

Research Article

Deregulation of microRNAs in Head and Neck Cancer Patients

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Abstract

Objectives: Head and neck cancer is amongst the most prevalent malignancies in the world. The condition is also alarming in Pakistan. The main aim of the current research was to examine the expression profiling of microRNAs which are miR-105-5p, miR-10a-5p, miR-3658, miR-3160-3p, miR-4795-5p and miR-431-5p in HNC patients. Furthermore, the expression was also correlated with the clinical parameters, and prognostic significance was evaluated.

Methods: 300 HNC patients tumor samples and an equal number of healthy control samples were collected from different hospitals in Pakistan. The expression analysis of the selected microRNAs was carried out using real-time PCR (qPCR).

Results: The results showed that the microRNAs were significantly deregulated in HNC patients compared to the controls. miR-105-5p ($p < 0.0004$), miR-10a-5p ($p < 0.0001$), miR-3658 ($p < 0.003$), miR-3160-3p ($p < 0.0001$) were found significantly downregulated, while miR-4795-5p ($p < 0.0001$) and miR-431-5p ($p < 0.0001$) were found upregulated in HNC patients as compared to controls. The Kaplan-Meier analysis showed that the deregulation of these microRNAs was found associated with decreased survival in HNC patients.

Conclusion: Our results suggested that the selected microRNAs were found deregulated in HNC patients and this deregulation was also found associated with significantly increased risk of HNC not only that it was also linked to decreased survival of HNC patients.

Keywords: HNC, microRNAs, real-time PCR, expression analysis, survival analysis

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Cancer in the head and neck region consists of malignancies of the pharynx, paranasal sinuses, larynx, oral cavity, and nasal cavity and is attributed to tumor aggression, and early lymph node metastases.^[1] It is the world's sixth most prevalent cancer, with a survival ratio of less than 50% to be 5 years, one of the lowest among malignant tumors.^[2] Head and neck cancer (HNC) affects 11% of females and 21% of males in Pakistan each year. Oral cancer is reported to be the second most occurring malignancy after breast cancer in females and lung cancer in males.^[3] Tobacco usage, alcohol intake, abnormalities in genetic and epigenetic pathways,

and specific environmental factors all contribute to the development of HNC.^[4] A multidimensional transcriptome data analysis could give important information about how cancer progresses, how cells communicate, and how long they live.^[5] Numerous markers for HNC have been found, but most of them have failed to predict advanced disease, due to a lack of correlation with lymph node metastases.^[6] The short non-coding RNAs, which are 18–22 nucleotides in length, have recently been found to be important predictors of a wide range of health outcomes. They have also shown that they can be used as therapeutic biomarkers, as well.^[7]

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Many miRNAs control gene expression by blocking individual genes or gene networks, affecting a whole biological cascade in the process. More than 2600 human miRNAs have been identified in the miRbase (Release 21), a database that maintains miRNAs data. It has been found that 60% of human protein-coding genes are influenced by these miRNAs.^[8] Yang et al., (2019) has showed that different miRNAs are regulated in HNC in a way that affects many molecular pathways and cell activities, which can lead to tumor development and metastasis. A study reported to use the real-time PCR to look at the expression levels of 236 different miRNAs in 104 people who had HNSCC.^[9] According to one of the studies, people who have downregulation of miRNA-205 and Let-7d in their bodies were more likely to get localized cancer and low mortality rate. Some 51 head and neck cancers tumor samples that had been formalin-fixed were found to have higher expression of miR-423, miR-106b, miR-20a, and miR-16, while miR-10a was down-regulated.^[10] Considering their important role modulation of changing miRNA concentrations using compounds that replace downregulated miRNAs or antagonists that bind to overexpressed miRNAs is a potential future prospect.^[11]

Many malignancies, including HNC, have been shown to use miRNAs as pawns in the genesis of cancer. Several prior studies have found significant differences in miRNA levels between malignancies and their matching normal tissues. Furthermore, a difference in miRNA expression has been seen in primary disease against metastatic areas, implying that miRNAs are involved in tumorigenesis. Current study was organized to check the relative expression of miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p in head and neck cancer tissues and adjacent controls through qPCR.

Methods

Sample Collection

In the present research study, 300 HNC tissue samples were collected along with adjacent healthy tissues taken as controls, for the analysis (Table 1). All the patients were treated at the Pakistan Institute of Medical Sciences (PIMS). Each participant was asked for an interview after obtaining informed permission during the recruiting period, and the interviewer utilized a standardized questionnaire to collect demographic information. The TNM classification of malignant tumors employed by the International Union Against Cancer was used to establish pathological or clinical staging as well as the anatomical location of the lesion. Ethical committees of both PIMS and COMSATS University Islamabad approved the study.

Table 1. Demographic and clinical parameters of HNC patients

Parameter	n (%)
Total HNC Patients	300
Adjacent Controls	300
Gender	
Male	183 (61)
Female	117 (39)
Mean Age at Diagnosis	
≤46	123 (41)
>46	177 (59)
Smoking History	
Smoker	141 (47)
Non-Smoker	159 (53)
Anatomical Site	
Larynx	141 (47)
Oral Cavity	117 (39)
Pharynx	42 (14)
Grades	
Poorly differentiated	60 (20)
Moderately differentiated	90 (30)
Well differentiated	150 (50)
Clinical Staging	
C1-C2	174 (58)
C3-C4	126 (42)
T Staging	
T1-T2	168 (56)
T3-T4	132 (44)
N Staging	
N0	141 (47)
N1-N3	159 (53)
M Staging	
M0	222 (74)
M1	78 (26)

RNA Extraction and Real time PCR

The RNA extraction procedure of tumor samples and controls was performed using Trizol reagent method^[12] and later was stored at -80 °C. A Nanodrop spectrophotometer measured total RNA in each sample (ND- 1000,USA). The SuperScript First-Strand Synthesis System was used to perform reverse transcription polymerase chain reaction (RT-PCR) (Invitrogen, USA). To manufacture first-strand cDNA from purified poly (A) + or whole RNA, the SuperScript III First-Strand Synthesis System for RT-PCR was improved. It has a high yield of cDNA, as well as sensitivity and specificity.

