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MICRORNAS AS PREDICTORS OF ORAL CANCER

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ABSTRACT

Background: Oral cancer is defined as uncontrollable growth of cells seen in the oral cavity. It appears as a growth or sore in the mouth that does not cure. Oral cancer can affect the mouth, palate, sinuses, and pharynx. Squamous cell carcinoma is the most common type of oral cancer. MicroRNAs (miRNAs) have been shown to be involved in a wide range of biological processes. A significant role for miRNA in cancers is to target gene expression level through their respective signaling pathways. The current study analyzed gene expression profiles of a few microRNAs such as miR-21, miR-137, miR-200c and miR-205 in pathogenesis of oral squamous cell carcinoma(OSCC). **Methods:** Biopsy samples were collected from 50 patients recently diagnosed with oral cancer along with corresponding non malignant portions, with the approval of Institutional Ethics Committee. Quantitative real time PCR (qRT PCR) was used to quantify the levels of miRNAs expression. The association between miRNA expression levels and clinico-pathological parameters was analyzed using MedCalc software. **Findings and interpretation**: This study found miRNA-21 was up-regulated (in 54% cases) whereas miR-137 (48%), miR-200c (46%), miR-205 (42% of cases) was down-regulated in OSCC. Among these four microRNAs, only miR-137 was differently associated with the risk of OSCC, whereas all other microRNAs were found to be directly associated with OSCC. This

study demonstrated an association of miR-21, miR-137, miR-200c and miR-205 in OSCC with altered gene expression, suggesting that in spite of varying expressions in miRNAs' its role in the development of oral cancer was very much evident. It is suggested that these miRNAs could probably serve as biomarkers for oral cancer management.

KEYWORDS: Oral cancer, microRNAs, Demographics, biomarkers, economic status, miR-21, miR-137, miR-200c, miR-205, Gene expression, qRTPCR, reverse transcription.

INTRODUCTION

Recent times have witnessed the progress in early detection of cancers, and how it can be treated with full recovery. With new technologies coming in at the molecular level it is seen that we have been able to develop new biomolecules from the human tumor materials a species of new RNAs which are called microRNAs (miRNAs), which is another new diagnostic tool or biomarker for cancer detection. The molecular regulators like messenger RNAs, miRNAs and proteins plays a vital role in cancer metastasis. Among which miRNAs are thought to be involved in the post-transcriptional modulation of target mRNAs. It is understood that the alterations in miRNA expression are associated with carcinogenesis due to its involvement in various biological pathways. miRNAs can modulate the expression of genes by binding to the 3' UTR region of mRNAs resulting in translational inhibition or cleavage.[1] Each miRNA targets hundreds of transcripts thus placing miRNAs as largest family of gene regulators.[2] Conversely epigenetic mechanisms such as DNA methylation, miRNA activity, and histone acetylation, cause gene silencing, which promote the development of cancers of varying molecular subtypes.[3]

According to the statistics, in 2012 the incidence of oral cancer in India was 53,842 in males and 23,161 in females, with an age standardized incidence rate of 12.6 per 100,000 people.[4] The nationwide incidence could be as high as 20 per 100,000 population, with variations depending on the study designs, sampling, case ascertainment, gender and location. Variations in age-specific incidence rates also increased with age, dropping at the age of 70, a trend that was consistent in studies from 1990 to 2012.[5]

Oral squamous cell carcinoma (OSCC) has become the sixth most commonly occurring neoplasm globally,[5] which accounts for 50-70% of cancer deaths. Ninety percent of the oral cancers occur in the oral cavity.[6] Significant association has been reported between OSCC and the risks such as alcohol consumption, tobacco chewing and smoking.[7] Therefore,

consistent molecular markers that can provide early and more precise OSCC diagnosis, prognosis and best suitable treatment are needed. The molecular regulators like messenger RNAs, miRNAs and proteins plays a vital role in cancer metastasis, among which miRNAs are thought to be involved in the post-transcriptional regulation of target mRNAs.

