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ORIGINAL ARTICLE

Salivary miRNA 18a-5p Expression Level in Oral Squamous Cell Carcinoma

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ABSTRACT

Aim: To determine the expression level of the salivary microRNA 18a-5p in Oral squamous cell carcinoma patients and healthy individuals
Methodology: This case-control study was conducted on 40 samples out of which 20 were OSCC patients and 20 were healthy control patients. Saliva was collected from patients with newly diagnosed OSCC and healthy controls after getting informed written consent. All samples were recruited from Abbasi Shaheed hospital and Ziauddin University Hospital, Karachi. We evaluated the miRNA 18a-5p expression level in the saliva by reverse transcriptase polymerase chain reaction (RT-PCR) after isolating RNA through TRIZOL method.
Results: microRNA 18a-5p expression level was significantly expressed and higher in OSCC samples compared with controls moreover in the OSCC samples with the progression of stages and grades, the expression increased suggesting it tumor-promoting role in OSCC.
Conclusion: Our study concludes that the microRNA18a-5p can be the potential non-invasive biomarker for early detection of OSCC.
Keywords: Oral squamous cell carcinoma, microRNA, salivary biomarker

INTRODUCTION

Oral Cancer is the most aggressive type of malignancy that causes early death due to its early metastatic ability[1]. In Pakistan, it is dominant cancer in males while it lies only behind breast cancer in. Research studies show that the incidence varies according to ethnicity, Age, lifestyle, culture, and socioeconomic background[1]. Poor prognosis and low survival rate of OSCC is due to its diagnosis in the later stages of diseases[2]. The gold standard for the diagnosis of oral cancer at this time is a tissue biopsy followed by histological analysis. However, it is painful, invasive, time-consuming and potentially dangerous because neoplastic cells from the main tumor site can disseminate into nearby normal tissues during biopsy[3]. To overcome these risks a reliable biomarker should be isolated through non-invasive approach thus increasing the survival rate and improving the diagnosis of cancer in early stages[4]. Saliva has become a possible liquid biopsy media for the detection of malignancies and other systemic illnesses[5,6]. Saliva constantly bathes oral tissues, making saliva liquid biopsy superior to tissue and blood biopsy for diagnosing oral diseases. Saliva liquid biopsy is also non-invasive, economical, practical, painless, and repeatable[7].

MicroRNAs have been examined in numerous cancers as significant biomarkers which play a significant role in the genesis of tumors, tumor invasion, and distant metastasis[8]. Small non-coding RNA molecules known as microRNAs, which have 18 to 22 nucleotides, regulate the expression of genes by attaching to the 3' region of the UTR[9]. Their function in numerous crucial sub-cellular biological processes, including cell differentiation, proliferation, and metabolism, has been widely studied[10]. The important physiological processes which are regulated by miRNAs can be deregulated by abnormal miRNA expression leading to the initiation neoplastic changes. Numerous human malignancies, including nasopharyngeal carcinoma, pancreatic cancer, gastric cancer, and prostate cancer, have been linked to abnormal expression of miRNA 18a-5p[11,12,13].

The purpose of our research was to determine the expression level of the salivary microRNA 18a-5p in Oral squamous cell carcinoma patients and healthy individuals. The results of this study may help in identifying the role of miR 18a-5p in early detection of OSCC and its progression through non-invasive technique. Therefore, it can be used as a potential therapeutic target in improving the survival rate of patients. By identifying genetic biomarkers for oral cancer, early detection can be achieved, potentially reducing the incidence of OSCC in this region. Ho

The miRNA 18a-5p has not been well researched in oral cancer therefore it is dire need of time to explore it as a prognostic, diagnostic and therapeutic agent.