Primer Designing

Primers for miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p (internal control) were acquired from Mir Base and Ensemble Genome Browser for quantitative PCR. Primers were designed and confirmed

using NCBI Primer Blast and UCSC In Silico PCR using the primer quest tool of the Integrated DNA Technology (IDT) software. Each qPCR was carried out in a 20 μ l reaction mixture including around 1 μ l of RT reaction product, 10 μ l of 2X Syber Green, 1 μ l of each primer, and 7 μ l of RNase-free water. The 7900HT Fast Real-Time PCR machine (Applied Biosystems) was used to perform qPCR under standard circumstances. The 2-delta delta Ct analysis technique was used to calculate the relative mRNA levels of microRNAs,^[13] where U6 was used as reference.

Statistical Analysis

The student t-test, chi-square test and One-way ANOVA were used to analyze the relationship between targeted miRNA expression and clinical and histological variables such as tumor type, grade, TNM stage, and so on. Kaplan Meier analysis was performed to check the correlation between expression deregulation and survival status of HNC patients. The statistical analysis was carried out using GraphPad Prism5.

Results

The expression levels of miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p in HNSCC tumor and healthy tissues were determined using qPCR.

The expression of miR-105-5p was observed to be significantly downregulated ($p < 0.0004$) in HNC patients compared to controls. M stages of tumors showed a statistically significant ($p < 0.02$) reduction in miR-105-5p expression. The tumors had a significant ($p < 0.02$) variation in histopathological grading. The expression of miR-105-5p was significantly downregulated in M1 metastatic tumors compared to M0 metastatic tumors, and in tumors which were well differentiated as compared to tumors which were poor to moderately differentiated. While in case of clinical stages, T-staging and N-staging, non-significant results were obtained in case of clinical staging, stage I-II were compared with stage III-IV, for T-staging T1-T2 were compared with T3-T4 staging and same kind of non-significant results were obtained for N-staging N0 verses N1-N2, results are shown in Figure 1a. The qPCR analysis of survival status of patients, location of tumor and histopathological grading was also performed, and significant results were obtained for histopathological grading ($p < 0.02$), graphical presentation as shown in Figure 1b.

In HNC tumors, the expression of miR-10a-5p was found significantly downregulated ($p < 0.0001$) than in normal tissue samples. Expression of miR-10a-5p in early clinical stages (I-II) was significantly downregulated ($p < 0.04$) than late clinical stages (III-IV). In early T-stage (T1-T2) tumor tissues,

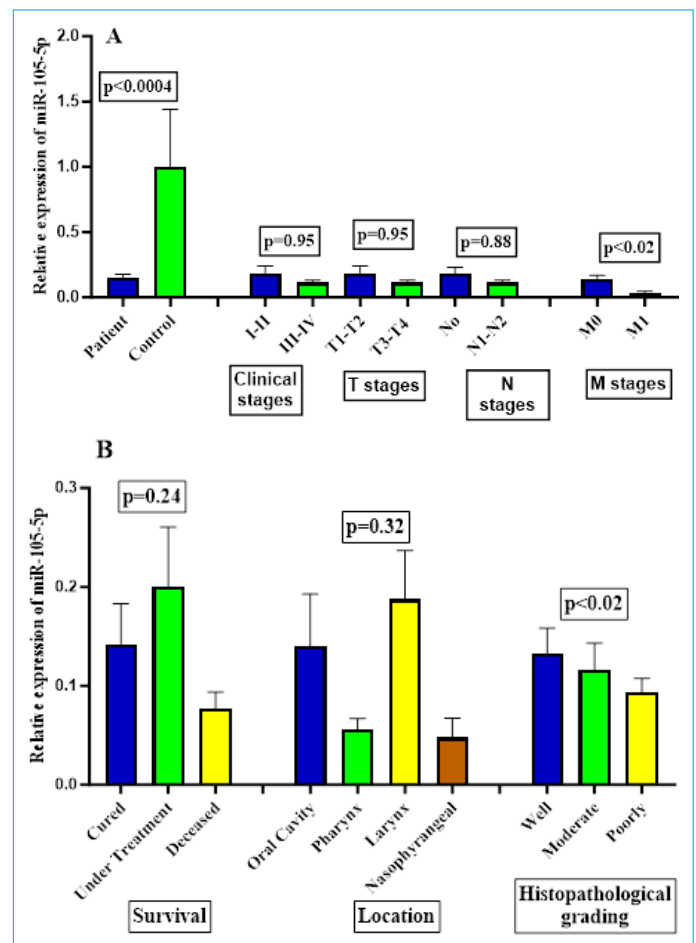


Figure 1. miR-105-5p relative expression in HNSCC tumor tissues. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1-N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I-II and clinical stage III-IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-105-5p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

miR-10a-5p expression was observed to be significantly high ($p < 0.04$) than in advance stage (T3-T4) tumor tissues. While results for M and N staging were non-significant, as shown in Figure 2a. The miR-10a-5p expression was downregulated in the tumor location i.e. pharynx than in the oral cavity and larynx, which was significant ($p < 0.05$). As demonstrated in Table 1, a significant difference ($p < 0.05$) was also seen in deceased patients as compared to alive patients. While the result for histopathological staging was non-significant, shown in Figure 2b.

