RESEARCH

MicroRNA 21as a novel biomarker in hepatitis C virus-related hepatocellular carcinoma

Maha Assem Hussein[1*] , Amal Fathy Mohammed Radwan[1], May Mohsen Fawzi[1], Laila Ahmed Rashed[2] and Eman Hassan Ahmed Ibrahim Saad

Abstract

Background: Hepatocellular carcinoma is considered one of the most common cancers occurring in human population all over the world. It became an increasingly threatening malignancy due to both morbidity and mortality. Chronic viral hepatitis B and hepatitis C are two risk factors, which account for 80–90% of all HCC cases worldwide. Alfa Feto protien is used as a tumor marker for HCC diagnosis and prognosis prediction; however, its false negative rate when used alone is as high as 40% for patients with early-stage HCC. AFP levels remain normal in 15–30% of all the patients, even patients with advanced HCC. It has been demonstrated that miRNAs (MicroRNAs) are an important class of non-coding RNAs. They act as tumor oncogenes or suppressors and are involved in the HCC development. MiRNAs are endogenous nucleotides that can be found in intra- and extracellular spaces, such as the blood, urine, and saliva.

The study evaluated the miRNA 21 as a novel biomarker in patients with HCV related hepatocellular carcinoma.

Results: The study was conducted on three groups. Group (1) included 25 patients with liver cirrhosis due to hepatitis C virus infection. Group (2) included 25 patients with hepatocellular carcinoma (HCC) on top of liver cirrhosis due to hepatitis C virus infection. Group (3) included 10 normal control subjects. There was a significant difference in the mean level of miRNA between the three groups with p value < 0.001 with the highest value in group 2 (8.28 ± 2.55), then in group1 (5.04 ± 2.11) and the lowest in group 3 (control) (1.02 ± 0.07). MiRNA 21 has a sensitivity of 68% and a specificity of 96%, to differentiate between the liver cirrhosis group and HCC group.

Conclusion: miRNA 21 can be a promising marker for detection of patients with HCV-related hepatocellular carcinoma, with higher specificity compared to α feto protein; however, its cost is higher.

Keywords: Hepatocellular carcinoma, HCV, MicroRNA 21

Background

Hepatocellular carcinoma (HCC) is one of the most common cancer in humans all over the world [1]. Hepatocellular carcinoma (HCC) has become an increasingly threatening malignancy due to both morbidity and mortality [2].

HCV infection leads to chronic inflammation in the liver, which initiates several changes as a production of oxidative stress, steatosis, progressive fibrosis, cirrhosis, and finally HCC [1].

AFP is used as a tumor marker for HCC diagnosis and prognosis prediction; however, its false negative rate when used alone is as high as 40% for patients with early-stage HCC. It may remain normal in 15–30% of all the patients, even patients with advanced HCC [3, 4].

*Correspondence: maha.assem@cu.edu.eg

[1] Internal Medicine - Faculty of Medicine, Cairo University, Cairo, Egypt
Full list of author information is available at the end of the article

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Despite the major advances achieved in the diagnostic management of HCC, only one third of the newly diagnosed patients can receive curative treatments [5]. Better understanding of HCC biology in addition to advances in technology have led to the discovery of novel biomarkers [5].

MiRNAs are considered an important class of noncoding RNAs which act as tumor oncogenes or suppressors and are involved in the HCC development [6]. They are endogenous nucleotides that are found in intra- and extracellular spaces, such as the blood, urine, and saliva [7, 8].

MiRNA-21 has been involved in the development of fibrosis in multiple organs and has also been suggested to act as an "oncomir." MiRNA 21 was the microRNA that showed the strongest upregulation in activated hepatic stellate cells (HSCs) in multiple models of fibrogenesis [9]. MiRNA 21 is closely related to hepatic malignancy.

