Archives of Gastroenterology and Hepatology

ISSN: 2639-1813

Volume 3, Issue 1, 2020, PP: 21-29

MiRNA-330 and MiRNA-621 as Significant Tools for Hepatocellular Carcinoma Prediction

Amal Ahmed Mohamed[1]*, Dina M. Abo-Elmatty[2], Noha M. Mesbh[2], Omnia ezzat[3], Eman T. Mehanna[2], Ahmed A. Youssef[3], Ossama A. Ahmed[4], Eman Alsayed Abouahmed[5], Ahmed Farouk[6], Shrook mousa[7], Reda S. Abdelghany[8], Mahmoud Maamoun Shaheen[7], Gina G. Naguib[4]

[1]*Department of Biochemistry National Hepatology and Tropical Medicine Research Institute, Egypt.

[2]Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.

[3]Department of Biochemistry, Faculty of Pharmacy, Egyptian Russian University, Egypt.

[4]Internal medicine department, Ain Shams University, Egypt.

[5]Department of Clinical Pathology, Minia University Hospital, Minia, Egypt.

[6]Radiology Department, National Institute of Diabetes and Endocrinology.

[7]Internal medicine department, Faculty of Medicine, Cairo University, Egypt.

[8]Tropical Medicine Department, Ahmed Maher Teaching Hospital, Cairo, Egypt.

***Corresponding Author:** Dr. Amal Ahmed Mohamed, Department of Biochemistry National Hepatology and Tropical Medicine Research Institute, Egypt.*

Abstract

***Background and Aim:** Hepatocellular carcinoma is ranked as the second most frequent cause of cancer-related death globally. In Egypt, liver cancer forms 70.48% of all liver tumors. The role of alphafetoprotein (AFP) in the diagnosis of hepatocellular carcinoma (HCC) is getting smaller owing to the advances in new markers and imaging modalities. Serum micro RNAs are full of non-invasive biomarkers. MiRNA-330 and MiRNA -621 could be novel potential diagnostic and prognostic biomarkers for HCC. Our study observed the significance of miRNA 330 and miRNA 621 in cases of HCC patients and comparing these results with those in cirrhotic patients preparing to use them for prediction of HCC development.*

***Methods:** This study included two hundred and one candidates; 67 healthy volunteers, 67 patients with cirrhosis and 67 patients with hepatocellular carcinoma. HCC diagnosis was confirmed using imaging techniques and tumor staging was also done. MiRNA 330 and miRNA 621 levels expression were measured by real-time quantitative PCR (RT-qPCR).*

***Results:** HCC patients had significantly higher miRNA 330 and miRNA 621 levels than patients with cirrhosis and healthy controls. There is a statistically significant difference between different grades of hepatocellular carcinoma patients in their microRNA 330 levels, where on applying pairwise comparison we found that grade I had significantly higher miRNA 330 folds than grade III.*

***Conclusions:** Our study concluded the significance of miRNA 330 and miRNA 621 in cases of HCC patients as they had significantly higher mRNA 330 and mRNA 621 levels than patients with cirrhosis and healthy controls. MiRNA 330 and miRNA 621 are considered as significant tools for prediction of HCC occurrence.*

Keywords: *HCC, Human Genome, MicroRNAs, miRNA, Biomarkers.*

Introduction

Hepatocellular carcinoma (HCC) is ranked as the third leading cause of cancer mortality and it is a major cause of death in patients with liver cirrhosis [1].

In Egypt, liver cancer compromises 11.75% of the tumors of all digestive organs and 1.68% of the total malignancies. Egyptian patients with HCC constitute 70.48% of all liver tumors [2]. Upon this, HCC represents

the main complication of cirrhosis in Egypt with high potential incidence. Subsequently, HBV and HCV have a greater attention as primary risk factors [3], and improvements in screening programs and diagnostic tools [4].

The sequence of the genome of the human has been finished in 2003, and it was informed that only 20,000-25,000 genes, about 1.5% of all human genome, can encode protein [5].

About one third of the human genome is found to be regulated by miRNAs which are short RNA molecules which are found in all eukaryotic cells. MiRNAs regulate many important functions such as growth, metabolism and differentiation [7].