In HNC tumor tissues, there was a significant downregulation ($p < 0.003$) in miR-3658 expression (third microRNA selected in the current study) as compared to controls. Ex-

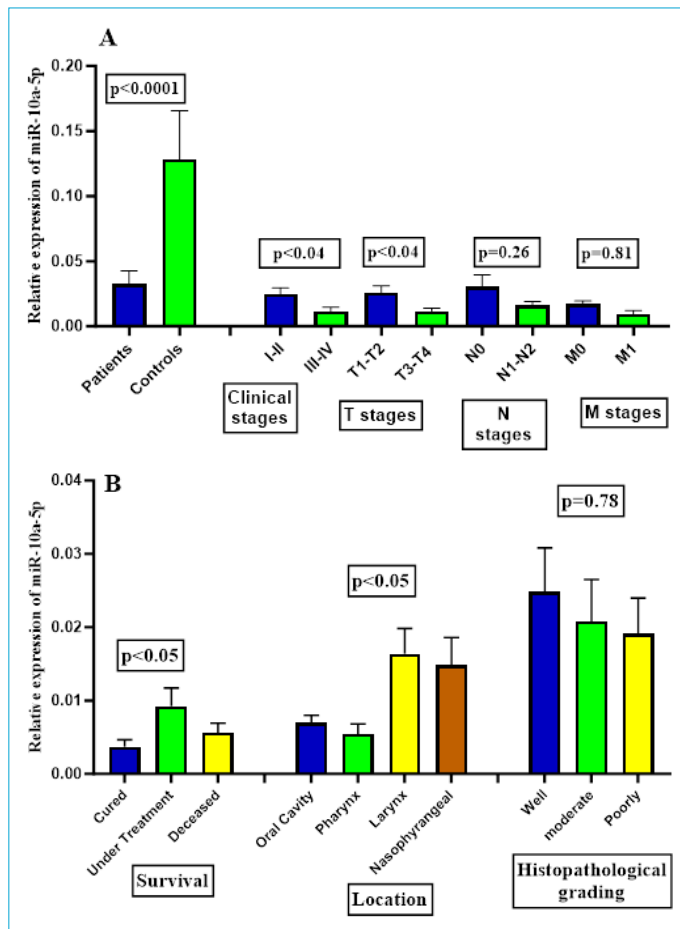


Figure 2. miR-10a-5p relative expression in HNSCC tumor tissues. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1–N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I–II and clinical stage III–IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-105-5p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

pression of miR-3658 in early clinical stages (I–II) showed significant upregulation ($p < 0.03$) as compared to late clinical stages (III–IV). T1–T2 tumor tissues showed a statistically significant ($p < 0.03$) increase in miR-3658 expression compared to T3–T4 tumor tissues. In the case of N stages, N1–N2 showed statistically significant ($p < 0.005$) downregulation in miR-3658 expression. M1 stage tumors showed a statistically significant ($p < 0.0001$) decrease in miR-3658 expression when compared to M0 stage tumors, as shown in Figure 3a. The tumors had a significant ($p < 0.04$) variation in histopathological grading, while non-significant results were obtained for tumor location and survival status, graphical presentation is shown in Figure 3b.

In HNC tumors, miR-431-5p expression was significant-

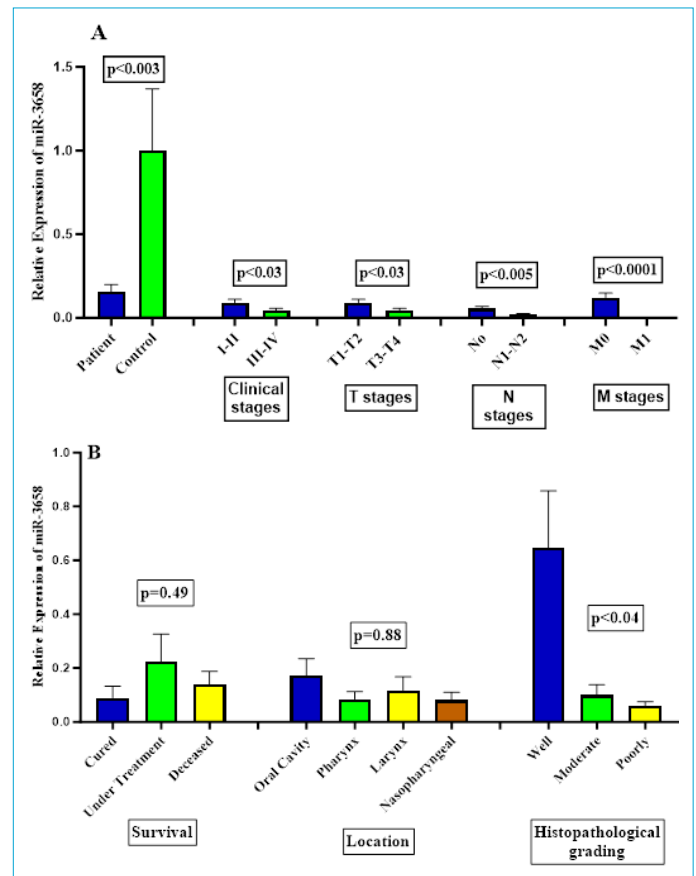


Figure 3. The expression of miR-3658 in HNSCC tumor tissues was compared. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1–N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I–II and clinical stage III–IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-105-5p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

ly ($p < 0.0001$) increased relative to controls. The results demonstrated dysregulation in all other groups, in clinical staging stage III–IV showed significant upregulation ($p = 0.0004$). The T3–T4 stages showed significant upregulation ($p = 0.0001$) than in T1–T2 stages while in the case of N stages in N1–N2 significant ($p = 0.0001$) upregulation was seen and in M1 stage significant upregulation ($p = 0.0001$) was observed as compared to M0. The results are shown in Figure 4a. In the case of relative expression in survival status, location of tumor, and histological grading non-significant deregulation was observed, as shown in Figure 4b. When compared to controls, miR-3160-3p expression was significantly ($p < 0.0001$) downregulated in HNSCC tumors, as shown in Figure 5a. In early clinical stages (I–II) miR-3160-3p expression was significantly upregulated ($p < 0.01$)