Methods

The study was a prospective case-control study conducted on patients with HCV related liver cirrhosis and HCC. The study included 50 Egyptian patients with HCV infection recruited from the Internal Medicine department, [BLINDED FOR PEER REVIEW] University Hospital during the period January 2018 to January 2019. They were 35 males and 15 females with their age ranged from 45 to 82 years with a mean age of 62.22 ± 8.7. Group 1 included 25 patients with liver cirrhosis due to hepatitis C virus infection. Group 2 included 25 patients with hepatocellular carcinoma (HCC) on top of liver cirrhosis due to hepatitis C virus infection. Group 3 included 10 volunteer normal control subjects. We excluded patients with hepatitis B virus infection, subjects with significant alcohol consumption (over 20 g per day in men or 10 g per day in women), patients with liver cirrhosis due to other causes than HCV infection, patients with hemodynamic instability, hepatorenal syndrome, hepatic encephalopathy, and spontaneous bacterial peritonitis. The study protocol was approved by the Cairo University ethical committee. All participants provided a written informed consent. All participants underwent complete screening panel, including medical history, clinical examination, and biochemical profile including complete blood count, liver and kidney function tests, coagulation profile, and serum AFP. Abdominal ultrasonography was done for diagnosis of liver cirrhosis, and detection of HCC which was confirmed by triphasic C.T. Serum samples from all participants were obtained for detection and quantification of the miRNA using reverse transcription-polymerase chain reaction (RT-PCR).

Statistical analysis

Data were coded and entered using the SPSS (Statistical Package for the Social Sciences) version 25. Data was summarized using the mean, standard deviation, median, minimum, and maximum or median and interquartile range in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003a). For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5 [10]. Correlations between quantitative variables were done using Spearman correlation coefficient. The ROC curve was constructed with area under curve analysis performed to detect best cutoff value of miRNA 21 for detection of HCC and liver cirrhosis. *P*-values less than 0.05 were considered as statistically significant.

Results

The mean level of platelet count, ALT, AST, and GGT were significantly higher in group 2 (patients with hepatocellular carcinoma (HCC) on top liver cirrhosis) in comparison to group 1 (patients with liver cirrhosis) (P <0.001). The mean level of total and direct bilirubin were significantly higher in group 2 in comparison to group 1 (P <0.004). There was no significant difference between the two groups in age, hemoglobin, albumin, prothrombin time, or INR (Table 1).

Regarding the mean level of miRNA, there was a significant difference between the three groups with *p* value < 0.001 with the highest value in group 2 (8.28 ± 2.55), then in group 1 (5.04 ± 2.11) and the lowest in group 3 (control) (1.02 ± 0.07) (Table 2).

There was a positive correlation between miRNA and platelet count, AST, ALT, and α feto protein (Table 3)

The ROC curve showed that the miRNA 21 has sensitivity 68% and specificity 96%, to differentiate between liver cirrhosis group and HCC group with an area under the curve of 0.834 and a cutoff value of 7.85. While alfa feto protein sensitivity was 84% and specificity 84% for detection of HCC from liver cirrhosis in our studied patients Fig. 1.

Discussion

In Egypt, there was a remarkable increase in the proportion of HCC among patients with chronic liver diseases. This is due to the increasing risk factors including the emergence of HCV over the equal time frame, the contribution of (HBV) infection, improvement of the screening programs, and diagnostic tools for liver cancer as well as the improved survival rates among patients with cirrhosis

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Table 1 Demographic and laboratory data of the patients

Parameter	Group 1: Patients with liver cirrhosis (mean ± SD)	Group 2: Patients with liver cirrhosis and HCC group (mean ± SD)	P value
Age (year)	63.44 ± 9	61 ± 7.76	0.472
Platelets (/cmm)	$95.8 \times 10^3 \pm 38.5$	$148.40 \times 10^3 \pm 55.24$	**0.001**
Haemoglobin (g/dl)	9.62 ± 1.58	10.4 ± 1.42	0.053
Albumin (mg/dl)	2.52 ± 0.62	2.57 ± 0.61	0.719
ALT (U/ml)	35.08 ± 25.37	81.28 ± 72.27	**< 0.001**
AST (U/ml)	59.24 ± 31.36	138.20 ± 93.02	**< 0.001**
GGT (U/ml)	48.56 ± 45.48	155.44 ± 159.04	**0.001**
Total bilirubin (mg/dl)	1.98 ± 2.79	3.91 ± 4.20	**0.004**
Direct BIL. (mg/dl)	0.89 ± 1.72	1.84 ± 1.88	**0.004**
Prothrombin time (second)	17.15 ± 3.49	16.17 ± 2.21	0.392
INR	1.53 ± 0.40	1.50 ± 0.36	0.923
Alfa feto protein (AFP)	5.77 ± 6.08	4762.32 ± 13800.58	**< 0.001**
miRNA	5.04 ± 2.11	8.28 ± 2.55	**< 0.001**

P values <0.05 were considered statistically significant

to allow time for some of them to develop HCC [11]. Early detection of HCC is important as it improves overall survival, especially for patients who are able to receive potentially curative therapy [12].