Each miRNA targets hundreds of transcripts thus placing miRNAs as largest family of gene regulators.[2] It is believed that the alterations in miRNA expression are associated with carcinogenesis due to the involvement of miRNAs in various biological pathways. miRNAs can regulate the expression of genes by binding to the 3' UTR region of mRNAs resulting in translational inhibition or cleavage.[1] miRNAs processed within the nucleus as long primary transcripts are subsequently cleaved to produce pre-miRNAs by an RNase III enzyme, pre-miRNAs are exported to the cytoplasm, where they are processed by the Dicer to form mature miRNAs (figure 1). Expression analysis of miRNA may be used to distinguish several cancer types and subtypes. These miRNA expression results provide remarkable diagnostic implications.[8] The present study is the first to comprehensively explore the role of miR21, miR137, miR200c and miR205 in patients diagnosed with OSCC in South India.

miR-21 controls apoptosis, cell proliferation and transition of epithelial tissue to mesenchymal tissue during the tumorigenesis and was found to be up regulated in several cancers.[9,13] It was verified that miRNA may influence tumor progression by the down-regulation of various targeted tumor suppressor genes namely phosphatase tensin homolog (PTEN) located on chromosome 10. It was reported that miR-21 was often up regulated in various solid tumors like breast, pancreas, lung, gastric, colon and esophageal cancers. miR-21 plays an important role in carcinogenesis by interfering with the oncogene function.[9] Circulating miR-21 was reported as a potential biomarker in hepatocellular carcinoma, glioma and sarcoma considering it as a vital parameter for early diagnosis. miR-21 is an important molecular regulator responsible for carcinogenesis,[14] proliferation[15] and anti-apoptosis.[16] Various studies defend the hypothesis that miR-21 is being overexpressed in different tumors and one of the most relevant oncogene-like factor.[17]

miR-137 located on human chromosome 1p22 was shown to act as tumor suppressor in various cancers which include squamous cell carcinoma, colorectal cancer and melanoma through cell cycle control.[18] Cyclin dependent kinase 6 (CDK 6) plays a vital role in cell cycle regulation and enable the cells to pass through the G1 check point of the cell cycle. CDK6 overexpression may results in elevated progression via G1/S phase of cell cycle

leading to elevated proliferation and reduced DNA repair capacity. CDK4 and CDK6 are the main targets of miR-137 in the regulation of cell cycle during G1 phase. CDK6 over expression was reported in various other soft and solid tissue tumors such as glioblastoma, lung adenocarcinoma and medulloblastoma.

miR-200c family influences distant metastatic biology by down regulating transcriptional programme which inhibits angiogenesis by different pathways by targeting interleukin 8.[19] Studies have reported that the significant reduction in metastasis, angiogenesis and induced vascular normalization have been observed with the release of miR-200c into the tumor endothelial cells.

miR-205 is known to work as a therapeutic agent[20] for its well known blocking angiogenesis function. miR-205 is also believed to have the ability to maintain and play a prominent role in biogenesis. It is considered as a tumor suppressor miRNA, due to its down-regulation in several cancers.[21] The tissue remodeling by the epithelial to mesenchymal transition (EMT) during embryonic development is considered as an initiation step in tumor metastasis. miR-200c also has a different capability to inhibit TGF-β induced EMT. The repression of E-cadherin transcriptional repressors namely ZEB1 and ZEB2 factors are associated with EMT as well as tumor metastasis are regulated by combination of miR-200c and miR-205. In the process of EMT induction, the miRNA inhibition is an essential process, which also requires the upregulation of ZEB1 and ZEB2. The abnormal expression of these miRNAs with their involvement in regulation of EMT represent their prominent role in tumor progression.

miRNA and cancer related studies reveal that miR-21, miR-137, miR-200c and miR-205 can functions as regulatory molecules in post-transcriptional gene silencing for the phenomenal characteristics by regulating cellular proliferation to apoptosis (figure 2), which have great impacts on the malignant transformation. The expression analysis of miRNA was significantly associated with cancers. Hence, the objective of this study was to analyse the expression levels of these miRNAs in patients diagnosed with OSCC and correlate these studies with the clinical and pathological features of the study population.