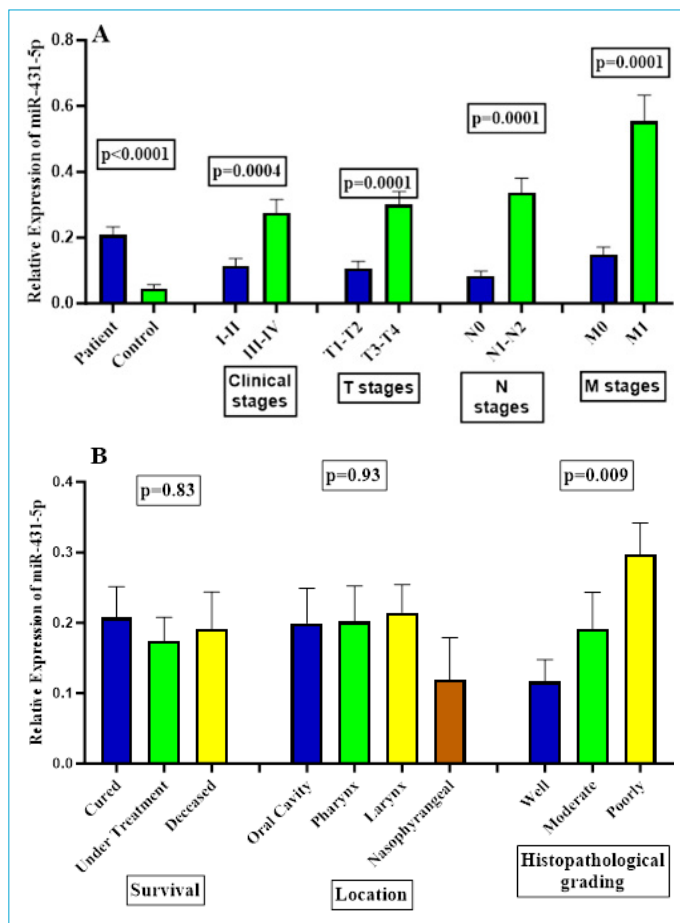


Figure 4. miR-431-5p relative expression in HNSCC tumor tissues. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1–N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I–II and clinical stage III–IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-105-5p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

than in late clinical stages (III–IV). Early T-stage (T1–T2) tumor tissues had a similar significant ($p < 0.01$) upregulation in miR-3160-3p expression as advanced T-stage (T3–T4) tumor tissues. Advanced N stages (N1–N2) showed a statistically significant ($p < 0.03$) increase in miR-3160-3p expression, as seen in Figure 5a. The relative expression was also analyzed in survival status, tumor location and histopathological grading, statistical significant results were obtained as shown in Figure 5b.

When compared to controls, the expression of miR-4795-5p was significantly ($p < 0.0001$) elevated in HNSCC tumors. In the case of later clinical stages (III–IV) significant upregulation was observed as compared to early stages (I–II), the same kind of expression was observed

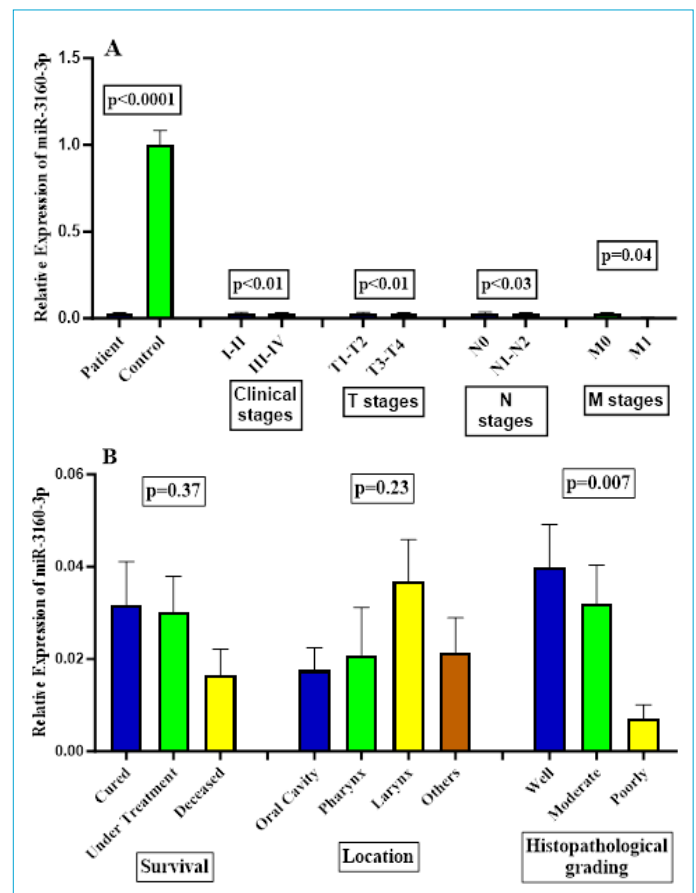


Figure 5. In HNSCC tumor samples, relative expression of miR-3160-3p. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1–N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I–II and clinical stage III–IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-3160-3p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

in T staging where T3–T4 showed significant upregulation ($p = 0.0001$) as compared to T1–T2. In N staging N1–N2 showed significant upregulation ($p = 0.02$) as compared to N0, in M staging M1 was also found significantly upregulated ($p = 0.0001$) as compared to M0 as shown in Figure 6a. In case of survival status, location of tumor and Histopathological grading no significant results we obtained, as demonstrated in Figure 6b.

Spearman Correlation of Selected miRNAs and Clinical Features in HNC Patients:

Additionally, to explore miRNA-miRNA relationship, we did not observe any significant Spearman correlation between miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p in HNSCC cases. In case of miR-