Therefore, searching for new biomarkers became mandatory.

MiRNA-21 gene is located on human chromosome 17, which is a highly conserved microRNA. Micro RNA-21 is upregulated in various solid tumors, including breast cancer, lung cancer, gastric cancer, and colorectal cancer [13].

Micro-RNA-21 has been related to pathophysiological processes such as inflammation, fibrosis, cardiac hypertrophy, and ventricular remodeling [14]. The level of circulating microRNA-21 in patients with hypertensive heart disease was higher than that in the control group and was positively correlated with the expression of serum myocardial fibrosis markers [15].

The study evaluated miRNA 21 as a novel biomarker in patients with HCV-related hepatocellular carcinoma.

In our study we found that the plasma level of miRNA 21 was significantly higher in liver cirrhosis and HCC groups in comparison to the healthy control group, being highest among HCC patients compared to the other two groups with *p*-value < 0.001. Our results were similar to Guo et al. [16], El Gedawy et al. [17], Karakatsanis et al. [18], and Demerdash et al. [19].

Regarding the relation between miRNA 21 and tumor burden, our data showed that there was no statistically significant difference in the level of neither α feto protein nor miRNA 21 in relation to focal lesion number, site, or size of the tumor. On the other hand, Yi and Li reported that high expression of miRNA-21

was correlated with a tumor size of >5 cm but was not significantly correlated with tumor number or differentiation [20]. Hegazy et al. also reported no significant impacts of tumor characters as regards the number, site, size, and microscopic features on serum or tissue expression of miRNA 21 [21].

ROC analysis showed that miRNA 21 has a cutoff value of 7.85 to differentiate between the liver cirrhosis group and HCC group with a sensitivity of 68% and specificity of 96% and an area under the curve of 0.834, while alfa feto protein sensitivity was 84% and specificity was 84% for detection of HCC from liver cirrhosis.

Guo et al. study showed that serum miRNA-21 maintained its diagnostic efficiency in AFP-negative HCC patients. Their optimal diagnostic cutoff for miR-21 was 8.023-fold. They reported that AFP showed lower sensitivity and specificity and suggested that miRNA-21 could have a greater diagnostic performance in discriminating HCC, liver cirrhosis, and chronic hepatitis than AFP [16], while in our study, the sensitivity of miRNA 21 was lower than AFP (68% for miRNA 21 compared to 84%), but with higher specificity. El Gedawy et al. showed that miRNA-21 revealed that, at a cut-off value of 3.93 (fold expression), the sensitivity and specificity for differentiation of HCC cases were 93% and 90%, respectively [17]. Hegazy et al. found that the specificity of miRNA-21 was superior to AFP in diagnosis of HCC [21].

There was statistically significant difference between the liver cirrhosis group and HCC group as regards the median of α feto protein being higher in the HCC group with *p*-value < 0.001. These results were similar to studies done by Sacco et al. (2014). Tsai et al. (2014), Chen et al. (2006), and Changchien et al. (2008) [22–25].

Table 2 Comparison between 3 groups regarding serum micro-RNA level

	Cirrhosis					HCC					Control					P value
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
mRNA	5.04	2.11	4.90	1.50	9.03	8.28	2.55	8.70	2.70	12.10	1.02	0.07	1.00	0.93	1.20	< 0.001

Table 3 The correlation between miRNA 21 and other parameters (ALT, AST, platelet count, and α feto protein)

All patients		mRNA
platelets x 10^3 (/cmm)	Correlation coefficient	0.424
	P value	**0.002**
	N	50
ALT (U/ml)	Correlation coefficient	0.289
	P value	**0.042**
	N	50
AST (U/ml)	Correlation coefficient	0.398
	P value	**0.004**
	N	50
α feto protein (*N* up to 10) ng/ml	Correlation coefficient	0.384
	P value	**0.006**
	N	50

Also, the miRNA 21 level was positively correlated with platelet count, AST, α feto protein, and ALT in all patients. El Gedawy et al. reported that there was a significant positive correlation between miRNA-21 and AFP in the HCC group and a significant positive correlation was detected between miRNA-21 and ALT in chronic liver disease group [17]. Demerdash et al. revealed a positive correlation between miRNA-21 and serum transaminase levels (ALT and AST), in both CHC and HCC groups [19].