Economic burden

Despite advances in OSCC treatment strategies, patients with advanced stages of disease are still suffering from cosmetic and functional morbidity. Unlike other malignancies, the OSCC is usually diagnosed at advanced stages, which lead to very poor survival rate. Oral cancer is

often diagnosed when it has metastasized to another location, making prognosis difficult and patients are at a higher risk of developing secondary tumours. Besides the metastasis, at these later stages, the primary tumour has had time to invade deeper into local structures of the oral cavity or its place of origin, hence its discovery in late stages becomes almost impossible for appropriate treatment. In addition, in certain locations, surgery is impossible and patients are at 20 times higher risk of developing a secondary tumors. This heightened risk factor can last for 5 to 10 years or even less in many delicate areas of the nose or the throat or the oral cavity, after the first occurrence. Every year th United States spends an estimated $3.2 billion on treatment of head and neck cancers.[22] Hence, in developing countries one can imagine the economic burden. Every year we find growing number of cancer cases, although our knowledge on prevention and treatment has increased through research and clinical trials. Incidentally, due to personal habits, oral cancer is amongst the most prevalent cancers worldwide and its incidence appears to be higher in males than in females. The reason could be due to the use of tobacco and its products and consumption of alcohol. Historically a higher death rate is associated with this cancer due to its late diagnosis.

MATERIALS AND METHODS

Tissue Samples, Ethics Statement and Consent form

Fifty tumors and corresponding non-tumor tissues from adjacent regions were obtained from patients diagnosed with OSCC from MNJ Cancer Hospital, Hyderabad, between 2013 and 2014. Tumor and non-tumor tissue samples were verified as tumor and non-tumor by histopathological evaluation. The study was approved by the Institutional Ethics Committee and informed consent was obtained from all patients. A group of candidate miRNAs were chosen, and the expression was analyzed by qRT-PCR.

RNA extraction

The total RNA was isolated from the biopsy samples obtained from OSCC patients, by using “mirVana” miRNA Isolation Kit (Ambion). Its integrity was verified by 1.5% agarose gel plus ethidium bromide and ratio of optical density (OD) at 260 nm and 280 nm was also measured. RNA was quantified with the help of Nano Drop ND-1000 Spectrophotometer (Thermo Scientific). Single-stranded cDNA was synthesized from 300 ng of RNA in each sample.

Reverse transcription

Reverse transcription was carried out in a personal Master Cycler (Bio-Rad CFX 96), by using 1µg of total RNA in the presence of a Random Hexamer (50ng/µL) and a reverse transcriptase (50 U/µL) in total volume of 20µlL, including 10x TaqMan RT buffer, $MgCl_2$ solution (25 mM), dNTPs mixture (10 mM), RNAse inhibitor (20 U/µl) and nuclease-free water. The reaction mixture was incubated for 10 minutes at 25°C and 60 minutes at 42°C, heated for 5 minutes to 95°C and then at 4°C for a minimum of 2 minutes. The resulting cDNA was stored at –20° C till further use.

SYBR – Green Quantitative Reverse Transcription-PCR (qRT-PCR)

The miR-21, miR-137, miR-200c and miR-205 levels were quantified using quantitative reverse transcription-PCR (qRT-PCR) using SYBR-Green. Quantification of expression of miR-21, miR-137, miR-200c and miR-205 genes were performed using Bio-Rad CFx96. The relative quantification was done, where the gene expression was compared in each tumor with the gene expression in the non tumor group. In assessing the relative gene expression levels, quantitative Real time - polymerase chain reaction (qRT-PCR) was carried out with 1 µL of cDNA, 12.5 µL SYBR Green, and with specific primers (table 1) which were synthesized at Bioserve Biotechnologies Ltd. (Hyderabad, India).

Table 1: Oligonucleotide primer sequence of miR-21, miR-137, miR-200c and miR-205, GADPH used for qRT-PCR.

S. No.	Gene	Forward Primer 5'	Reverse Primer 5'	Annealing Temp
1.	miR-21	TAGCTTATCAGACTG ATG TTGA	AACGCTTCACGAATTTGCGT	54
2.	miR-137	CTTTCCGGTGGAACC AGTG	GCACAGCTTTGGATCCTTCT	54
3.	miR-200c	TAATACTGCCGGGTA ATGATGG	TCGTATCCAGTGCAGGGTC	57
4.	miR-205	CTTGTCCTTCATTCCA CCGGA	TGCCGCCTGAACTTCACTCC	58
5.	GAPDH	TGCACCACCAACTGC TTAG	AGTAGAGGCAGGGATGATGAT GTTC	

RT-PCR program used had: 5 min of initial denaturation and enzyme activation at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 60°C for annealing and 40 s at 72°C for elongation. Amplification and melting curve analysis were carried out to verify the specific product according to its specific melting temperature (Tm). Each reaction was performed in

triplicate. miRNA expression was defined based on the threshold cycle (Ct), and relative expression levels were calculated as 2-[(Ct of miRNA) – (Ct of reference gene)] after normalization with reference to expression of U6. The results were analyzed by the melting curve analysis software (figure 3). Gene expression values were expressed as Ct, Ct being the point at which the fluorescence rises significantly above baseline or background fluorescence, and comparing the Ct of the genes in tumors with the Ct of the genes in controls.