MicroRNAs abnormalities have been associated with a number of clinically important diseases (i.e. myocardial infarction and autoimmune disease) [6]. Abnormal miRNA expression participates in hepatic diseases such as polycystic liver disease and viral hepatitis [8,9]. Also, miRNAs can affect NAFLD through several pathways including inflammation, fibrosis, insulin resistance, lipid metabolism and the metabolic syndrome. Recently, altered miRNA expression has been announced in animal and human liver samples with NAFLD [10].

MiRNAs up-regulation or down-regulation are found in many human cancers. MiRNAs pattern alterations are responsible for changes which turn cells malignant [7]. MiRNAs act as gene regulators by targeting tumor-suppressor genes or oncogenes and therefore they are considered as oncogenes or tumor suppressors [11].

Recently, scientific advances in miRNAs will provide a great potential in the diagnosis and treatment of many diseases [7].

Aim of the Study

To correlate between miRNA-330 and miRNA-621 expression and development of hepatocellular carcinoma and liver cirrhosis.

Methodology

This study was approved by ethical committee in Faculty of Medicine, Ain shams university and suez canal university. Blood samples were collected from a total number of two hundred one subjects. All patients were recruited from MASRI (faculty of medicine ain shams research institute) Ain shams university treatment and research unit in the period between 2/2019 to—10 /2019 and have signed consent documents allowing their clinical information to be gathered and analyzed for research purpose.

Two hundred and one subjects were involved in the current study. Subjects were divided into 3 groups: control group (n=67), cirrhotic patients (n=67) and HCC patients group (n=67). The staging of HCC patients was done using okuada, CLIP and VISUM staging systems respectively. Severity of liver disease was assessed by modified Child-Pugh score, Model for end stage liver disease score (MELD) and updated MELD score (uMELD).

Venous blood samples (10 ml) were withdrawn from enrolled subjects by trained laboratory technicians. Three ml were collected in tubes containing EDTA for processing RNA extraction and miRNA while three ml of this quantity were left to clot at the temperature of room then centrifuged and sera were separated for determination of biochemical parameters. The following biochemical tests were done for all involved subjects: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (T. bilirubin) and albumin were assayed using Olympus diagnostic GmbH.

Two ml in Edta tube for complete blood picture (CBC) by Sysmex machine. The rest 2ml in sodium citrate vacutainer tube for PT/ INR measurements by KCL Delta. Serum AFP level was determined by using sandwich Enzyme Linked Immunosorbent Assay (ELISA) using washer (state fax) reader (state fax chromate -3033).

Determination of Serum Level of MiR-330 and MiR-621 by RT-qPCR

Total RNA extraction and purification were done using a miRNeasy Mini Kit; cat no: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Reverse Transcription

cDNA was synthesized by reverse transcription reaction using TaqMan MicroRNA Reverse Transcription Kit; cat no: 4366596 (Applied Biosystems, Foster city, USA) and the thermal cycler (Quanta Biotech).

Gene Expression Analysis

The quantification of miR-330and miR-621 levels was amplified from cDNA using TaqMan universal Master Mix and TaqMan assay(has-miR-330-5p; Catalog

number: 4427975, Assay ID: 002230) and (hsa-miR-621; Catalog no: 4427975;Assay ID: 001598). The U6 RNA was used as housekeeper gene (Catalog no: 4427975; Assay ID: 001973).

All samples were analyzed using the 5 plex Rotor- Gene PCR Analyzer (Qiagen, Germany). The $2^{\Delta\Delta Ct}$ method was conducted for the analysis of gene expression levels using TaqMan microRNA Control Assays U6 snRNA as an endogenous reference control for normalization purposes.

Statistical Analysis

All analyses were performed using statistical Package for social sciences (SPSS) for windows version 22.0 (SPSS, Chicago, IL, USA). Continuous data were expressed as mean ± standard deviation and categorical data as frequencies and percentages. For assessment of data homogeneity, all variables showed a skewed distribution with a prior confirmation through Kolmogorov–Smirnov (KS) test. As data were not normally distributed, Mann Whiney U test and Kruskal Wallis test were used for analysis of continuous variables and the pairwise comparison was estimated using Bonferroni correction. Spearman's correlation analysis was used to assess the correlations between continuous variables. Fisher's exact test and chi-square test were used for statistical analysis of categorical variables. Receiver operating characteristic (ROC) curves were applied to compare the diagnostic Performance of different diagnostic tests; mRNA 330, mRNA 621and AFP in Prediction of different clinical outcomes. For all tests a Probability value of less than 0.05 was considered statistically significant.