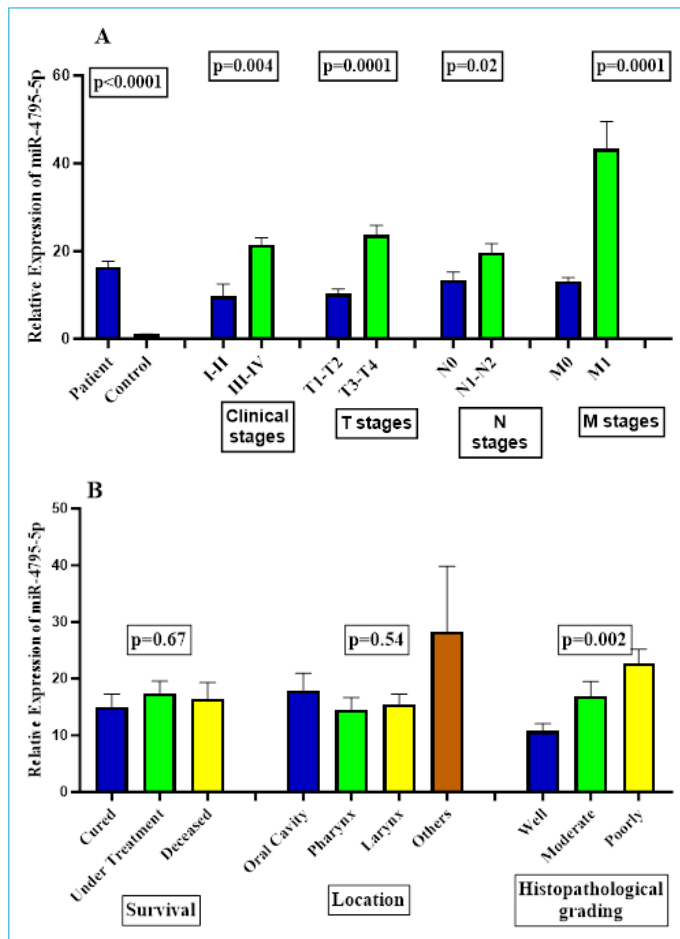


Figure 6. In HNSCC tumor samples, relative expression of miR-4795-5p. HNSCC tumors and normal control samples, HNSCC tumor samples with lymph node metastases (N1–N2) and without lymph node metastasis (N0), HNSCC tumor samples with distinct T stages, clinical stage I–II and clinical stage III–IV, and HNSCC tumor samples with M stage are all shown in column plot (a). The expression level of miR-4795-5p in HNSCC tumor samples in different areas of malignancy, together with survival data and histological grading, is shown in a column plot (b). One-way ANOVA and the t-test were used to get the p values.

NA–clinicopathological characteristic relationship, significantly positive correlation between miR-10a-5p with location ($r=0.316^{**}$, $p<0.002$) and significantly negative correlation with grade ($r=-0.216^{*}$, $p<0.04$) was observed. A positive correlation was observed between age against area ($r=0.275^{**}$, $p<0.006$), gender against area ($r=0.332^{**}$, $p<0.001$), C-stage against survival ($r=0.391^{**}$, $p<0.0001$), C-stage against T stage ($r=0.986^{**}$, $p<0.0001$), survival against T stage ($r=0.380^{**}$, $p<0.0001$), C-stage against M stage ($r=0.312^{**}$, $p<0.002$), survival against M stage ($r=0.211^{*}$, $p<0.04$), T stage against M stage ($r=0.330^{**}$, $p<0.001$). A negative correlation was observed between C-stage against N stage ($r=-0.726^{**}$, $p<0.0001$), survival against N stage ($r=-0.336^{**}$, $p<0.007$), N stage against T

Table 2. Correlations among clinical features and miRNA (miR-105-5p, miR-10a-5p, miR-3658, and miR-431-5p) expression of primary HNSCC

Age	Gender	Area	Clinical Stage	Survival	Grade	T Stage	N Stage	M Stage	miR-105-5p	miR-10a-5p	miR-3658	miR-431-5p	miR-3160-3p	miR-4795-5p
Age	0.059	0.275**	0.061	0.070	-0.072	0.061	0.032	0.050	-0.054	-0.007	0.063	-0.072	0.131	0.052
Gender		0.332**	-0.007	-0.086	0.089	-0.047	-0.090	-0.116	0.026	0.189	0.011	0.000	0.091	-0.094
Location			-0.138	-0.021	0.016	-0.148	0.101	-0.092	0.051	0.316**	0.007	-0.101	0.022	-0.184
Clinical Stage				0.391**	0.007	0.986**	-0.726**	0.312**	-0.097	-0.101	-0.101	-0.084	0.004	0.076
Survival					-0.056	0.380**	-0.336**	0.211*	0.006	0.073	0.124	-0.108	0.072	-0.037
Grade						-0.003	-0.191	-0.044	0.072	-0.216*	0.011	0.024	0.053	0.127
T Stage							-0.723**	0.330**	-0.053	-0.076	-0.109	-0.076	-0.015	0.098
N Stage								-0.077	0.120	0.043	0.140	0.031	0.024	-0.021
M Stage									0.017	0.006	0.028	-0.047	-0.020	0.107
miR-105-5p														-0.009
miR-10a-5p										0.180	-0.144	-0.042	-0.090	0.012
miR-3658												0.178	-0.074	0.019
miR-431-5p												-0.132	0.200	0.071
miR-3160-3p													0.016	0.159
miR-4795-5p														

*Spearman correlation coefficients; The expression levels of miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p were based on the relative miRNA level; The p-values were computed using one-way ANOVA and chi-square test; *, Correlation is significant at the 0.05 level (2-tailed); **, Correlation is significant at the 0.01 level (2-tailed).

stage ($r=-0.723^{**}$, $p<0.0001$) (Table 2).

Survival analysis

Kaplan-Meier analysis was performed to assess the prognostic value of selected microRNAs in HNC patients. The analysis showed that the significant downregulation of miR-105-5p ($p<0.0001$), miR-10a-5p ($p<0.0001$), miR-3160-3p ($p<0.0001$) and miR-3658 ($p<0.0001$) was found associated with the poor survival of HNC patients, as shown in Figure 7 (a-d). While the significant upregulation of miR-431-5p ($p=0.008$) and miR-4797-5p ($p<0.0001$) were associated with the poor survival of the HNC patients, as shown in Figure 7 (e, f).