Statistical Analysis Statistical analyses for the RT-PCR results were performed on ΔCT data. p values $\leq$0.05 were considered as statistically significant. MedCalc software for Windows (version 7.4.1.0; Mariakerke, Belgium) was also used for correlating with clinico-pathological parameters.

RESULTS

Patients' clinical data

A total number of 50 patients diagnosed with OSCC were included in this study. The demographic details of these patients' and clinical characteristics are mentioned in Table 2. The age range of OSCC male patients was between 26–74 years and female patients were in the age group between 21-63 years. The highest percentage of OSCC patients were identified between 46–65 years. Regarding the primary tumor site, there was a neat predominance on the BM (buccal mucosa) totaling 24 patients (48%), followed by the tongue totaling 17 patients (34%), then the mandible and oral cavity at 12%, and 9%. The stage of a cancer is a descriptor of how much the cancer has spread (figures 4a–d). The stage often takes into account the size of a tumor. Five (10%) cases were stage-I, 11 (22%) cases were stage-II, 24 (48%) cases were stage-III and 10 (20%) cases were stage- IV. Among the cases, 16% were alcoholics while 32% were smoker's and 32% were chewers (table 2).

Table 2: Clinico- pathological characteristics of patients.

Clinical Characteristics	n = 50
Gender	
Males	33(66%)
Females	17 (34%)
Age Distribution	
26-45 years	10(20%)
46-65years	31(62%)
66 years and above	9 (18%)
Site of Diagnosis	
Tongue	17(34%)
Buccal mucosa (BM)	24(48%)
Mandible	6(12%)
Oral Cavity	3(9%)
Staging	
Stage 1	5(10%)
Stage 2	11(22%)
Stage 3	24(48%)
Stage 4	10(20%)
Habitual Risk	
Alcoholics	8(16%)
Smokers	16(32%)
Chewing	26 (52%)

Expression of miR-21, miR-137, miR-200c and miR-205 in OSCC Patients

To define the role of these miRNAs in OSCC, the expression of these four miRNAs in 50 OSCC tumors and corresponding non tumor tissues were investigated. It was identified that miRNA-21 was up-regulated in OSCC compared with normal samples. Other three miRNAs miR-137, miR-200c, miR-205 were found to be down-regulated. Over expression of miR-21 was found in 54% (27 of 50) samples; there was significant difference in miR-21 expression between tumoral tissues and non tumoral tissues (p=0·0001). miR-137 and miR-200c was down regulated in 24 (48%) and 23 (46%) samples. There was no significant difference in miR-137 between OSCC tumor and non tumoral tissues (p=0·104), whereas miR-200c was significantly associated with OSCC (p=0·002) In this study it was also found that miR-205 was down regulated in 42% (21 of 50 samples) miR-205 is significantly associated with OSCC (p=0·001). (table 3).

Table 3: Analysis of miR-21, miR-137, miR-200 & miR-205 expression in OSCC tumor and non tumor tissues.

Gene	Expression Status	Tumor (n=50)	Non tumor (n=50)	P- Value
miR-21	Altered expression	27(54%)	7(14%)	0.0001
miR-137	Altered expression	24(48%)	16(32%)	0.104
miR-200	Altered expression	23(46%)	0(0)	0.002
miR-205	Altered expression	21(42%)	6(12%)	0.001

Correlation of miR-21, miR-137, miR-200c and miR-205 status with Clinico-pathological characteristics of OSCC patients

We investigated associations between miR-21, miR137, miR200c and miR-205 and the clinicopathologic features of the OSCC patients. There was no significant correlation correlation to demographic parameter, but were associated with tumorogenesis between miR21 and miR205 with clinicopathological features. miR-137 and miR-200c expression had significant difference in the clinical stage III of OSCC (p=0·001 and p=0·04; figure-1). miR-137 also showed significant difference in clinical stage II and buccal mucosa site of OSCC (p=0·007 and p=0·05; figure 2), but there was no significant difference in age, gender and habitual risk