Results

Table1. *Baseline characteristics of the study groups*

Variables	Healthy controls (n=67)	Cirrhotic patients (n=67)	HCC patients (n=67)	p-value
Age (years), mean ± SD	45.16 ± 17.15	54.90 ± 9.82 $^{\beta}$	60.42 ± 8.28 $\alpha,^{\beta}$	**<0.001** a
Gender, n (%)				
Male	46 (68.7)	37 (55.2)	45 (67.2)	0.21 b
Female	21 (31.3)	30 (44.8)	22 (32.8)	
BMI (kg/m2)**, mean ± SD**	22.21 ± 3.45	26.56 ± 2.84 $^{\beta}$	25.94 ± 2.21 $^{\beta}$	**<0.001** a
History of alcohol, n (%)				
Absent	61 (91)	67 (100)	66 (68.5)	**0.018** c
Present	6 (9)	0	1 (1.5)	
History of smoking, n (%)				
Absent	60 (89.6)	55 (82.1)	27 (40.3)	**<0.001**
Present	7 (10.4)	12 (17.9)	40 (59.7)	
Grading, n (%)				
I	-	10 (14.9)	11 (16.4)	
II	-	13 (19.4)	12 (17.9)	0.96 b
III	-	44 (65.7)	44 (65.7)	

a p-values are based on Kruskal Wallis test. Statistical significance at P < 0.05

b p-values are based on Chi-Square test. Statistical significance at P < 0.05

c p-values are based on Fisher Exact test. Statistical significance at P < 0.05 Values with superscript

$^{\beta}$ are different from healthy control.

Values with superscript α are different from cirrhotic patients.

HCC: hepatocellular carcinoma; BMI: body mass index.

Baseline characteristics of the studied groups were summarized in table 1. HCC patients were found to have significantly elder age than patients with cirrhosis (p=0.048) and healthy controls (p<0.001). Moreover, history of smoking was significantly higher among patients with hepatocellular carcinoma. Healthy participants had significantly lower BMI than cirrhotic and HCC patients (p<0.001).

Table2. *Comparison of different study outcomes among different study groups*

Variables	Healthy controls (n=67)	Cirrhotic patients (n=67)	HCC patients (n=67)	p-value
miRNA, mean ± SD				
miRNA 330	$2^{2.44} \pm 2^{3.09}$	$2^{3.49} \pm 2^{4.13}$	$2^{4.32} \pm 2^{4.67\ \alpha,\beta}$	**0.001** [a]
miRNA 621	$2^{4.23} \pm 2^{4.25}$	$2^{5.62} \pm 2^{7.99}$	$2^{2.12} \pm 2^{3.16\ \alpha,\beta}$	**<0.001** [a]

[a] p-values are based on Kruskal Wallis test. Statistical significance at P < 0.05 Values with superscript $^{\beta}$ are different from healthy control. Values with superscript $^{\alpha}$ are different from cirrhotic patients.

HCC: hepatocellular carcinoma; miRNA: micro RNA.

Table 2 shows that HCC patients had significantly higher miRNA 330 and miRNA 621 levels than patients with cirrhosis and healthy controls, whereas there was no statistically significant difference between patients with cirrhosis and healthy controls in their miRNA 330 and miRNA 621 levels.

Table3. *Correlation analysis between mRNA 330 and mRNA 621 with different clinical variables in HCC patients*

Variables	miRNA 330		miRNA 621	
	r	p-value	r	p-value
Age	0.038	0.76 [a]	-0.109	0.38 [a]
BMI	-0.229	0.06 [a]	-0.095	0.45 [a]
Size of lesion	0.268	**0.028** [a]	-0.127	0.31 [a]
MELD	-0.218	0.08 [a]	0.016	0.89 [a]
UMELD	-0.346	**0.004** [a]	-0.023	0.85 [a]

[a] p-values are based on Spearman's correlation test. Statistical significance at P < 0.05

BMI: body mass index; miRNA: micro RNA

In HCC patients, there was a positive significant correlation between size of the lesion and miRNA 330 levels (r= 0.268) (p=0.028). On the other hand, there was a negative significant correlation between patients' UMELD score and their miRNA 330 levels (r= -0.346) (p=0.004). (Table 3)

Table4. *Areas under the curve for analysis of miRNA 330, miRNA 621 and Alpha fetoprotein for prediction of hepatocellular carcinoma patients from healthy individuals*

