tissue was done according the standards. The quality and concentration of the extracted RNA was evaluated using the nano-drop instrument in wave-lengths of 260 and 280 nanometers (nm).

Over the next phase, cDNA was built from the extracted RNA using Zist Royesh kit. RT-PCR was performed using Zist Royesh kit and Rotor-Gene Qiagen. For each sample, a housekeeping gene was named U6 was used as a calibrator to normalize miR-494 expression. According to the kit instructions, Real time PCR was adjusted. It included an initiation phase with a 15-minute cycle at 95°c, an elongation phase with 35-

40 phases each containing a 15-30 second sub-phase at 95°c for denaturation and a 60-second sub-phase at 55-60°c for annealing and extension. Finally, there is a melting analysis using a melting curve with a cycle at 55-95°c. The results were finally interpreted based on amplification and melting peak curves. The average expression level of miR-494 was calculated in relation to the average expression level of the housekeeping gene U6 snRNA using the $\Delta\Delta Ct$ method. Then the $2^{\Delta\Delta Ct}$ formula was used to determine the multiple expression of miR-494.

Statistical Analysis

The results were analyzed with SPSS version 20 and all the means and standard deviations were calculated. Paired t test was used to analyze the relation between the expression level of miR-494 and the clinical and pathological features in both healthy and affected individuals regarding $P \leq 0.05$.

Result

Both the control and affected groups were homogenized regarding age. The age range of the affected and the control group were respectively 25-66 and 24-66. Having crunched the age average using the t test in both groups, a mean of 43.78 and an SD of 8.04 was reached for the affected and a mean of 44.22 and an SD of 8.12 was achieved for the control group showing no significant relation (p-value 0.398 and SD: Standard Deviation). Therefore, the age factor cannot interfere with the analysis.

Two cDNA vials were made for each group and all the Real-time RT-PCR reactions were duplicated and interpreted using melting curve. Regarding the affected group, 70% of the positive cases related to the miR-494 were seen in the patients 'peripheral blood (28 out of 40 individuals), whereas 6 out of 40 cases of the healthy group showed an increase in miR-494 expression. Using the two-sample binomial test, a statistical comparison was made between the two groups regarding the increase in miR-494 biomarker, which proved a significant relation between the two groups (P-value<0.001). (Figure 1).

The biomarker index was also positive in 23 cancer tissues out of a total of 30 (76.66%), while the healthy samples showed only 5 positive cases out of 30 (16.66%), which means a significant relation between the two group (p<0.001). (Figure 2).

Calculating the difference in miR-494 expression in both groups:

With the Ct of each sample having been determined, the 2ΔΔCt formula was used to calculate the relative difference of miR-494 expression of the test group versus the control group. Finally, it was revealed that miR-494 expression was 1.12 times as much in affected people compared to healthy individuals. The miR-494 biomarker expression was also found to be 1.28 times bigger in tissue samples of patients with oral squamous cell carcinoma compared to healthy participants. (Figure 3).
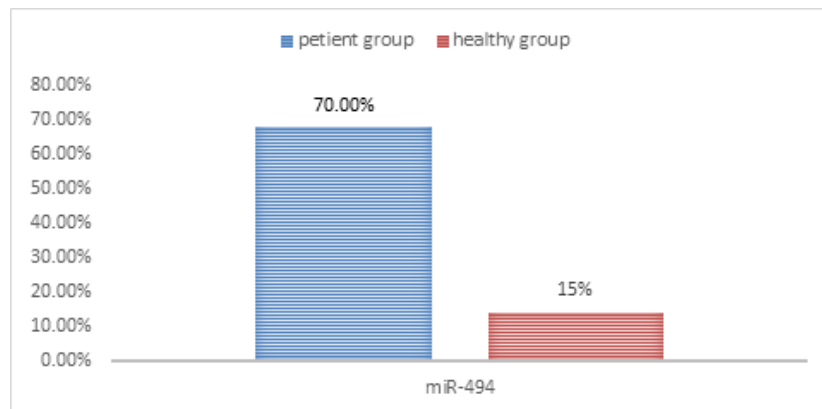

Figure 1. The percentage of positive miR494 biomarker results in peripheral blood of both healthy and affected individuals.
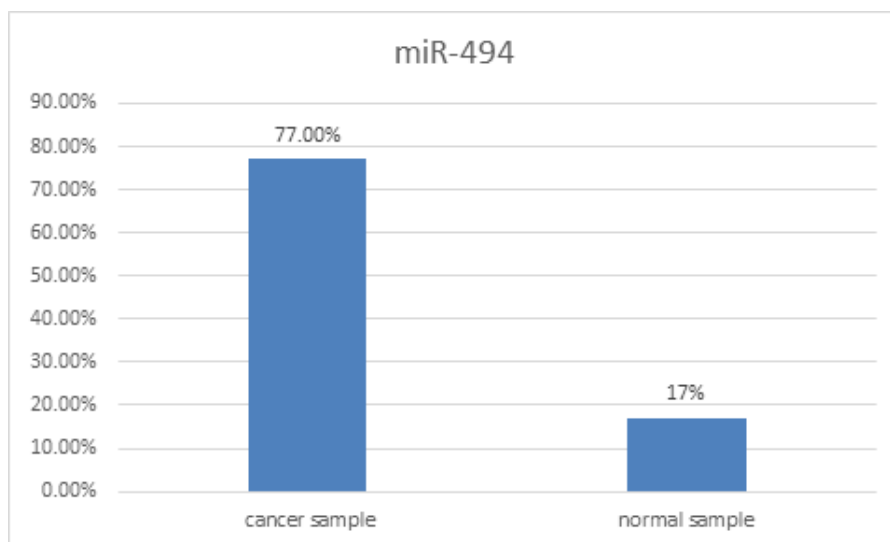