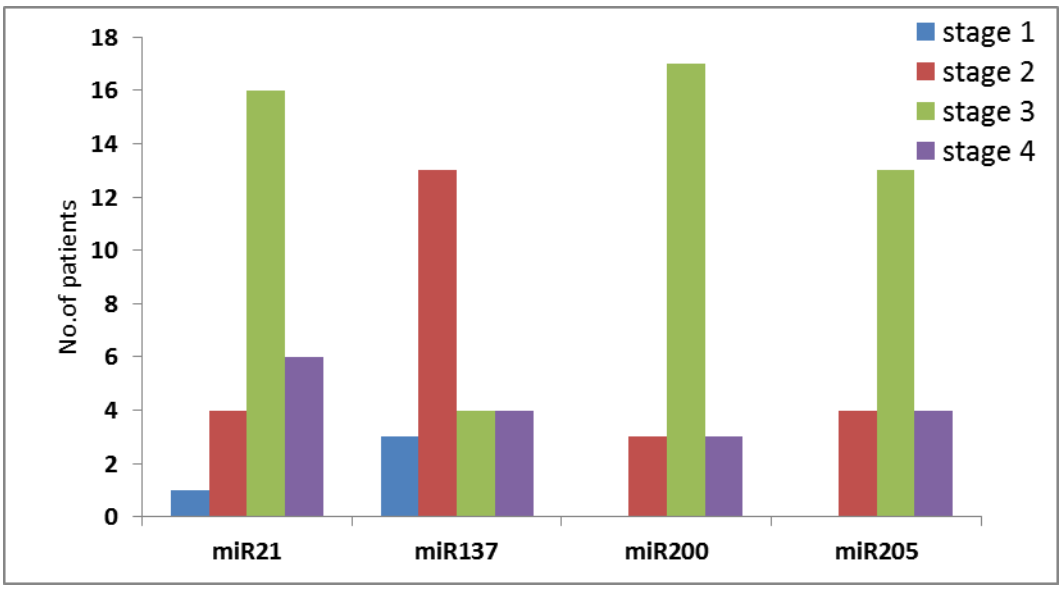

Figure 1: Altered expression of miRNAs according to the clinical grading.

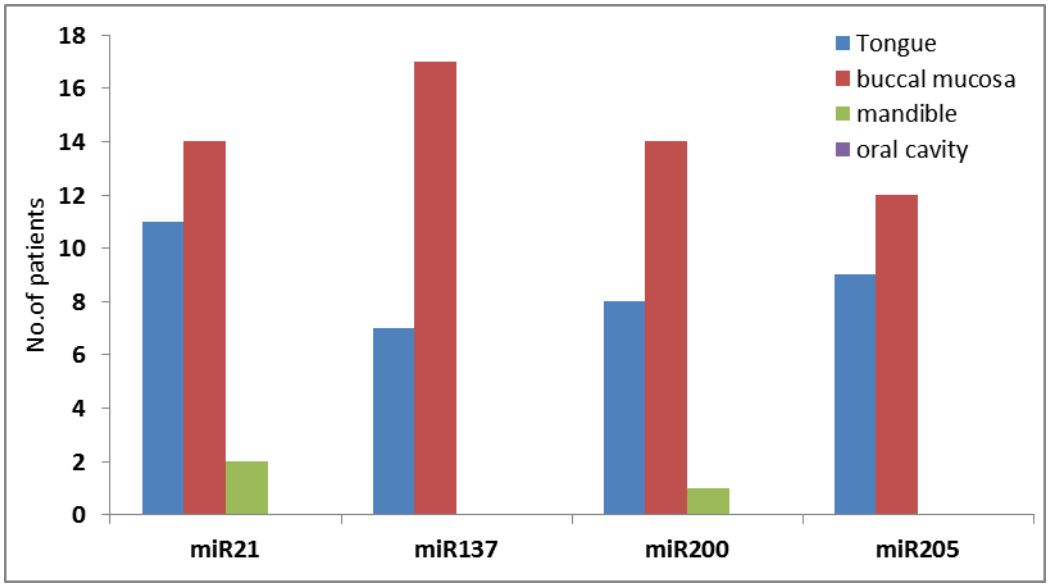

Figure 2: Altered expression of miRNAs according to their location of occurrence.

