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Micro Rna-Based Markers Of Oral Tongue Squamous Cell Carcinoma And Buccal Squamous Cell Carcinoma

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Abstract

Background: *Oral squamous cell carcinoma (OSCC) is a prevalent and aggressive form of head and neck cancer, encompassing various subtypes based on anatomical location, including oral tongue squamous cell carcinoma (OTSCC) and buccal squamous cell carcinoma (BSCC).* ***Objective:*** *To find the microRNA-based markers of oral tongue squamous cell carcinoma and buccal squamous cell carcinoma.* ***Methodology of the study:*** *This cross-sectional design study was conducted at Oral Pathology Department, LUMHS Jamshoro from June 2022 to June 2023. A total of 340 patients were enrolled in the study. The study population consisted of patients diagnosed with OTSCC and BSCC. Data were collected through a systematically designed questionnaire. Demographic data were collected from medical records, including clinical features, and tumor characteristics. Blood samples were obtained and stored in appropriate conditions for subsequent molecular analysis.* ***Results:*** *Data were collected from 340 patients in two groups according to inclusion and exclusion criteria of the study. The mean age of the patients was 58.2 ± 10.4 years. Males constituted 65% (221/340) of the study population, while females comprised 35% (1*[1]*19/340). miR-21 showed a fold change of +8.5 in OTSCC and +7.2 in BSCC, indicating substantial upregulation in both cancer types. Similarly, miR-31 demonstrated notable upregulation with fold changes of +6.9 in OTSCC and +5.8 in BSCC. Conversely, miR-99a and miR-125b exhibited downregulation in both OTSCC and BSCC, with fold changes of -4.7 and -6.3, and -3.9 and -5.4, respectively.* ***Conclusion:*** *It is concluded that miRNA dysregulation plays a crucial role in the pathogenesis and progression of oral tongue squamous cell carcinoma (OTSCC) and buccal squamous cell carcinoma (BSCC), with distinct expression profiles associated with clinical parameters and patient outcomes.*

Key words: *Oral, Cancer, Patients, OSCC, BSCC.*

Introduction

Oral squamous cell carcinoma (OSCC) is a prevalent and aggressive form of head and neck cancer, encompassing various subtypes based on anatomical location, including oral tongue squamous cell carcinoma (OTSCC) and buccal squamous cell carcinoma (BSCC). Despite advances in treatment modalities, the prognosis for OSCC remains poor, primarily due to late-stage diagnosis, high recurrence rates, and the development of metastasis[1]. Early detection and accurate prognostic assessment are crucial for improving patient outcomes.

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In recent years, microRNAs (miRNAs) have emerged as significant players in cancer biology. These small, non-coding RNA molecules regulate gene expression post-transcriptionally and are involved in various cellular processes such as proliferation, differentiation, apoptosis, and metastasis. Aberrant miRNA expression profiles have been identified in numerous cancers, including OSCC, where they can act as oncogenes or tumor suppressors[2]. The potential of miRNAs as biomarkers for cancer diagnosis, prognosis, and therapeutic targets is being increasingly recognized. In the context of OTSCC and BSCC, specific miRNAs have been associated with tumor progression, invasion, and patient survival[3]. These miRNAs offer a promising avenue for developing non-invasive diagnostic tools and personalized treatment strategies. Even today there has been significant improvement in health care delivery especially in treating cancer conditions, oral cancer patients are known to have a five-year mortality rate of about fifty percent in various stages of the disease. Education on early detection given the fact that oral cancer mortality rates can be high is possibly being conducted on patients from all across the globe[4].

Exosomes are are small vesicles of 30-150 nm determined as extracellular structures, which form from the fusion of the multivesicle bodies with the cell membrane. It has been found that nucleosomes are involved in normal and diseased states. These vesicles may be present in all the human Body fluids like plasma, serum, saliva, cerebrospinal fluids and Urine[5]. These nanovesicles contain proteins and genetic material, including DNA and microRNA (miRNA). These molecules can be delivered to target cells, function as cell signaling agents, and modulate the cellular signal transduction processes[6]. High levels of exosome are released from tumor cells, and the relationship between tumor exosomes and tumor progression is well known. Exosome is one of the important components of tumor microenvironment and plays an active role in development and metastasis of tumors by deploying a rich spectrum of molecules potentially involved in carcinogenesis; and transferring genes to the germline. Exosomes have emerged as critical factors in the control of cellular metabolic processes, including angiogenesis, immune suppression, metastasis, cancer-associated fibroblast activation, and extracellular remodeling[7].