Variable		Area under the curve	Stand. error	p-value	95% CI
miRNA 330	In	0.682	0.046	**<0.001**	(0.591 – 0.773)
miRNA 621	Dec	0.801	0.04	**<0.001**	(0.723 – 0.878)
AFP	In	0.865	0.037	**<0.001**	(0.793 – 0.937)

miRNA: Micro RNA; AFP: Alphafetoprotein

Figure (1) shows the ROC curve analysis of miRNA 330, miRNA 621 and α-fetoprotein for differentiating hepatocellular carcinoma patients from healthy participants, where the areas under the curve (AUC) were 0.682, 0.801 and 0.865 respectively (Table 4).

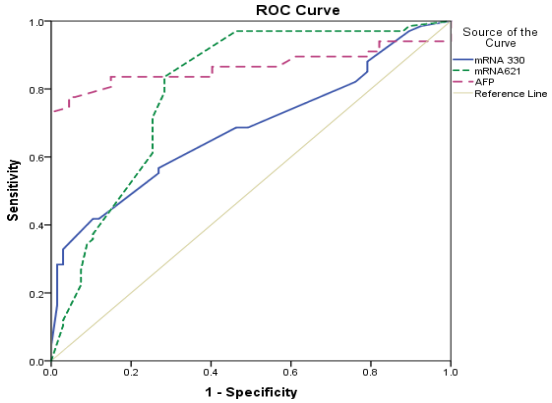

Fig1. *ROC for prediction of hepatocellular carcinoma patients from healthy individuals using miRNA 330, miRNA 621 and Alpha fetoprotein*

Table5. *Sensitivity, specificity, PPV, NPV and diagnostic accuracy of different parameters for prediction of hepatocellular carcinoma patients from healthy individuals*

Cut-off points	Sensitivity	Specificity	PPV*	NPV*	accuracy
miRNA 330					
$2^{1.7}$	68.66	52.24	58.97	62.5	60.45
$2^{1.9}$	68.66	53.73	59.74	63.16	61.19
$2^{2.27}$	56.72	73.13	67.86	62.82	64.93
miRNA 621					
$2^{1.81}$	71.64	74.63	73.85	72.46	73.13
$2^{2.27}$	79.1	71.64	73.61	77.42	75.37
$2^{2.77}$	83.58	71.46	74.67	81.36	77.61
AFP					
7.1	83.58	83.58	83.58	83.58	83.58
7.5	83.58	85.07	84.85	83.82	84.33
7.9	80.6	85.07	84.38	81.43	82.84

PI; Pulsatility index, CS; Cesarean section, IFC; intrapartum fetal compromise

miRNA: Micro RNA; AFP: Alphafetoprotein; PPV: positive predictive value; NPV: negative predictive value

For miRNA 330, values ≥ $2^{2.27}$ were found to have sensitivity = 56.72% and specificity = 73.13% in differentiating hepatocellular carcinoma patients from healthy participants. On the other hand, regarding mRNA 621, values ≤ $2^{2.77}$ were found to have sensitivity = 83.56 % and specificity = 71.46 % in differentiating hepatocellular carcinoma patients from healthy participants. Moreover, α-fetoprotein was found to have the best predictive ability (AUC= 0.865), where values ≥ 7.5 were found to have sensitivity = 83.58% and specificity = 85.07% in differentiating hepatocellular carcinoma patients from healthy participants (Table 5)

Table6. *Areas under the curve for analysis of miRNA 330, miRNA 621 and Alpha fetoprotein for differentiating hepatocellular carcinoma patients from cirrhotic patients*

Variable		Area under the curve	Stand. error	p-value	95% CI
miRNA 330	In	0.619	0.048	**0.017***	(0.525 – 0.714)
miRNA 621	Dec	0.685	0.47	**<0.001***	(0.594 – 0.777)
AFP	In	0.575	0.051	0.133	(0.223 – 0.406)

miRNA: Micro RNA; AFP: Alphafetoprotein

Areas under the curve (AUC) for curve analysis of miRNA 330, miRNA 621 and α-fetoprotein for differentiating hepatocellular carcinoma patients from cirrhotic patients were 0.619, 0.685 and 0.575 respectively (Table 6).