DISCUSSION

This study of miRNAs provides new insights into OSCC diagnosis. It was reported that single miRNA regulates simultaneously multiple target genes, hence it is possible to assume that miRNAs may serve as efficient regulators of tumor-related genes. Numerous studies have mentioned the expression of miRNAs in tumors and have revealed that miRNAs are frequently down regulated in tumor tissues compared with nontumor tissues.[23,24] Further, miRNAs can function as either oncogenes or tumor suppressors, based on the genes that they regulate. The changes in expression of miRNAs was observed in our earlier studies in OSCC.[25] This study analyzed the associations between miR-21, miR-137, miR-200c, miR-205 in OSCC and its clinicopathological correlations.

miR-21 was found to be up-regulated in a majority of cancers, including breast, lung, pancreatic and prostate cancers. Few investigations identified elevated expression of miR-21 in hepatocellular carcinomas,[26] gastric cancer,[27] ovarian cancer,[28,29] cervical carcinoma,[30] multiple head and neck cancer cell lines,[31] papillary thyroid carcinoma[32] and other neoplasias. miR-21 is highly up-regulated in leukemia patients with up to 10-fold expression reported in patients with chronic lymphocytic leukemia (CLL).[33] It was also over expressed in aggressive diffuse large B-cell lymphoma (DLBCL), follicular center lymphoma patients[34] and acute myeloid leukemia (AML).[35] Navvarro and colleagues documented the over expression of miR-21 in Hodgkin lymphoma lymph nodes and human Hodgkin lymphoma cell lines.[36] In general, miR-21 expressions are also high in numerous cancers, and sometimes accounts for up to 15–25% of total cellular miRNA.[37] Therefore, very high

expression of miR-21 may be the feature of malignant cells. According to the reports, miR-21 was vigorously upregulated in Epstein–Barr virus-infected human B lymphocytes[38] and heap-dna virus-associated hepatocellular carcinoma,[39] proposing the possibility of miR-21 involvement in OSCC progression.

PTEN is an identified target of miR-21 with increased expression of miR-21 resulting in reduction of PTEN expression. Our earlier studies on PTEN clarifies that its inactivation causes accumulation of phosphatidylinositol 3,4,5-triphosphate (PIP3) and increased the activity of serine/threonine protein kinase PDK-1 and AKT which promotes cell cycle progression, proliferation and inhibit apoptosis.[40] Clinically, over expression of miR-21 is associated with a poorer prognosis in carcinoma of tongue and other tumors such as breast tumors, head and neck tumors[41] and pancreatic cancer.[23] Hsu et al.[41] reported that miR-21 level was significantly reduced in post-operative samples from head and neck squamous cell carcinoma (HNSCC) patients as compared to the preoperative samples. These findings suggest that miR-21 may serve as a novel prognostic and diagnostic biomarker in patients with HNSCC.

miR-137, a short non-coding RNA molecule, regulates the expression of multiple genes by diverse mechanisms and acts as tumor suppressor in different cancers like colorectal cancer, melanoma and squamous cell carcinoma via cell cycle control. The post-transcriptional process of miR-137 varies by alteration in 15-bp variable nucleotide tandem repeat in primary miRNA transcript, leading to change in folding of secondary structure of miR-137. This change results in aberrant processing of miR-137, and results in downregulation of miR-137 in several malignant cell lines. The target genes of miR-137 are documented and presumed to play significant roles in cell cycle signaling. Cancer-initiating events like oncogenic Ras activation leading to the induction of cellular senescence, is a tumor suppressor response. During Ras-induced senescence, miR-137 targets KDM4A mRNA, and is responsible for activating retinoblastoma (pRb), a tumor suppressor pathway.[42]

miR-137 was down-regulated in this study in OSCC samples. Significant difference was not observed between OSCC tumor and non-tumor tissues (p=0·104), suggesting miR-137 may serve as tumor suppressor gene, and under expression of miR-137 results in stimulated cell proliferation and also invasion. miR-137 is often down-regulated in different cancers and is found to be a negative regulator of CDC42, CDK6 and E2F6 the prominent targets for miR-

137. Decrease in miR-137 expression was described in colorectal cancer[43] and central nervous system tumor cell lines.[44] miR-200c, miR-137 expression represented significant difference in the clinical stage III of OSCC (p=0·04 and p=0·001).

