

# Serum microRNA 143 as a potential biomarker for the diagnosis of hepatitis C virus-related hepatocellular carcinoma

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## Background

Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer worldwide. Early recognition of the onset of HCC would help to select more effective therapies for patients leading to a better prognosis and life span. So, the development of effective markers for the diagnosis of HCC could have an impact on HCC-related cancer mortality. MicroRNAs (miRNAs) are reported as a group of small noncoding RNAs that can function as endogenous RNA interference to regulate the expression of targeted genes.

## Aim

To study serum miR-143 expression level in patients with hepatitis C virus (HCV)-related HCC.

## Patients and methods

The present study was conducted on 60 participants classified into group A: 30 patients with HCV-related cirrhosis with HCC; group B: 15 patients with HCV-related cirrhosis without HCC; and group C: healthy participants as control. Expression of miR-143 in the serum of all participants was obtained in all groups. Total serum RNA was extracted with small RNA enrichment followed by reverse transcription real-time PCR. Expression of miR-143 in the serum of all participants was obtained using the comparative cycle threshold method ( $2^{-\Delta\Delta CT}$ ) after normalization for the expression of Syn-cel-miR-39 mi script miRNA mimic as control. MiR-143 expression levels were then compared in different groups.

## Results

The mean serum miR-143 levels were significantly higher in cirrhotic patients without and with HCC than in healthy participants ( $1.69 \pm 0.64$ ,  $13.0 \pm 6.23$  vs.  $0.56 \pm 0.29$ ,  $P < 0.001$ , respectively) and in patients with HCC than in patients without HCC ( $P < 0.001$ ). The mean serum miR-143 levels were significantly higher in cirrhotic patients with HCC Barcelona Clinic Liver Cancer stage D than stage B ( $19.91 \pm 2.40$ ,  $13.41 \pm 1.80$ ,  $P < 0.001$ , respectively) and in stage B compared with stage A ( $13.41 \pm 1.80$ ,  $5.68 \pm 1.86$ ,  $P < 0.001$ , respectively).

## Conclusion

The significantly higher serum level of miR-143 in cirrhotic patients with HCC compared with those without and to normal participants may suggest its role in hepatocarcinogenesis and may have a value as a potential biomarker for early diagnosis of HCV-related HCC.

## Keywords:

hepatitis C, hepatocellular carcinoma, microRNA

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## Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer with an estimated incidence of one million cases per year. It is the fifth most common cancer worldwide and the third leading cause of cancer death after lung and stomach cancer [1].

Because of detection at an advanced, nonoperable stage, the prognosis of HCC is usually poor. This limits the potential for curative surgical treatment only for cases with small HCC malignancies. This problem calls for more surveillance strategies targeted to the population at risk, to be used for

early detection of HCC [2]. Currently, the diagnosis of HCC depends on clinical information, hepatic ultrasonography, and measurement of serum alpha-fetoprotein (AFP) at 6–12 month intervals. However, neither of the aforementioned is adequate for detecting very small (<2 cm) HCC tumors. The sensitivity of AFP is low particularly in the detection of early-stage HCC. As such, no marker has been proven

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to be reliable to detect small HCC tumors (2 cm or less). Therefore, it is urgently needed to identify novel biomarkers with high efficacy for early detection and therapeutic monitoring of HCC [3].

MicroRNAs (miRNAs) are small noncoding RNAs that can function as post-transcriptional regulators of expression of their targeted genes [4]. In addition to their presence in tissues, miRNAs were recently shown to be secreted from the cells and can thereby gain access to different body fluids such as plasma, serum, urine, saliva, sputum, etc. [5]. Nevertheless, they were shown to remain highly stable upon exposure to conditions such as boiling, extremes of pH, and long-duration storage that induce immediate degradation of free RNA [6].

The advent of highly sensitive real-time (RT) PCR assay detection methods aided the detection of miRNAs in blood. They were also proven as minimally invasive and their results showed high reproducibility. Circulating miRNAs can therefore be used as noninvasive, sensitive disease biomarkers. Today, prediction and diagnosis of HCC via determining disease-specific circulating miRNAs has become the focus of many studies. Researchers have previously reported the potential clinical application of circulating miRNAs (such as miR-122) as diagnostic and prognostic HCC biomarkers. Nonetheless, further investigation of aberrant miRNA expression could lead to the discovery of novel miRNA biomarkers for HCC [7].