Similarly, Bihrer et al. found that miRNA-21 serum levels were strongly correlated with ALT and AST activities [26].

Hegazy et al. reported that the serum miRNA 21 was directly correlated to α-fetoprotein [21].

In our study, the platelet count was significantly higher in HCC patients than liver cirrhosis patients, and this could be explained by being part of paramalignant syndrome associated with HCC or as hepatic malignancies can synthesize thrombopoietin, a major factor in platelet production. Platelets have been reported to interact with cancer cells and be involved in their growth enhancement [27]. Tumor microenvironment, needed for the development of tumor cells, is a complex system, which includes stromal, endothelial and immune cells, cytokines and growth factors, proteolytic enzymes like

Fig. 1 ROC curve for detection of HCC from liver cirrhosis using miRNA 21
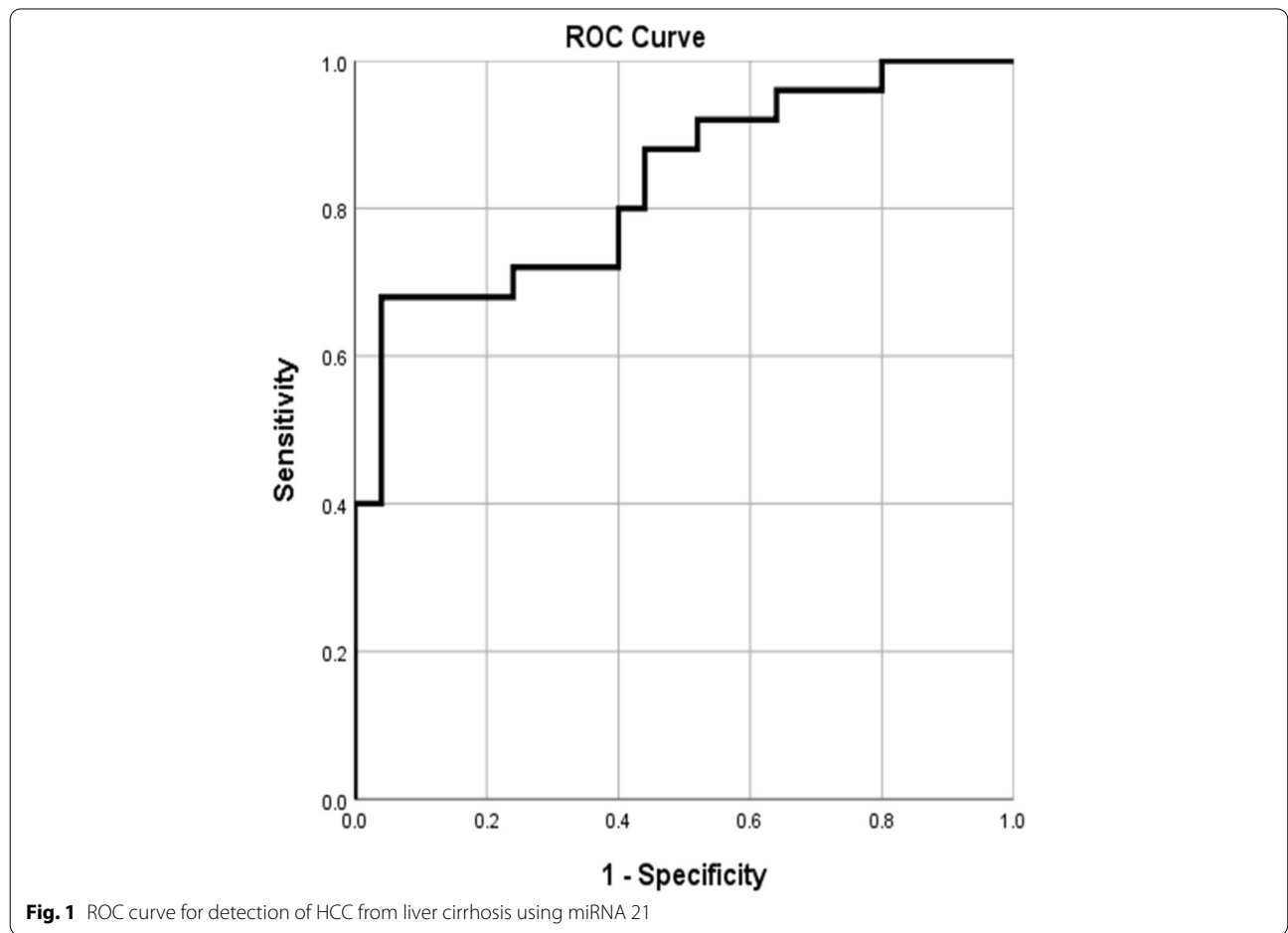
matrix metalloproteinases (MMPs), extracellular matrix (ECM) proteins, and microvesicles and platelets [28, 29]. As tumor cells activate platelets, activated platelets in turn contribute to several steps of carcinogenesis ,such as the secretion of granules containing growth factors (IGF-1, transforming growth factor-β (TGF-β) and PDGF), pro-angiogenic, and anti-angiogenic factors like (angiopoietin-1, angiostatin) [30].