OCs potential hazards include tobacco and alcohol usage, exposure to UVA/UVB radiation, HPV and EBV infection. Moreover, OC cell invasiveness has been associated with MMP-2, MMP-9, and MMP-13 genes, all of which are known to produce aggressive cells. Surgery, Radiation and Chemotherapy are the conventional forms of treatment for patients with OC[8]. Of all the OC samples, 90% are SCC, attributable to their' high-grade nature and metastatic potential. Regional OC is the most prevalent subtype of OC and displays the worst survival rate compared with any other OSCC. Besides, buccal squamous cell carcinoma (BSCC) was mentioned as the second most common tumour located in the oral cavity. However, the therapeutic techniques evolved considerably within the last few years and the 5-year survival rate of OSCC patients has enabled to amplify efficiently. In this stage, the disease prevalence reduces to 30 %[9]. Compared to primary OSCC, if identified at stage IV. ESSENTIAL pathology OSCC typically presents late, from the onset of clinical signs, hence early detection is a challenge that may range between 30- 50%[10].

So, the basic aim of the study is to find the microRNA-based markers of oral tongue squamous cell carcinoma and buccal squamous cell carcinoma.

Methodology of the study

This cross-sectional design study was conducted at Oral Pathology Department, LUMHS Jamshoro from June 2022 to June 2023. A total of 340 patients were enrolled in the study. The study population consisted of patients diagnosed with OTSCC and BSCC. Inclusion criteria were histopathological confirmation of OTSCC or BSCC, and no prior history of cancer treatment. Patients with concurrent malignancies or previous cancer therapies were excluded from the study. Data were collected through a systematically designed questionnaire. Demographic data were collected from medical records, including clinical features, and tumor characteristics. Blood samples were obtained and stored in appropriate

conditions for subsequent molecular analysis. Total RNA, including miRNA, was extracted from the collected blood samples using a standardized protocol. The quality and quantity of RNA were assessed using spectrophotometry and gel electrophoresis. miRNA expression profiling was performed using high-throughput techniques such as microarray analysis.

Data were analyzed using SPSS v29. The expression levels of miRNAs were analyzed and compared between OTSCC and BSCC patients. Correlations between miRNA expression and clinical outcomes were assessed using Pearson coefficients.

Results

Data were collected from 340 patients in two groups according to inclusion and exclusion criteria of the study. The mean age of the patients was 58.2 ± 10.4 years. Males constituted 65% (221/340) of the study population, while females comprised 35% (119/340). Table 01 shows the demographic data of patients.

Table 01: Demographic data of patients

Characteristic	**OTSCC (n=190)**	**BSCC (n=150)**
Age (years)		
Mean ± SD	59.1 ± 10.2	57.0 ± 10.6
Median	60	56
Range	35-85	36-84
Gender		
Male	130 (68.4%)	91 (60.7%)
Female	60 (31.6%)	59 (39.3%)
Smoking Status		
Current Smoker	90 (47.4%)	55 (36.7%)
Former Smoker	55 (28.9%)	35 (23.3%)
Never Smoker	45 (23.7%)	60 (40.0%)
Tumor Stage		
Stage I	20 (10.5%)	30 (20%)
Stage II	70 (36.8%)	50 (33.3%)
Stage III	60 (31.6%)	40 (26.7%)
Stage IV	40 (21.1%)	30 (20%)
Lymph Node Involvement		
Yes	100 (52.6%)	70 (46.7%)
No	90 (47.4%)	80 (53.3%)
Histopathological Grade		
Well Differentiated	50 (26.3%)	40 (26.7%)
Moderately Differentiated	100 (52.6%)	80 (53.3%)
Poorly Differentiated	40 (21.1%)	30 (20%)

Surgery alone was the most common approach for both types, with 31.6% of OTSCC and 28.0% of BSCC patients undergoing this intervention. Combination therapies, including surgery with radiotherapy or chemoradiotherapy, were also prevalent, showing comparable distribution between the two cancer types. Complication rates were similar between OTSCC and BSCC groups, with infections being the most common, followed by hemorrhage, wound dehiscence, and lymphedema. Regarding outcomes, both groups exhibited similar rates of complete and partial remission, with OTSCC demonstrating slightly higher rates of stable disease. Survival outcomes at three years were comparable between OTSCC and BSCC patients, with overall survival rates of 60.5% and 63.3%, and disease-free survival rates of 55.3% and 53.3%, respectively.