Discussion

There are a lot of different locations in the head and neck region where the cancer could arise, like those that start in the oral cavity, oropharynx, nasal cavity, hypopharynx, larynx, pharynx and the lymph nodes in your neck and head. Head and neck cancers (HNC) are among the top 10 malignancies in the world.^[1, 14] More than six hundred thousand incidents of HNC are identified each year, with three hundred thousand individuals dying from the cancer. The second most common cancer in Pakistan is head and neck cancer. It is responsible for 21% of male malignancies and 11% of female cancers in Pakistani population.^[15] It rep-

resents 18.74% incidence rate among all recent reported malignancies.^[16] Oral cancer is the second most frequent malignancy, accounting for 15% of all new cancer cases.^[17] Oral cancer is the most frequently occurring cancer in Pakistan, after breast cancer in women and lung cancer in men. Karachi, Pakistan's largest city, has a high incidence of HNC, with 28.9%, followed by 22.7 %. Jamshoro had 17.2%, Multan had 17.2%, and Peshawar had 8.2% incidence rate of HNC.^[18]

MicroRNAs with a length of 19 to 25 nucleotides are single-stranded noncoding RNAs. These microRNAs have been found to undergo differentiation, alter gene regulation, cell death, cell proliferation, and stress responses.^[19] MicroRNAs play an important role in pathological state such as cancer by acting as oncogenic microRNAs and tumor suppressor miRNAs.^[20] MicroRNAs have been implicated and indicated to have a part in a range of malignancies, including lung, stomach, colon, breast, prostate, and thyroid cancers.^[21] Any dysregulation in these microRNAs will lead to abnormality in cell function causing carcinogenesis. MiRNA either down-regulate tumor suppressor genes by suppressing their expression or up-regulate the gene's expression, making it an oncogene, depending on its type and target.^[22] So far, substantial scientific study findings have indicated that miRNAs can be biomarkers, and can be utilized for prediction, diagnosis, and prognosis. Furthermore, research

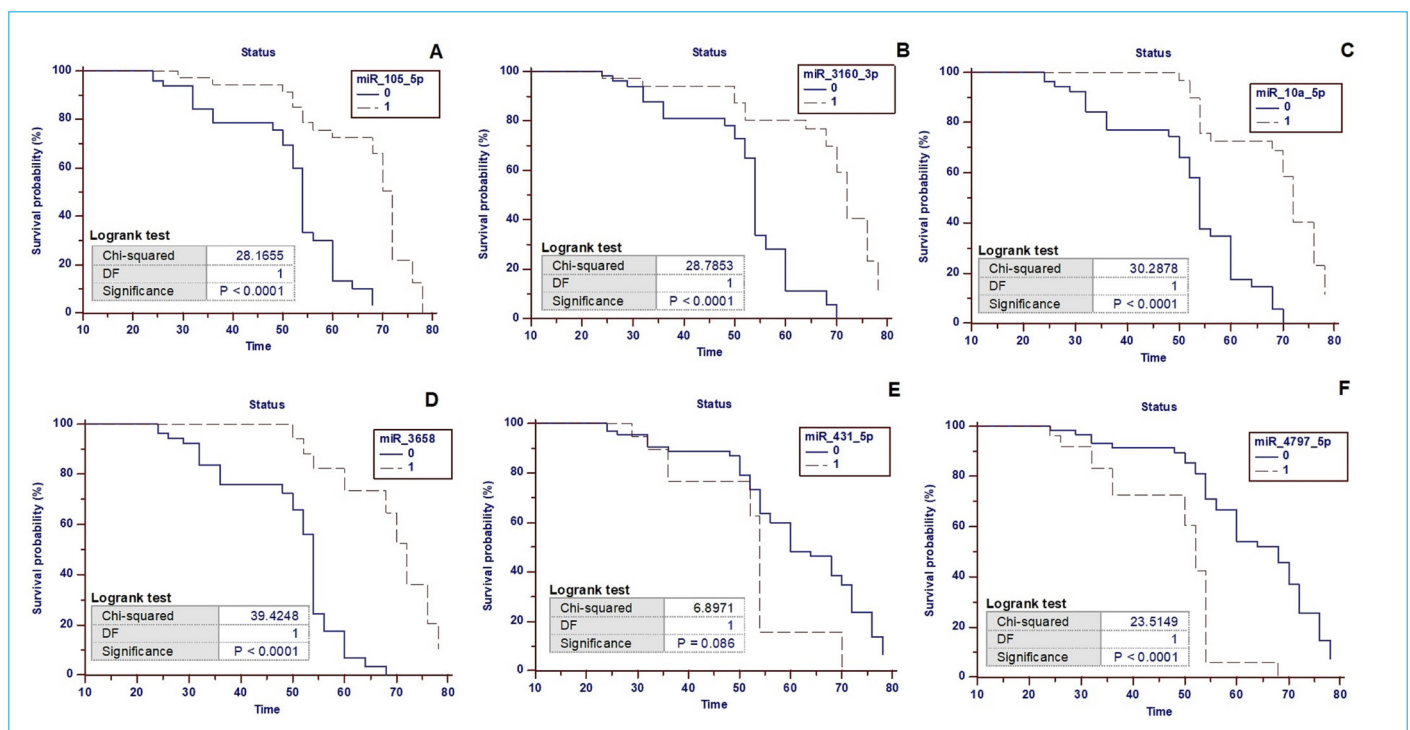


Figure 7. Kaplan-Meier analysis of microRNAs in HNC patients. Survival curve of (a) miR-105-5p, (b) miR-3160-3p, (c) miR-10a-5p, (d) miR-3658, (e) miR-431-5p and (f) miR-4797-5p in HNC patients where "0" represents down regulation and "1" represents upregulated expression of microRNAs. Level of significance $p<0.05$.

employing slightly insidious techniques in order to collect blood, saliva, and urine is critical for the growth of accurate and low-cost miRNA-based technologies for routine usage in clinics for early cancer detection/diagnosis and therapy evaluation/prognosis.^[23]