The miR-200c family and miR-205 function in regulation of epithelial to mesenchymal transition (EMT). EMT promotes tissue remodelling in the course of embryonic development and is considered as a primary step in metastasis. The members of miR-200 family and miR-205 were distinctly down-regulated in cells that confront EMT in response to TGF-β or to ectopic expression of the protein tyrosine phosphatase Pez. Requisite expression of miR-200 family solely was sufficient to prevent TGF-β-induced EMT. These miRNAs together cooperatively regulate the expression of E-cadherin transcriptional repressors ZEB1 and SIP1 factors previously involved in EMT as well as tumour metastasis. Repression of the miRNAs was required to induce EMT in process of upregulating ZEB1 and/or SIP1. Conversely the abnormal expression of these miRNAs in mesenchymal cells initiates mesenchymal to epithelial transition (MET). Manifesting the role of these miRNAs in regulation of EMT, their expression was believed to be lost in invasive breast cancer cell lines with the mesenchymal phenotype. Expression of miR-200 family was lost prominently in regions of metaplastic breast cancer specimens deficient in E-cadherin.[45] These evidences support that the down regulation of these miRNAs may be a salient step in cancer progression.

In this study, the down regulation of miR -200c was observed in OSCC tumor tissues compared non-tumor tissues in the same patient (p=0·0002). miR-200 family functions as a master regulator of various cancer hallmarks. miR-200 family contains a similar seed sequence with a single base difference between two groups. miR-200c is one of the short non-coding RNAs which play prominent roles in tumor development and metastasis. We suggest that miR-200c may suppress the invasion, migration as well as proliferation of OSCC, suggesting the tumor suppressive role of miR-200c in the cancer type. In the saliva samples of oral cancer patients, miR-200c was significantly under expressed compared to controls.[46] miR-200c was significantly down regulated in a panel of 30 spindle cell carcinomas compared with the normal mucosa as determined by qRT-PCR.[47] High expressions of miR-200c was associated with the poor survival in non-small cell lung cancer (NSCLC) patients. On the other hand, however, reports suggest that miR-200c was a direct target of p53 transcription factor, which could inhibit cell proliferation by the cell cycle arrest.[48,49] Justifying the fact that miR-200c expression goes down during EMT and up again

during MET, make the miR-200 family a key regulator of cancer progression and a probable therapeutic target.[49] These findings suggest a dual role of miR-200c as a tumor-promoting or suppressive miRNA, Thus, it is important to understand the molecular mechanisms mediating targets of miR-200c in oral squamous cell carcinoma.

In the present study, the miR-205 expression was found to be significantly down regulated in tumor tissue in comparison with non - tumor tissues, suggesting that miR-205 is a powerful tumor suppressor in oral cancer. However, there are some controversies with regard to the expression of miR-205 in several other cancers. Sempere et al.[43] and Feber et al.[44] reported in their studies that miR-205 was significantly down-regulated in breast and esophageal cancer. In contrast, Gottardo et al.[46] and Iorio et al.[47] reported in their studies that miR-205 was up-regulated significantly in kidney and ovarian tumors. These studies reveal that the observed changes in expression of miR-205 are tissue-specific. This study indicates that the down-regulation of miR-205 may be considered as a prognostic marker for oral squamous cell carcinoma. The associations between miR-21 and miR-205 with clinicopathologic features of OSCC patients represented no significant correlation, but were associated with tumorigenesis through their signaling pathways.

CONCLUSIONS

Oral cancer rates increases with age with a rapid increase after age 50 and peaks between 60 to 70 years. We believe that these findings may be important for precision medicine, which aims to consider each patient's genetic, environmental, and lifestyle characteristics when developing and assigning treatment. miRNAs being key regulators of various cellular processes like proliferation, differentiation, apoptosis, survival, motility and morphogenesis, they can influence gene expression in cancers. It is concluded from our results that alterations in the expression of miR-21, miR-137, miR-200c and miR-205 could influence target genes hence can be useful biomarkers for early detection of oral cancer.

Contributors: KJ, and SPS, were involved in the design of the study, SPS performed all the analyses, KJ, SPS, UK, & RM contributed equally to the development of the manuscript.

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Conflict of Interest: None declared.

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