Among the large number of miRNAs, miR-143, owing to its association with malignancies, has been widely studied. Mir-143 is a short RNA molecule highly conserved in vertebrates and has been implicated in several cancers [8]. The expression of miR-143 was shown to be downregulated in breast, gastric, and colorectal cancers [9–11]. However, it was found that miR-143 was overexpressed in other cancers such as pancreatic ductal adenocarcinoma [12] and during the differentiation of prostate cancer stem cells and could promote prostate cancer metastasis by repressing fibronectin-type III domain containing 3B expression [13].

Few previous studies were focused on the relationship between miR-143 expression and the diagnosis of HCC. So, the aim of this study was to study serum miR-143-expression level in patients with hepatitis C virus (HCV)-related HCC.

## Patients and methods

An informed consent was obtained from all participants included in the study. The study

protocol conformed to the ethical guidelines of the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Alexandria Faculty of Medicine Human Research Committee. This study was conducted on 60 participants allocated into three groups as follows:

Group A: 30 patients with HCV-related cirrhosis with HCC classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system into 10 patients with early-stage BCLC A; 10 patients with intermediate-stage BCLC B; and 10 patients with end-stage BCLC D. Group B: 15 patients with HCV-related cirrhosis without HCC. Group C: 15 age-matched and sex-matched healthy participants with no evidence of liver disease were included as the control group.

The diagnosis of chronic HCV infection with cirrhosis was based on the following criteria: (a) positive test for HCV antibody and (b) detectable serum HCV RNA and the diagnosis of cirrhosis was determined by clinical, biochemical, and ultrasonographic evidence.

The diagnosis of HCC was based on serum levels of AFP, ultrasonography, and triphasic computed tomography. All the patients of groups A and B were selected from the Hepatobiliary Unit of Alexandria University Hospital.

Patients who have the following criteria: seropositivity for HBV infection, other causes of chronic liver diseases, chronic illness, for example, collagen diseases, diabetes mellitus, autoimmune diseases, cardiac, respiratory, renal diseases, chronic alcoholism, and other malignancies were excluded from the study.

All patients included in the study were evaluated clinically as regards history taking and clinical examination focusing on symptoms and signs of chronic liver disease (previous upper gastrointestinal bleeding, jaundice, ascites, hepatic encephalopathy, and bleeding diathesis), presence of palpable focal hepatic lesions, and liver and spleen size. Blood samples were collected from all patients and healthy participants and the following tests were performed: complete blood picture, serum creatinine, liver test profile (serum aspartate aminotransferase) and alanine aminotransferase, serum albumin, serum bilirubin, serum gamma glutamyl transpeptidase and prothrombin time (PT), serum HCV RNA levels using RT-PCR and serum AFP levels using standardized enzyme-linked immunosorbent assay kit. The severity of the liver disease in patients with HCV-related cirrhosis was graded according to Child–Pugh

classification. Radiological examination using ultrasonographic/triphasic computed tomography examination was performed for the assessment of liver echo pattern, liver and spleen size, presence of ascites, and tumor characteristics in patients with HCC (maximum diameter, echo pattern, number of nodules, extension, portal vein invasion, and intrahepatic metastasis). The stage of HCC was determined according to the BCLC staging system.

#### RNA extraction to perform expression of serum microRNA 143

The blood samples were collected and centrifuged at 3000 rpm for 5 min to separate serum and then stored at  $-80^{\circ}\text{C}$  until RNA extraction. Total RNA was isolated from 100  $\mu\text{l}$  of serum previously thawed on ice using an miRNAeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The RNA was eluted with 40  $\mu\text{l}$  of RNase-free water and was stored at  $-80^{\circ}\text{C}$  until RT-PCR reaction. The concentration of all RNA samples was quantified using NanoDrop 1000 (Thermo Scientific, Wilmington, Delaware, USA). Thereafter, TaqMan miRNA assay was used to perform the expression levels of serum miRNA-143. All reagents, primers, and probes were obtained from Applied Biosystems (Foster City, California, USA). A measure of 5  $\mu\text{l}$  of total RNA was used from each sample for individual assays in 15  $\mu\text{l}$  reactions containing reverse transcription mixture and primers. The mix was incubated at  $16^{\circ}\text{C}$  for 30 min,  $42^{\circ}\text{C}$  for 30 min, and  $85^{\circ}\text{C}$  for 5 min; miRNA expression levels were quantified using Applied Biosystems Step One RT-PCR System, thermal cycling (Applied Biosystems). For this purpose, 1  $\mu\text{l}$  of reverse transcription reaction was mixed with 10  $\mu\text{l}$  of  $2\times$  TaqMan PCR mixtures, 1  $\mu\text{l}$  of TaqMan primer mix and probe and 8  $\mu\text{l}$  water to obtain a final volume of 20  $\mu\text{l}$ . An RT-PCR was done in triplicate, including nontemplate controls. The relative expression of mature miRNA was calculated using the comparative cycle threshold ( $2^{-\Delta\Delta\text{CT}}$ ) method. Syn-cel-miR-39 mi script miRNA mimic was used as a control to normalize the data.