In present study we checked the expression of miR-105-5p, miR-10a-5p, miR-3658, miR-431-5p, miR-3160-3p, and miR-4795-5p in 300 tumor samples as well as adjacent controls of head and neck cancer patients, to assess whether they act as oncogenic or tumor suppressor. There is no proper study conducted for the miRNA's expression in head and neck cancer before and a very little knowledge of their role in different cancers is present, making them a perfect candidate to check as biomarkers in HNC patients. The qPCR of miRNA-105-5p showed that it is significantly down regulated in cancerous tissues of patients as compared to adjacent controls ($p < 0.0004$). In contrast, other studies have been reported that miR-105-5p is up-regulated in non-small cell lung cancer (NSCLC)^[24] and colorectal cancer.^[25] In one of the reported studies overexpression of miR-105-5p was found inhibiting the cell proliferation in glioma cells and HCC. This differential expression of miR-105-5p was linked to the different microenvironment of the cells.^[24] We found that miRNA-10a-5p expression levels were significantly downregulated ($p < 0.0001$) in cases compared to adjacent controls. Some studies showed similar results that miR-10a-5p was significantly down regulated in renal cell carcinoma (RCC) associated with the poor prognosis^[26] and in ovarian carcinoma.^[27] In contrast some studies showed over expression of miR-10a-5p in NSCLC^[28] and in cell lines of Cholangiocarcinoma (CCA).^[29] These findings suggested that the miR-10a-5p expression was tissue specific and its role varies from cancer to cancer. It was observed that miR-3658 was significantly downregulated ($p < 0.003$) in cancerous tissues of patients as compared to adjacent controls. Some studies have supported the fact as miR-3658 reported to be downregulated in colorectal cancer.^[30] In contrast, other studies have been reported that hsa-miR-3658 was upregulated in various cancer including bladder cancer^[31, 32] and multiple myeloma.^[33] These findings have suggested that hsa-miR-3658 expression was tissue specific and had a tumor suppressor role as also reported by another study where miR-3658 acted as tumor suppressor by reducing cell migration.^[30] However^[32] have reported that upregulation of miR-3658 in bladder cancer was significantly associated ($p < 0.05$) with TNM staging and histological grade. An expression of miR-431-5p was significantly upregulated ($p < 0.0001$) in cancerous tissue samples compared to adjacent controls. In line with our findings, another study shows that, in colon cancer upregulation of the miR-431-5p prohibited cell proliferation.^[34] While in another study miR-

431-5p was observed to be down-regulated in the serum of diffused large beta cell lymphoma patients.^[35] Recent studies suggested that the deregulation of miR-431-5p is involved in initiating and progressing different cancers. Significant downregulation ($p < 0.0001$) of miR-3160-3p expression was observed in head and neck cancer tissues compared with adjacent controls. The miR 3160 3p has a function in the regulation of exosomal gene DE miRNAs in obesity. The downregulation of miR-3160-3p was associated with the upregulation of some genes in obesity.^[36] In human umbilical vein endothelial cells the expression of miR-3160-3p were overserved significantly upregulated when exposed to melatonin, miR-3160-3p is likewise up-regulated.^[37] The expression of miR-3160-3p in cutaneous Melanoma, miR 3160-3p has a role in the primary dysregulation.^[38] Expression analysis indicated the significant up-regulation ($p < 0.0001$) of miR-4795-5p, in cancerous tissues as compared to adjacent controls. The upregulation of miR-4795-5p was also observed in brain tumor metastasis.^[39] In contrast, other studies have reported the downregulation of miR-4795-5p in upper urinary tract urothelial carcinoma^[40] and in colorectal cancer.^[41]

The expression analysis of all the miRNAs showed that they behaved differently among themselves as well as comparing with previous studies in other cancers. Significant downregulation in cancerous tissues as compared to controls was showed by miR-105-5p, miR-10a-5p, miR-3658, and miR-3160-3p while miR-431-5p, and miR-4795-5p showed significant upregulation, thus proving them to be useful as biomarkers for head and neck cancer.

Conclusion

Our study showed that these microRNAs can easily be used for expression analysis as biomarkers for early detection of head and neck cancer. Combinations of biomarkers can be used to verify the results as some showed significant down-regulation (miR-105-5p, miR-10a-5p, miR-3658, and miR-3160-3p) while some were upregulated (miR-431-5p and miR-4795-5p). Early detection of the head and neck cancer through these miRNA biomarkers will enable the prevention of advance stages of cancer. This study has some limitations, but the main one is smaller sample size. In future, studies should be done with large sample size and with fresh tumor or blood samples for proper evaluation of these biomarkers to be used for early detection of cancer.