Figure 2. The percentage of positive miR494 biomarker results in cancer and healthy tissue samples.
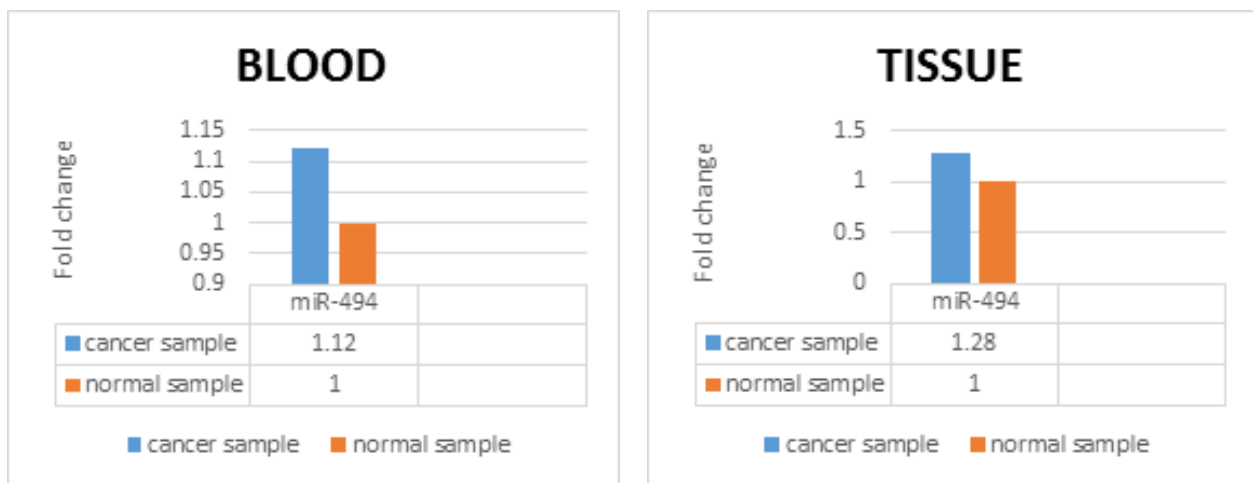
Figure 3. Expression differences in cancer and healthy groups.

Discussion

Changes in miR-494 expression are related to pathogenicity of most cancers and particularly oral squamous cell carcinoma [15]. miRNAs relation with various types of cancer is due to genetic changes, single-nucleotide polymorphisms and defections in miR-NAs biogenesis pathways resulting in immune system escape, apoptosis resistance, invasion, metastasis and sustainable angiogenesis [16].

An effective and non-invasive diagnostic method could be the analysis of abnormal expression patterns of miRs towards early diagnosis and awareness [17]. The present study observed a 1.12-fold increase (P value<0.001) in miR-494 in peripheral blood samples of the patients with oral squamous cell carcinoma compared to healthy ones and a 1.28-fold increase (P value<0.001) in miR-494 cancer-affected tissue samples in comparison with healthy tissues. The role played by miR-494 in various cancers, as well as changes in its expression, has been analyzed and observed in several research studies. It has been revealed that overexpression (up-regulation) of miR-494 in cancer tissues and NSCLC cell line acts as an onco-miR in Nsclc lung cancer development [18]. Also, in colon cancer, an increase in miR-494 expression results in permanent activation of Wnt/β-catenin signaling pathways, tumorgenesis and over-proliferation through deactivating APC and finally the development of colon cancer [19]. A rise in miR-494 levels in MDA-MB231 and MDA-MB-468 cell lines in breast cancer has been reported to promote proliferation, cell migration and metastasis power [20]. In addition to the above-mentioned, this increase has also been seen in other types of cancer like hepatocellular carcinoma (HCC) [7], which approves of our results about oral squamous cell carcinoma.

However, some studies have indicated that down-regulation of miR-494 was related to the development of gastric cancer where a decrease in the expression of miR-494 works as an anti-oncogene addressing c-myc and its down-regulation finally delaying the transition from G1 to S phase in cell cycle and interfering with the development of gastric cancer cells [10]. In a study, the expression level of miR-494 down-regulate and HOXA10 up-regulate resulting in faster cell proliferation in oral cancer cells [11]. Regarding chemotherapy resistant Acute Myeloid Leukemia (AML), miR-494 expression decreased (as a c-myc regulator) leading to an increase in c-myc expression and promote AML cell proliferation [21]. Moreover, down-regulation of miR-494, followed by an overexpression in SOCS6, has also been detected in cervical cancer cases [22]. In another study, a decreased pattern of miR-494 expression and a rise in SDC1 protein level was found in Pancreas cancer and pancreatitis [23]. Up-regulation of miR-494 leads to reduced survival of cell lines in renal tumors [12], which is in contrast with our results.

Conclusion

The results of this study show a relation between miR-494 expression and oral squamous cell carcinoma and can be used as a reliable biomarker and a noninvasive method of screening and early diagnosis of oral squamous cell carcinoma.

Conflict of Interest

There is no conflict of interest to declare.

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