Table7. *Sensitivity, specificity, PPV, NPV and diagnostic accuracy of different parameters for prediction of hepatocellular carcinoma patients from cirrhotic patients*

Cut-off points	Sensitivity	Specificity	PPV*	NPV*	Accuracy
miRNA 330					
$2^{1.9}$	68.66	47.76	56.79	60.38	58.21
$2^{2.27}$	56.72	58.21	57.58	57.35	57.46
miRNA 621					
$2^{1.81}$	71.64	62.69	65.75	68.85	67.16
$2^{2.27}$	79.1	50.75	61.63	70.83	64.93
AFP					
23.5	64.18	52.24	57.33	59.32	58.21
25.5	61.19	58.21	59.41	60	59.7

miRNA: Micro RNA; AFP: Alphafetoprotein

For miRNA 330, values ≥ $2^{1.90}$ were found to have sensitivity = 68.66% and specificity = 47.76% in differentiating hepatocellular carcinoma patients from cirrhotic patients. On the other hand, regarding miRNA 621, values ≤ $2^{1.81}$ were found to have sensitivity = 71.6% and specificity =62.69% in differentiating hepatocellular carcinoma patients from cirrhotic patients. Moreover, α-fetoprotein values ≥ 25.5 were found to have sensitivity = 61.2% and specificity = 58.21% in differentiating hepatocellular carcinoma patients from cirrhotic patients (Table 7)

A total of two hundred and one participants were involved in the study. 67 were healthy controls, 67 were cirrhotic patients and 67 were hepatocellular carcinoma patients. Baselines characteristics were recorded including age, gender, BMI, history of smoking and alcohol intake.

HCC patients were found to have significantly elder age than patients with cirrhosis (p=0.048) and healthy controls (p<0.001) with mean age 45.16 for healthy controls, 54.9 for cirrhotic patients and 60.42 for hepatocellular carcinoma patients.

Moreover, history of smoking was significantly higher among patients with hepatocellular carcinoma (40 hepatocellular carcinoma patients versus 7 healthy controls). Healthy participants had significantly lower BMI than cirrhotic and HCC patients (p<0.001).

There was a statistically significant difference between cirrhotic patients, HCC patients and healthy controls in all laboratory measures (as bilibubin, albumin, INR, serum creatinine, haemoglobin, platelets, AFP and HCV Ab) in the form of significantly lower laboratory values for healthy controls compared to patients with cirrhosis and hepatocellular carcinoma except WBC count which showed no statistical significance.

On the other hand, HCC patients had significantly higher ALT and AST levels than patients with cirrhosis and healthy controls (p<0001).

HCC patients had significantly higher miRNA 330 and miRNA 621 levels than patients with cirrhosis and healthy controls.

Correlation of miRNA 330 and miRNA 621, of hepatocellular carcinoma patients with their baseline characteristics, showed that there is a statistically significant difference between different grades of hepatocellular carcinoma patients in their miRNA 330 levels (p=0.032), where on applying pairwise comparison we found that grade I had significantly higher miRNA 330 than grade III (p=0.026).

Regarding association of miRNA 330 and miRNA 621 of hepatocellular carcinoma patients with different staging systems, there was no statistically significant difference in the levels of miRNA 330 or miRNA 621 between various stages of Okuda staging, CLIP staging or VISUM HCC Vienna staging.

In HCC patients, there was a positive significant correlation between size of the lesion and miRNA 330 levels (r= 0.268) (p=0.028). On the other hand, there was a negative significant correlation between patients' UMELD score and their miRNA 330 levels (r= -0.346) (p=0.004).

Correlation analysis between miRNA 330 and miRNA 621 with different laboratory variables in HCC patients, we found that there was a positive significant correlation between albumin level and miRNA 330 levels (r= 0.379) (p=0.002). On the other hand, there was a negative significant correlation between total serum bilirubin and their miRNA 330 levels (r= -0.280) (p=0.019).

ROC curve analysis of miRNA 330, miRNA 621 and α-fetoprotein for prediction of hepatocellular carcinoma occurrence showed that the areas under the curve (AUC) were 0.682, 0.801 and 0.865 respectively.