Disclosures

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References

1. Cho JK, Hyun SH, Choi N, Kim MJ, Padera TP, Choi JY, et al. Significance of lymph node metastasis in cancer dissemination of head and neck cancer. *Trans Oncol* 2015;8(2):119–25.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer*. 2010;127(12):2893–917.
3. Mohsin Z, Faiq A, Naqvi T, Rehman S, Ahmed SI, Farrukh K, et al. Evaluation of head and neck cancer knowledge among the at-risk population of Karachi, Pakistan: A cross-sectional survey. *J Health Res* 2021;36(4):725–37.
4. Nagadia R, Pandit P, Coman WB, Cooper-White J, Punyadeera C. miRNAs in head and neck cancer revisited. *Cell Oncol* 2013;36(1):1–7.
5. Cieřlik M, Chinnaiyan AM. Cancer transcriptome profiling at the juncture of clinical translation. *NatrRev Gen* 2018;19(2):93–109.
6. Tran N, O'Brien CJ, Clark J, Rose B. Potential role of micro-RNAs in head and neck tumorigenesis. *Head Neck* 2010;32(8):1099–111.
7. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: Biomarkers, functions and therapy. *Trend Mol Med* 2014;20(8):460–9.
8. Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Gen Res* 2009;19(1):92–105.
9. Childs G, Fazzari M, Kung G, Kawachi N, Brandwein-Gensler M, McLemore M, et al. Low-level expression of microRNAs let-7d and miR-205 are prognostic markers of head and neck squamous cell carcinoma. *Am J Pathol* 2009;174(3):736–45.
10. Hui AB, Lenarduzzi M, Krushel T, Waldron L, Pintilie M, Shi W, et al. Comprehensive MicroRNA profiling for head and neck squamous cell carcinomas. *Clin Cancer Res* 2010;16(4):1129–39.
11. Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, et al. Treatment of HCV infection by targeting microRNA. *New England J Med* 2013;368(18):1685–94.
12. Masood N, Malik FA, Kayani MA. Expression of xenobiotic metabolizing genes in head and neck cancer tissues. *Asian Pac J Cancer Prev* 2011;12(2):377–82.
13. Chen Z, Jin Y, Yu D, Wang A, Mahjabeen I, Wang C, et al. Down-regulation of the microRNA-99 family members in head and neck squamous cell carcinoma. *Oral Oncol* 2012;48(8):686–91.
14. Suh Y, Amelio I, Urbano TG, Tavassoli M. Clinical update on cancer: Molecular oncology of head and neck cancer. *Cell Death Dis* 2014;5(1):e1018.
15. Masood N, Kayani MA. Mutational analysis of xenobiotic metabolizing genes (CYP1A1 and GSTP1) in sporadic head and neck cancer patients. *Gen Mol Biol* 2011;34:533–8.
16. Pervez S, Jabbar AA, Haider G, Ashraf S, Qureshi MA, Lateef F, et al. Karachi Cancer Registry (KCR): Age-standardized incidence rate by age-group and gender in a Mega city of Pakistan. *Asian Pac J Cancer Prev* 2020;21(11):3251.
17. Akram S, Mirza T, Mirza MA, Qureshi M. Emerging patterns in clinico-pathological spectrum of oral cancers. *Pakistan J Med Sci* 2013;29(3):783.
18. Chaudhry S, Khan AA, Mirza KM, Iqbal HA, Masood Y, Khan NR, et al. Estimating the burden of head and neck cancers in the public health sector of Pakistan. *Asian Pac J Cancer Prev* 2008;9(3):529–32.
19. Zeng Y, Cullen BR. The biogenesis and function of MicroRNAs. *Gene Expression and Regulation*. Springer, 2006, p. 481–92.
20. Svoronos AA, Engelman DM, Slack FJ. OncomiR or tumor suppressor? The duplicity of microRNAs in cancer. *Cancer Res* 2016;76(13):3666–70.
21. Michael MZ, O'Connor SM, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 2003;1(12):882–91.
22. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nat Rev Gen* 2008;9(2):102–14.
23. Mahn R, Heukamp LC, Rogenhofer S, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. *Urology* 2011;77(5):1265. e9–16.
24. Dong X, Chang M, Song X, Ding S, Xie L, Song X. Plasma miR-1247-5p, miR-301b-3p and miR-105-5p as potential biomarkers for early diagnosis of non-small cell lung cancer. *Thorac Cancer* 2021;12(4):539–48.
25. Shen Z, Zhou R, Liu C, Wang Y, Zhan W, Shao Z, et al. MicroRNA-105 is involved in TNF- α -related tumor microenvironment enhanced colorectal cancer progression. *Cell Death Disease* 2017;8(12):1–13.
26. Arai T, Okato A, Kojima S, Idichi T, Koshizuka K, Kurozumi A, et al. Regulation of spindle and kinetochore-associated protein 1 by antitumor miR-10a-5p in renal cell carcinoma. *Cancer Sci* 2017;108(10):2088–101.
27. Liu LJ, Sun XY, Yang CX, Zou XY. MiR-10a-5p restrains the aggressive phenotypes of ovarian cancer cells by inhibiting

- HOXA1. *Kaohsiung J Med Sci* 2021;37(4):276–85.
28. Bao M, Pan S, Yang W, Chen S, Shan Y, Shi H. Serum miR-10a-5p and miR-196a-5p as non-invasive biomarkers in non-small cell lung cancer. *Int J Clin Exp Pathol* 2018;11(2):773.
29. Gao L, Yang X, Zhang H, Yu M, Long J, Yang T. Inhibition of miR-10a-5p suppresses cholangiocarcinoma cell growth through downregulation of Akt pathway. *OncoTargets Therapy* 2018;11:6981.
30. Hosseini F, Soltani BM, Baharvand H, Hosseinkhani S. Hsa-miR-3658 down-regulates OCT4 gene expression followed by suppressing SW480 cell proliferation and migration. *Biochem J* 2020;477(12):2281–93.
31. Luan T, Zou R, Huang L, Li N, Fu S, Huang Y, et al. Hsa-miR-3658 promotes cell proliferation, migration and invasion by effecting LASS2 in bladder cancer. *Clin Lab* 2018;64(4):515–25.
32. Chen Y, Wang H, Liang M, Zou R, Tang Z, Wang J. Upregulation of miR-3658 in bladder cancer and tumor progression. *Genet Mol Res* 2016;21:2274–82.
33. Hao M, Zang M, Wendlandt E, Xu Y, An G, Gong D, et al. Low serum mi R-19a expression as a novel poor prognostic indicator in multiple myeloma. *Int J Cancer* 2015;136(8):1835–44.
34. Kong Q, Han J, Deng H, Wu F, Guo S, Ye Z. miR-431-5p alters the epithelial-to-mesenchymal transition markers by targeting UROC28 in hepatoma cells. *OncoTargets Therapy* 2018;11:6489.
35. Meng Y, Quan L, Liu A. Identification of key microRNAs associated with diffuse large B-cell lymphoma by analyzing serum microRNA expressions. *Gene* 2018;642:205–11.
36. Yang Z, Wei Z, Wu X, Yang H. Screening of exosomal miRNAs derived from subcutaneous and visceral adipose tissues: Determination of targets for the treatment of obesity and associated metabolic disorders. *Mol Med Rep* 2018;18(3):3314–24.
37. Son GW, Yang H, Park HR, Lee SE, Jin YH, Park CS, et al. Analysis of miRNA expression profiling in melatonin-exposed endothelial cells. *Mol Cell Toxicol* 2016;12(1):73–81.
38. Lorusso C, De Summa S, Pinto R, Danza K, Tommasi S. miRNAs as key players in the management of cutaneous melanoma. *Cells* 2020;9(2):415.
39. Li Z, Yang H, Ye L, Quan R, Chen M. Role of exosomal miRNAs in brain metastasis affected by radiotherapy. *Trans Neurosci* 2021;12(1):127–37.
40. Tao L, Zeng Y, Wang J, Liu Z, Shen B, Ge J, et al. Differential microRNA expression in aristolochic acid-induced upper urothelial tract cancers ex vivo. *Mol Med Rep* 2015;12(5):6533–46.
41. Bara Jr T, Gurzu S, Sugimura H, Bara T, Beleaua MA, Jung I. A systematic review of the possible carcinogenic role of the aristolochic acid. *Rom J Morphol Embryol* 2017;58(1):41–4.