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METHODOLOGY

This case-control study was commenced after getting an approval letter from the Ethical Review Board (ERC) Committee, (ERC CODE: 3580421MKPAT) Ziauddin university Karachi. Total 40 Patients, 20 biopsy-proven OSCC and 20 healthy controls were recruited from the dental OPD by consecutive sample technique. The written informed consent form was signed by all participants. Patients who had received chemotherapy, radiotherapy or having other cancer were excluded from the study.
Saliva Collection: Prior to saliva collection, all subjects abstained from smoking, drinking, and eating for at least one hour. After rinsing the mouth with sterile saline, unstimulated whole saliva (WS) samples were taken. To remove debris and cells, the obtained samples were centrifuged at 2,600 g for 15 min at 4°C.Saliva cell pellet was chosen for qPCR to evaluate the mirna18a-5p expression level following centrifugation.
RNA Extraction: RNA was extracted from saliva using the trizol Kit.(Kit 15596026)

Table 1: Primers used in the study for cDNA synthesis

Gene	Primer	Sequencing
	Stem loop	*5'TGATGCAGGTGTCGTGGAGTCGGCAA TTCTGCATCACTATCTGC'3*
miRNA 18a-5p	Forward primer	5' TAAGGTGCAT CTAGTGCAGA 3'
	Reverse primer	3'AACGGCTGAGGTGCTGTGAACTC5'
U6/RNA	Forward primer	5′-GCTTCGGCAGCACATATACTAAAAT-3'
	Reverse primer	5′-GTGCAGGGTCCGAGGT-3′

cDNA synthesis: Reverse transcription of miRNA was carried out using a Revert Aid first (catalogue# K162) in accordance with the manufacturer's instructions. Primers for miRNA-18a and U6 RNA were created by the primer 3 creation tools before being purchased from Penicon (Table 1).

RT-PCR: RT-PCR was carried out through temperatures of 95ºC, 60ºC, and 72ºC for denaturation, annealing and extension respectively for 40 cycles.

Statistical Analysis: All the data were analyzed on SPSS version 20. To compare the expression level between OSCC and controls, Kruskal Wallis and Post Hoc analysis were done. Results having a p-value of less than 0.05 were considered statistically significant.

RESULTS

Demographic parameters of participants: In the current study, we found that 22(55%) were females and 18(45%) were males. The Majority of the study participants (57%) had an age of more than 40 years. Most of them were Sindhi speaking and were smokers having the buccal mucosa as a common affected site by OSCC in our population as shown in table 2.

Comparison of the expression level of miRNA 18a-5p in Cases and Controls: The median and IQR of cases were found to be (0.9850 & 3.13) while in controls it was (4.725 & 4.57). MicroRNA level was significantly higher in smokers and Ghutka consumers as compared to other habits (P-Value 0.003). Though it showed insignificant statistical association, the expression level was higher in males of older age groups with p-value of (0.232) and (0.29) respectively as shown in Table 3.

Table 2: Demographic and clinicopathological characteristics of the study population

Study Variables	Frequency
Gender	
Male	(55%)
Female	(45%)
Age	
>40	(42%)
<40	(57%)
Grades	
Well Differentiated	(12.5%)
Moderately Differentiated	(27.5%)
Poorly Differentiated	(10%)
SITES	
Buccal Mucosa	(17.5%)
Tongue	(10%)
Retromolar Pad	(7.5%)
Palate	(10%)
Upper Lip	(5%)
Habits	
Smoking	18
Betel Nut	05
Ghutka	04
Ethnicity	
Punjabi	(12.5%)
Sindhi	(40%)
Urdu Speaking	(30%)
Balochi	(7.5%)
Kashmiri	(10%)

Table 3: Demographic features with the expression of microRNA

Variables	Frequency		ΔCT value		p-value
	Cases	Controls	Median	IQR	
Gender					
Male	07	15	1.08	9.5	0.232
Females	13	05	4.40	5.1	
Age					
<40	14	09	2.4	7.22	0.29
>40	06	11	4.73	9.15	
Habits					
Smoking	14	07	4.155	10.36	0.003
Ghutka	05	00	4.15	9.11	
Betel Nut	04	00	2.1	6.53	

Comparison of microRNA 18a-5p expression level with the clinic pathologic parameters: After comparing the expression of microRNA with clinic pathological parameters, the median; IQR of 1.11 (6.8) in stage I was observed while in stage II it was 0.9700 (2.41). Similarly, in comparison with histopathological grades, poorly differentiated cases showed the median; IQR of (4.66;8.48), in moderately (0.9200 ;5.56) and well-differentiated cases (1.80; 4.86). Whereas buccal mucosa was found to be most common site with a median; IQR of (1.03; 6.18) (Table 4).