For prediction of hepatocellular carcinoma occurrence, Sensitivity, specificity, PPV, NPV were calculated. For miRNA 330, values ≥ 22.27 were found to have sensitivity = 56.72% and specificity = 73.13% in differentiating hepatocellular carcinoma patients from healthy participants. On the other hand, regarding miRNA 621, values ≤ 22.77 were found to have sensitivity = 83.56 % and specificity = 71.46 % in differentiating hepatocellular carcinoma patients from healthy participants. Moreover, α-fetoprotein was found to have the best predictive ability (AUC= 0.865), where values ≥ 7.5 were found to have sensitivity = 83.58% and specificity = 85.07% in differentiating hepatocellular carcinoma patients from healthy participants.

ROC curve analysis of miRNA 330, miRNA 621 and α-fetoprotein for prediction of cirrhosis occurrence showed that the areas under the curve (AUC) were 0.557, 0.620 and 0.935 respectively.

For prediction of cirrhosis occurrence, Sensitivity, specificity, PPV, NPV were calculated. Alpha-fetoprotein was found to have the best predictive ability (AUC= 0. 935), where values ≥ 8.05 were found to have sensitivity = 82.09 % and specificity = 94.03 % in differentiating cirrhotic patients from healthy participants.

ROC curve analysis of miRNA 330, miRNA 621 and α-fetoprotein for differentiating hepatocellular carcinoma patients from cirrhotic patients showed that areas under the curve (AUC) were 0.619, 0.685 and 0.575 respectively.

For prediction of hepatocellular carcinoma patients from cirrhotic patients,

Sensitivity, specificity, PPV, NPV were calculated and showed that for miRNA 330, values ≥ 21.90 were found to have sensitivity = 68.66% and specificity = 47.76%. On the other hand, regarding miRNA 621, values ≤ 21.81 were found to have sensitivity = 71.6 % and specificity = 62.69 %. Moreover, α-fetoprotein values ≥ 25.5 were found to have sensitivity = 61.2% and specificity = 58.21 %.

Discussion

Hepatocellular carcinoma is ranked as the fifth most common cancer and the second most frequent cause of cancer-related death globally. It represents about 90% of primary liver cancers and considered a major global health problem [12].

In our study, hepatocellular carcinoma were related to elder age group, cigarette smoking with higher BMI. Also, HCC patients had significantly higher ALT and AST levels than patients with cirrhosis.

HCC patients had significantly higher miRNA 330 and miRNA 621 levels than patients with cirrhosis and healthy controls. Unlike other groups, there was no statistically significant difference between patients with cirrhosis and healthy controls in their miRNA 330 and miRNA 621 levels.

Similar to our results, a study was designed to observe the biological significance of miR-330 in HCC cases. MiR-330 level was significantly higher in HCC than normal specimens and that higher expression of miR-330 was significantly associated with more aggressive phenotypes and shorter overall survival in HCC [13].

Also another study was observing the biological significance of miR-330 in hepatocellular carcinoma. It demonstrated that the miR-330 level was significantly higher in HCCs than in normal tissues. MicroRNA -330 up-regulation has a prognostic impact on HCC cells and that this miRNA exerts its oncogenic activity in HCC cells. MiR-330 may represent a new prognostic marker and potential therapeutic target for HCC. Moreover, high expression of miR330 may be associated with some aggressive tumor parameters (i.e., tumor size, metastasis, and vascular invasion) and reduced overall survival in HCC patients. There are potential ability of miR-330 to promote HCC growth and progression. Over-expression of miR-330 was found to facilitate cell proliferation, invasion and tumorigenesis of HCC cells [14].

A study reported the critical role of miR-330-5p in enhancing HCC progression as miR-330-5p expression which was upregulated in HCC tissues and was associated with tumor size, number and node Metastasis stage in HCC patients. Therefore, miR-330-5p may provide a novel and promising prognostic and therapeutic target for HCC [15].

Another study included 90 patients to observe the expression of miR-621which found to be lower in HCC cells with association of poor prognosis in such cases. It showed also miR-621 can be used as a novel therapeutic target for HCC treatment as it significantly enhance radiosensitivity in HCC patients [11].

Unlike our study, a study showed that miR-621 was downregulated in the HCC cells and that poor survival was linked to lower expression of miR-621. Thus, these results revealed that miR-621 acts as a tumor suppressor in HCC via downregulation of CAPRIN1. MiR-621 could be a novel biomarker for diagnosis and prognosis for HCC [16].

Another study was observing the biological function of miR-621 in cases of bladder cancer. It demonstrated that miR-621 was downregulated in cancerous tissues and that miR-621 expression was negatively correlated with overall survival in such group [17].