Yalmaz and Erdal [30] in their study concluded that platelet count has a useful prognostic value in HCC and that it can be included in screening programs and can also be used to predict disease prognosis.

We found a statistically significant difference between the liver cirrhosis group and HCC group in the mean value of platelet count, AST, ALT, and GGT with (p-value 0.001), bilirubin (total and direct) with p-value 0.004 being higher in the HCC group. The elevation of liver function tests in HCC patients can be attributed to the on-going damage of the liver tissue due to malignant tissue growth, invasion, and subsequent loss of functioning liver cells.

Carr BI [31] reported that significantly higher aggressiveness score for HCC was associated with higher GGT, ALP, AST, and total bilirubin, as well as for platelets compared to other patients. It was also found to be associated with higher incidence of portal vein thrombosis, tumor multifocality, and higher AFP levels [32].

There was no significant difference between the two groups in age, hemoglobin, albumin, prothrombin time, or INR. Regarding the mean value of hemoglobin, it may be due to small sample size or may be due to hemoglobin drop as a result of blood loss from variceal bleeding which is more marked and frequent in HCC patients.

Bihrer et al. reported that the level of miRNA-21 in serum from patients with HCC on top of chronic hepatitis C (CHC) was elevated compared to healthy controls. However, they also reported increased levels of miRNA-21 in serum of patients with chronic hepatitis C without HCC with no difference between serum miRNA-21 level in both groups [26].

Hegazy et al. and Tomimaru et al. showed that miRNA 21 was overexpressed in the serum of patients with HCC compared to patients with chronic hepatitis [21, 33].

Conclusions

In conclusion, this study has shown that miRNA 21 is a promising marker for detection of patients with HCV-related hepatocellular carcinoma, with higher specificity and lower sensitivity compared to α feto protein; however, its cost is higher.

We recommend further studies of miRNA 21 in HCC patients to further evaluate miRNA 21 efficacy in detection of AFP-negative HCC cases as well as further studies of miRNA 21 in HCC patients with considering gender to detect if there is difference in its value between male and female sex and to detect performance of miRNA 21 in metastatic HCC as well as staging of HCC.

Authors' contributions
AF was responsible for the concept of the work. MF was responsible for the interpretation of the data. LR was responsible for the laboratory investigations. EH was responsible for collecting the data and its analysis. MA was responsible for drafting the work and its revision. The authors have read and approved the manuscript.

Availability of data and materials
Not applicable

Declarations

Ethics approval and consent to participate
This study was approved by Cairo University ethical committee. Written informed consent was obtained from all participants. Also, a consent was obtained from all participants for publication. Reference number is I-210316.

Consent for publications
All the authors approved.

Competing interests
The authors read and approved the final manuscript.

Author details
[1]Internal Medicine - Faculty of Medicine, Cairo University, Cairo, Egypt. [2]Medical Biochemistry and Molecular Biology - Faculty of Medicine, Cairo University, Cairo, Egypt.

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