Table 4: Clinic pathological parameters with the expression of microRNA 18a-5p

Variables	Frequency	ΔCT value		p-value
	Cases	Median	IQR	
Stages				
I	04	1.11	6.68	0.008
II	09	.9700	2.41	
III	05	.600	3.63	
IV	02	3.7	3.74	
Grades				
Well differentiated	05	1.80	4.86	0.001
Moderately	11	0.9200	5.56	
differentiated	04	4.666	8.48	
Poorly differentiated				
SITES				
Buccal Mucosa	07	1.03	(6.18)	0.004
Tongue	03	1.300	(0)	
Retromolar Pad	04	0.5300	(4.33)	
Palate	04	0.5500	(1.36)	
Upper Lip	02	4.56	(0)	
Habits				
Smoking	18	4.155	10.36	0.003
Ghutka	05	.0500	9.11	
Betel Nut	04	.975	6.53	

DISCUSSION

The discovery of novel, precise biomarkers may aid in the earlier detection and better prognosis of oral cancer patients. Saliva miRNAs have been thoroughly investigated as potential biological indicators in many other malignancies, including esophageal and pancreatic tumor. Saliva is an easily available medium to explore biomarkers especially in oral cancer because it is continuously bathing the oral cavity making it more authentic candidate for oral cancer diagnosis.

Our study revealed that level of miRNA-18a-5p expression in saliva was significantly higher in the saliva of OSCC patients as compared with healthy individuals. This study showed that miRNA18a-5pexpressionincreased significantly as tumors progressed. This upregulated expression may indicate a role form iRNA18a-5p in the pathogenesis of oral cancer.

Many other research studies on numerous tumors also aid our study. Up regulation of miRNA18a-5p expression level was noticed in lung cancer compared with normal lung tissue[14]. Similarly, up regulationofmiRNA18a-5 p showed positive effects on the growth of cells in renal cell carcinoma[15]. Another research showed that miRNA18a-5p augmented the spread nasopharyngeal cancer through suppression of SMG1 and activation of them TOR pathway[16]. These studies showed the tumor progressive role of miRNA18a-5p.

We also evaluated miRNA18a-5p expression in OSCC clinic pathological parameters and observed increased expression in stages III and IV, suggesting its role in the progression of OSCC. Similar findings were observed iinosteosarcoma,wheremiRNA18a-5p expression is significantly increased in tumor stages T3 and T4 compared to tumor stages T1 and T2[17]. These results augments the opinion that miRNA18a5p plays a significant role in cancer promotion.

However, few research articles point to its tumor-suppressive function in ovarian tumor, miRNA18a-5p suppressed tumor spread by targeting metal oproteinase-3and acted as a tumor suprressor[18]. Another study found that miRNA 18a-5p has a tumor inhibit or function in breast cancer by attaching with SNAIL[19].

This might be due to variable microenvironments in various tumors that influence tumor behavior. The controversial role of miRNA18a-5pin tumor genesis should be investigated soon.

CONCLUSION

This study provides evidence that expression of miRNA -18a-5p is significantly higher in patients of OSCC as compared to normal controls. Our study also shows that as oral cancer progresses the expression becomes high suggesting a potential role in the progression of oral cancer.

Limitations: Our study had small sample size. We didn't follow patients to assess the expression level after their treatment. In our research we only used qPCR to check the expression level. Specific microRNA isolations kits should be used to quantify the microRNA for better results. We could have compared the expression level of salivary microRNA with the serum.

Ethical Approval: IRB: Approved by Ethical Review committee, Ziauddin Medical University, ERC CODE:(3580421MKPAT)

Conflict of interest: Nil

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