A study included 52 patients with primary liver tumours with dysplastic nodules and hepatocellular carcinoma who were examined for microRNAs (miR-122, miR-100, miR-10a, miR-198, miR-145). In liver tumour cases some were overexpressed (which were: miR-122, miR-100 and miR-10a) while others were downregulated (which were: miR-198 and miR-145)[18].

In addition, another study identified seven miRNAs (hsa-miR-199a-3p, hsa-miR-199b-3p, hsa-miR-139-5p, hsa-miR-139-3p, hsa-miR-424-3p, hsa-miR-1269b and hsa-miR- 1269a) which showed different expressions in HCC and normal liver tissues. Some can function as tumour suppressors in cases of HCC (as hsa-miR-199a-3p, has-miR- 199b-3p, hsa-miR-139-5p, hsa-miR-139-3p, hsa-miR-424-3p), While others can considered as onco-miRNAs in cases of HCC (as hsa-miR-1269b and hsa-miR-1269a) [19].

In contrast to our results, a study was done to examine the effect of hsa-miR-330 on MCF-7 cell line. It showed that hsa-miR-330 has a tumor-suppressive effects on breast cancer cells by exerting antiproliferative and anti-migrative effects [20].

Another study evaluated the expression levels of miR-330 in prostatic epithelial cell lines, PZ-PHV-7, PWR-1E and RWPE-1, and prostate cancer lines, PC-3, LNCap, 22Rv1 and DU145, using miR-quantitative RT–PCR analysis. It showed that miR-330 can act as a tumor suppressor in prostate cancerous cells as its level was down regulated in prostate cancer tumors [21].

Another study included HCC, chronic HCV patients, liver cirrhosis and health controls to confirm that miR-122 can be used as diagnostic markers for HCV-related HCC [22]. These results go along with our study which confirmed the role of microRNA 330 and microRNA 621 in detection of hepatocellular carcinoma in comparison with hepatic cirrhosis patients and healthy subjects.

Also, a study investigated 119 patients diagnosed with HCC who underwent routine curative surgery. It showed that miR-21 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues. The results of this study suggested miR-21 expression level could be a novel potential biomarker for HCC prognosis [23].

Conclusion

MicroRNA 330 and microRNA 621 are considered as significant tools for prediction of HCC development, Our study concluded the significance of microRNA 330 and microRNA 621 in cases of HCC patients as they had significantly higher miRNA 330 and miRNA 621 levels than patients with cirrhosis and healthy controls.

References

[1] Ferrer-f J, Forner A, Liccioni A, Miquel R. 2016. Prospective Validation of Ab Initio Liver Carcinoma Upon Detection of Risk Factors for Recurrence After Resection. 63(3):839–849. doi:10.1002/hep.28339.

[2] Mokhtar N, Gouda I, Adel I. 2007. Cancer Pathology Registry 2003-2004 and Time Trend Analysis. :2007.

[3] Gomaa AI, Hashim MS, Waked I. 2014. Comparing staging systems for predicting prognosis and survival in patients with hepatocellular carcinoma in Egypt. PLoS One. 9(3): 1–11. doi:10.1371/journal.pone.0090929.

[4] El-Serag HB. 2001. Epidemiology of hepatocellular carcinoma. (2001). Clin Liver Dis.: 5 :87-107.

[5] Gonzaga-Jauregui, Claudia, James R. Lupski, Richard A. Gibbs. 2012. Human genome sequencing in health and disease. Annual review of medicine; 63: 35-61.

[6] Soifer, H. S., Rossi, J. J., & Sætrom, P. 2007. MicroRNAs in disease and potential therapeutic applications. Molecular therapy, 15(12), 2070-2079.

[7] Ardekani AM, Naeini MM. 2010. The role of microRNAs in human diseases. Avicenna J Med Biotechnol. 2(4):161–179.

[8] Chang MH, You SL, Chen CJ, Liu CJ, Lee CM, Lin SM, et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. J Natl Cancer Inst;101:1348-55.

[9] Chu, A. S., & Friedman, J. R. (2008). A role for microRNA in cystic liver and kidney diseases. The Journal of clinical investigation, 118(11), 3585-7.

[10] X. Jin, Y.-F. Ye, S.-H. Chen, C.-H. Yu, J. Liu, Y.-M. Li. 2009. MicroRNA expression pattern in different stages of nonalcoholic fatty liver disease. Digestive and liver disease, 41(4), 289-97.