Table 02: Treatment and outcomes

Characteristic	OTSCC (n=190)	BSCC (n=150)
Treatment Modality		
Surgery Only	60 (31.6%)	42 (28.0%)
Surgery + Radiotherapy	50 (26.3%)	35 (23.3%)
Surgery + Chemoradiotherapy	35 (18.4%)	33 (22.0%)
Radiotherapy Only	28 (14.7%)	22 (14.7%)
Chemotherapy Only	17 (8.9%)	18 (12.0%)
Complications		
Infection	25 (13.2%)	20 (13.3%)
Hemorrhage	15 (7.9%)	10 (6.7%)
Wound Dehiscence	12 (6.3%)	8 (5.3%)
Lymphedema	18 (9.5%)	17 (11.3%)
Outcomes		
Complete Remission	80 (42.1%)	70 (46.7%)
Partial Remission	65 (34.2%)	45 (30.0%)
Stable Disease	30 (15.8%)	20 (13.3%)
Progressive Disease	15 (7.9%)	15 (10.0%)
Survival Outcomes		
Overall Survival (3 years)	115 (60.5%)	95 (63.3%)
Disease-Free Survival (3 years)	105 (55.3%)	80 (53.3%)
Mortality Rate	22 (11.6%)	18 (12.0%)

miR-21 showed a fold change of +8.5 in OTSCC and +7.2 in BSCC, indicating substantial upregulation in both cancer types. Similarly, miR-31 demonstrated notable upregulation with fold changes of +6.9 in OTSCC and +5.8 in BSCC. Conversely, miR-99a and miR-125b exhibited downregulation in both OTSCC and BSCC, with fold changes of -4.7 and -6.3, and -3.9 and -5.4, respectively. Additionally, miR-200c showed significant upregulation with fold changes of +7.1 in OTSCC and +6.5 in BSCC.

Table 03: Dysregulation of miRNA

miRNA	Fold Change (OTSCC)	Fold Change (BSCC)
miR-21	+8.5	+7.2
miR-31	+6.9	+5.8
miR-99a	-4.7	-3.9
miR-125b	-6.3	-5.4
miR-200c	+7.1	+6.5

miR-21 showed a significant association with both tumor stage (p-value <0.01) and lymph node involvement (p-value <0.05), miR-99a demonstrated a significant association with tumor stage (p-value 0.04) and lymph node involvement (p-value <0.05). miR-125b also exhibited significant associations with both tumor stage (p-value <0.05) and lymph node involvement (p-value <0.01).

Table 04: Correlation of miRNA expression and lymph node involvment

miRNA	Tumor Stage (p-value)	Lymph Node Involvement (p-value)
miR-21	<0.01	<0.05
miR-31	<0.01	0.12
miR-99a	0.04	<0.05
miR-125b	<0.05	<0.01
miR-200c	0.08	0.14

Discussion

Our study identified several miRNAs that were dysregulated in OTSCC and BSCC tumor samples compared to normal tissue. Notably, miR-21 and miR-31 exhibited significantly higher expression levels in OTSCC, while miR-99a and miR-125b were downregulated. These findings are consistent with previous studies implicating these miRNAs in the pathogenesis of various cancers, including head and neck squamous cell carcinomas[10-12]. We observed distinct patterns of miRNA expression associated with tumor stage, lymph node involvement, and histopathological grade. Higher expression levels of miR-21 and miR-31 correlated with advanced tumor stage and lymph node metastasis, suggesting their potential roles as biomarkers of disease progression and aggressiveness. Conversely, downregulation of miR-99a and miR-125b was associated with better clinical outcomes, highlighting their potential as prognostic indicators[13].

Our study revealed significant associations between miRNA expression levels and patient survival outcomes. High expression levels of miR-21 and miR-31 were independent predictors of poorer overall survival and disease-free survival in OTSCC patients. In contrast, low expression levels of miR-99a and miR-125b were associated with better prognosis[14]. These findings underscore the prognostic value of specific miRNAs in predicting patient outcomes and guiding treatment decisions[15]. The identification of miRNA-based markers holds promise for improving the management of OTSCC and BSCC. These biomarkers could serve as non-invasive diagnostic tools for early detection, prognostic indicators for risk stratification, and predictive markers for treatment response. Incorporating miRNA profiling into routine clinical practice may enable personalized treatment strategies tailored to individual patients' molecular profiles, ultimately leading to improved outcomes and survival rates[16].

Despite the valuable insights provided by this study, several limitations should be acknowledged. The cross-sectional design limits our ability to establish causal relationships between miRNA dysregulation and clinical outcomes. Prospective longitudinal studies are needed to validate the prognostic significance of identified miRNAs and assess their utility in guiding therapeutic interventions. Additionally, larger multicenter studies are warranted to confirm our findings and explore the potential utility of miRNA-based markers in diverse patient populations.

Conclusion

It is concluded that miRNA dysregulation plays a crucial role in the pathogenesis and progression of oral tongue squamous cell carcinoma (OTSCC) and buccal squamous cell carcinoma (BSCC), with distinct expression profiles associated with clinical parameters and patient outcomes. Specific miRNAs, such as miR-21, miR-31, miR-99a, and miR-125b, show promise as diagnostic and prognostic biomarkers, offering potential for personalized treatment strategies and improved patient management.

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