#### Statistical analysis

Statistical analysis of the data was performed using SPSS program (version 20.0) (IBM-SPSS Inc., New York, New York, USA) for Windows. Statistical significance was assessed at  $P$  value less than 0.05. Sensitivity and specificity of serum miR-143 levels in discriminating cirrhotic patients with and without HCC were assessed by plotting a receiver-operating characteristic (ROC) curve and determining its cutoff value.

#### Results

Table 1 shows the demographic data of the studied groups. There was no statistically significant difference between the three groups regarding sex and age.

Table 2 shows that the mean hemoglobin level was significantly lower in the HCC group A patients than in healthy participants ( $9.22\pm 2.09$  vs.  $12.30\pm 0.44\text{g/dl}$ , respectively,  $P<0.001$ ). It was also significantly lower in cirrhotic group B patients than in healthy participants ( $10.26\pm 2.76$  vs.  $12.30\pm 0.44$  g/dl,  $P<0.008$ ), while there was no statistically significant difference between HCC group A and cirrhotic group B patients ( $9.22\pm 2.09$  vs.  $10.26\pm 2.76\text{g/dl}$ ). The mean platelet count was significantly lower in HCC group A patients and cirrhotic group B patients than in healthy participants ( $85.31\pm 53.49\times 10^3/\text{cmm}$  and  $119.40\pm 54.07\times 10^3/\text{cmm}$  vs.  $254.9\pm 31.6\times 10^3/\text{cmm}$ , respectively). Also there was a statistically significant decrease in platelet count in HCC group A patients compared with cirrhotic group B patients ( $85.31\pm 53.49\times 10^3/\mu\text{l}$  vs.  $119.40\pm 54.07\times 10^3/\mu\text{l}$ , respectively,  $P<0.021$ ).

Serum level of total and direct bilirubin, alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transpeptidase were significantly higher in group A and group B patients compared with group C, but no significant difference was found between group A and group B patients.

Mean serum albumin level was significantly lower in cirrhotic patients with and without HCC than in

**Table 1 Comparison between the studied groups according to the demographic data**

	HCC (N=30) [n (%)]	Cirrhosis (N=15) [n (%)]	Control (N=15) [n (%)]	Test of significance	P
Sex					
Male	18 (60.0)	9 (60.0)	9 (60.0)	$\chi^2=0.079$	1.000
Female	12 (40.0)	6 (40.0)	6 (40.0)		
Age (years)					
Minimum-maximum	38.0-67.0	45.0-65.0	48.0-65.0	F=0.157	0.855
Mean $\pm$ SD	56.0 $\pm$ 10.76	57.20 $\pm$ 4.84	56.67 $\pm$ 4.97		
Median	61.0	58.0	56.0		

$\chi^2$ ,  $\chi^2$  test; HCC, hepatocellular carcinoma. F, F value for analysis of variance test, significance between groups was done using post-hoc test (least significant difference).