[11] Shao Y, Song X, Jiang W, Chen Y, Ning Z, Gu W, Jiang J. 2019. MicroRNA-621 Acts as a Tumor Radiosensitizer by Directly Targeting SETDB1 in Hepatocellular Carcinoma. Mol Ther. 27(2):355–364. doi:10.1016/j.ymthe.2018.11.005. https://doi.org/10.1016/j.ymthe.2018.11.005

[12] Galle PR, Forner A, Llovet JM, Mazzaferro V, Piscaglia F, Raoul JL, Schirmacher P, Vilgrain V. 2018. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol. 69(1): 182–236. doi: 10.1016/j.jhep.2018.03.019. https://doi.org/10.1016/j.jhep.2018.03.019.

[13] Hu X, Feng Y, Sun L, Qu L, Sun C. 2017. Roles of microRNA-330 and Its Target Gene ING4 in the Development of Aggressive Phenotype in Hepatocellular Carcinoma Cells. Dig Dis Sci (2017) 62:715–722 DOI 10.1007/s10620-016-4429-2.

[14] Hu X, Feng Y, Sun L, Qu L, Sun C. 2017. Roles of microRNA-330 and Its Target Gene ING4 in the Development of Aggressive Phenotype in Hepatocellular Carcinoma Cells. Dig Dis Sci. 62 (3): 715–722. doi:10.1007/s10620-016-4429-2.

[15] Xiao S, Yang M, Yang H, Chang R, Fang F and Yang L. 2018. miR-330-5p targets SPRY2 to promote hepatocellular carcinoma progression via MAPK/ERK signaling. Oncogenesis (2018) 7:90. DOI 10.1038/s41389-018-0097-8.

[16] Zhang Y, You W, Zhou H, Chen Z, Han G, Zuo x, et al.2018. Downregulated miR-621 promotes cell proliferation via targeting CAPRIN1 in hepatocellular carcinoma. Am J Cancer Res 2018; 8(10):2116-2129www.ajcr.us/ISSN:2156-6976/ajcr0083151.

[17] Tian H, Wang X, Lu J, Tiand W, Chen P. 2019. MicroRNA-621 inhibits cell proliferation and metastasis in bladder cancer by suppressing Wnt/β-catenin signalling. Chemico-Biological Interactions 308 (2019) 244–251.

[18] Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, Dienes HP, Odenthal M. 2008. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology. 47(4):1223–1232. doi:10.1002/hep.22158.

[19] Wang X, Gao J, Zhou B, Xie J, Zhou G, Chen Y. 2019. Identification of prognostic markers for hepatocellular carcinoma based on miRNA expression profiles. Life Sci. 232(June). doi: 10.1016/j.lfs.2019.116596.

[20] Hosseinzadeh E, Mansoori B, Mohammadi A, Khaze V, Rezazadeh M, Baradaran B. 2019. The inhibitory effect of hsa-mir-330 replacement on the proliferation and migration of breast cancer MCF-7 cells. Int J Women's Heal Reprod Sci. 7(3):360–365. doi:10.15296/ijwhr.2019.59. https://doi.org/10.15296/ijwhr.2019.59.

[21] Lee KH, Chen YL, Yeh SD, Hsiao M, Lin JT, Goan YG, Lu PJ. 2009. MicroRNA-330 acts as tumor suppressor and induces apoptosis of prostate cancer cells through E2F1-mediated suppression of Akt phosphorylation. Oncogene. 28(38):3360–3370. doi:10.1038/onc.2009.192.

[22] Li J, Qiyu S, Wang T, Jin B, Li N. 2019. Improving the Detection of Hepatocellular Carcinoma using serum AFP expression in combination with GPC3 and micro-RNA miR-122 expression. Open Life Sci. 14(1):53–61. doi:10.1515/biol-2019-0007.

[23] Wang WY, Zhang HF, WangL, Ma YP, Gao F, Zhang SJ, et al. 2014. miR-21 expression predicts prognosis in hepatocellular carcinoma. Clinics and Research in Hepatology and Gastroenterology (2014) 38, 715—719.

Citation: **Amal Ahmed Mohamed, Dina M. Abo-Elmatty, Noha M. Mesbh, et al.** *Rectourethral Fistula in Children with Anorectal Malformation. Archives of Gastroenterology and Hepatology. 2020; 3(1): 21-29.*