**Table 2 The laboratory data of the studied groups**

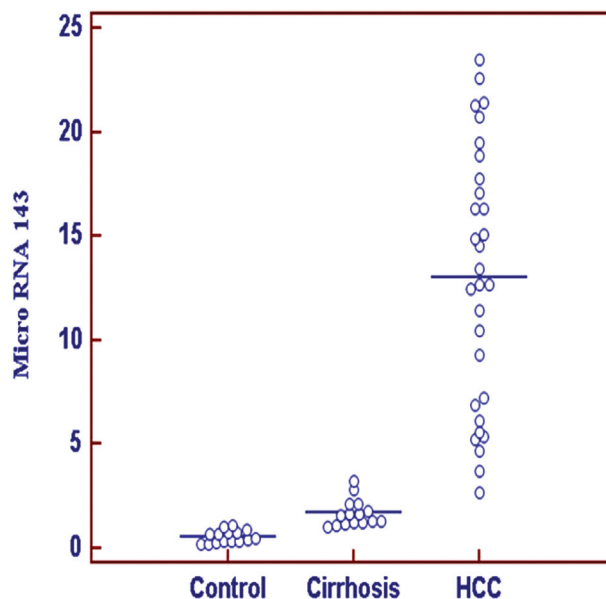
Laboratory test	HCC (N=30)	Cirrhosis (N=15)	Control (N=15)	P value
Hb (g/dl) (mean ±SD)	9.22 ±2.09	10.26 ±2.76	12.30 ±0.44	$P_1=0.111$ $P_2<0.001^*$ $P_3=0.008^*$
WBC×10 <sup>3</sup> /cmm (mean±SD)	6.0 ±2.63	5.44 ±2.92	7.6±1.2	$P_1=0.354$ $P_2<0.001^*$ $P_3<0.001^*$
Platelet×10 <sup>3</sup> /μl (mean±SD)	85.31 ±53.49	119.40 ±54.07	254.9 ±31.6	$P_1=0.021^*$ $P_2<0.001^*$ $P_3<0.001^*$
Total serum bilirubin (mg/dl) (mean±SD)	2.11 ±1.16	3.16 ±2.21	1.04 ±0.2	$P_1=0.181$ $P_2=0.001^*$ $P_3=0.003^*$
Direct serum bilirubin (mg/dl) (mean±SD)	1.05 ±0.65	1.58 ±1.54	0.17 ±0.04	$P_1=0.539$ $P_2<0.001^*$ $P_3<0.001^*$
ALT (U/l) (mean ±SD)	73.03 ±82.0	52.67 ±26.40	41.13 ±6.2	$P_1=0.791$ $P_2<0.001^*$ $P_3=0.003$
AST (U/l) (mean ±SD)	87.88 ±84.23	70.47 ±27.70	39.5 ±4.8	$P_1=0.791$ $P_2<0.001^*$ $P_3=0.003^*$
ALP (IU/l) (mean ±SD)	111.97 ±21.30	123.40 ±32.13	87.9 ±3.23	$P_1=0.107$ $P_2=0.001^*$ $P_3<0.001^*$
GGT (U/l) (mean ±SD)	114.0 ±19.0	126.07 ±32.03	86.33 ±4.6	$P_1=0.074$ $P_2<0.001^*$ $P_3<0.001^*$
Albumin (g/dl) (mean±SD)	3.08 ±0.79	2.81 ±0.98	3.99 ±0.4	$P_1=0.271$ $P_2<0.001^*$ $P_3<0.001^*$
PT (s) (mean ±SD)	17.04 ±5.02	21.90 ±10.62	11.1 ±0.5	<0.001*
AFP (ng/dl) (mean±SD)	922.4 ±2042.4	14.93 ±19.47	1.8±0.8	<0.001*

AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; Hb, hemoglobin; HCC, hepatocellular carcinoma; PT, prothrombin time; WBC, white blood cell count.  $P_1$ , P value for groups A and B.  $P_2$ , P value between groups A and C.  $P_3$ , P value between groups B and C. Statistically significant at P value of less than or equal to 0.05.

healthy participants (3.08±0.79 and 2.81±0.98 vs. 3.99 ±0.4 g/dl, respectively), while there was no statistically significant difference between cirrhotic patients with and without HCC. The mean PT showed significant increases in cirrhotic patients with HCC and without HCC (17.04±5.02 and 21.9±10.62 s) compared with healthy participants (11.1±0.5 s); also, there was statistically significant increases in PT in cirrhotic patients without HCC than with HCC. The mean serum AFP levels were significantly higher in cirrhotic patients with and without HCC than healthy participants (922.4±2042.4 and 14.93±19.47 vs. 1.8 ±0.8 ng/dl, respectively) ( $P<0.001$ ) and in patients with HCC than without HCC ( $P<0.001$ ).

Table 3 shows that mean serum miR-143 levels were significantly higher in cirrhotic patients with and without HCC than in healthy participants (13.0

**Figure 1**



Comparison between the studied groups according to miR-143. miR-143, microRNA 143.

±6.23 and 1.69±0.64 vs. 0.56±0.29, respectively,  $P<0.001$ ) and in patients with HCC than in patients without HCC (Kruskal–Wallis test,  $\chi^2=49.408$ ,  $P<0.001$ ) (Fig. 1).

Table 4 shows that mean serum miR-143 levels were significantly higher in cirrhotic patients with HCC BCLC stage D compared with stages A and B (19.91 ±2.40, 13.41±1.80, and 5.68±1.86, respectively) (Kruskal–Wallis test,  $\chi^2=25.689$ ,  $P<0.001$  and  $r_s=0.941$ ). These data indicate that miR-143 was positively correlating with HCC stage.

Table 5 The mean serum level of miR-143 was significantly higher in cirrhotic patients with early HCC than without HCC (5.68±1.86 vs. 1.69±0.64, respectively,  $P<0.001$ ).

ROC curve analysis (Fig. 2) shows that the sensitivity and specificity of serum miR-143 expression level in discriminating cirrhotic patients with HCC from those without were 96.67 and 100%, respectively, at a cutoff level of more than 3.26 and area under the curve (AUC) was 0.996 indicating good discriminative power with P value less than or equal to 0.001. These results show that miR-143 could serve as a valuable biomarker for the diagnosis of HCC in HCV-related cirrhosis.

ROC curve analysis (Fig. 3) shows that the sensitivity and specificity of serum miR-143 in discriminating cirrhotic patients from healthy control group was 100 and 93.33%, respectively, at a cutoff level of more than

**Table 3 Comparison between the studied groups according to microRNA 143**

	HCC (N=30)	Cirrhosis (N=15)	Control (N=15)	KW, $\chi^2$	P
MiR-143					
Minimum–maximum	2.70–23.50	1.04–3.26	0.18–1.07	49.408*	<0.001*
Mean±SD	13.0±6.23	1.69±0.64	0.56±0.29		
Median	13.05	1.56	0.47		

$\chi^2$ ,  $\chi^2$  test; HCC, hepatocellular carcinoma; miR-143, microRNA 143; KW, Kruskal–Wallis. F, F value for analysis of variance test, significance between groups was done using post-hoc test (least significant difference). Statistically significant at P value of less than or equal to 0.05.

**Table 4 The relation between the stage of hepatocellular carcinoma and serum microRNA 143**

	BCLC			KW, $\chi^2$	P
	A (early) (N=10)	B (intermediate) (N=10)	D (late) (N=10)		
MiR-143					
Minimum–maximum	2.70–9.30	10.50–16.30	16.30–23.50	25.689*	<0.001*
Mean±SD	5.68±1.86	13.41±1.80	19.91±2.40		
Median	5.50	13.05	20.10		
Significance between groups	$P_1<0.001^*$ , $P_2<0.001^*$ , $P_3<0.001^*$				
$r_s$ (P)	0.941(<0.001*)				

BCLC, Barcelona Clinic Liver Cancer; KW,  $\chi^2$ ,  $\chi^2$  for Kruskal–Wallis test; miR-143, microRNA 143. Significance between groups was done using Mann–Whitney test.  $P_1$ , P value for comparing between A and B.  $P_2$ , P value for comparing between A and D.  $P_3$ , P value for comparing between B and D.  $r_s$ , Spearman's coefficient. \*Statistically significant at P value less than or equal to 0.05, significance between groups

**Table 5 Serum microRNA 143 in cirrhotic patients with early hepatocellular carcinoma and without hepatocellular carcinoma**

	BCLC stage A HCC (N=10)	Cirrhosis (N=15)	Z	P
MiR-143				
Minimum–maximum	2.70–9.30	1.04–3.26	4.049*	<0.001*
Mean±SD	5.68±1.86	1.69±0.64		
Median	5.50	1.56		

BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma; miR-143, microRNA 143; Z, P, Z and P values for Mann–Whitney test for comparing between the two groups. \*Statistically significant at P value less than or equal to 0.05.

1 and AUC equal to 0.996 indicating good discriminative power and statistically significant at P value less than or equal to 0.001. These results indicated that miR-143 could serve as a valuable biomarker for differentiating HCV-related cirrhosis from healthy participants.

ROC curve (Fig. 4) compares serum miR-143 and AFP in cirrhotic patients with HCC and patients without HCC. It shows a significant difference between AUC of miR-143 and AFP ( $P<0.001$ ) with a sensitivity and specificity of 96.67 and 100% for miR-143, respectively, and 80 and 100% for AFP, respectively, AUC=0.996 for miR-143 and 0.929 for AFP at a cutoff level of more than 3.26 for miR-143 and more than 72 for AFP. These results indicate that miR-143 has better diagnostic performance than AFP in the detection of HCC.

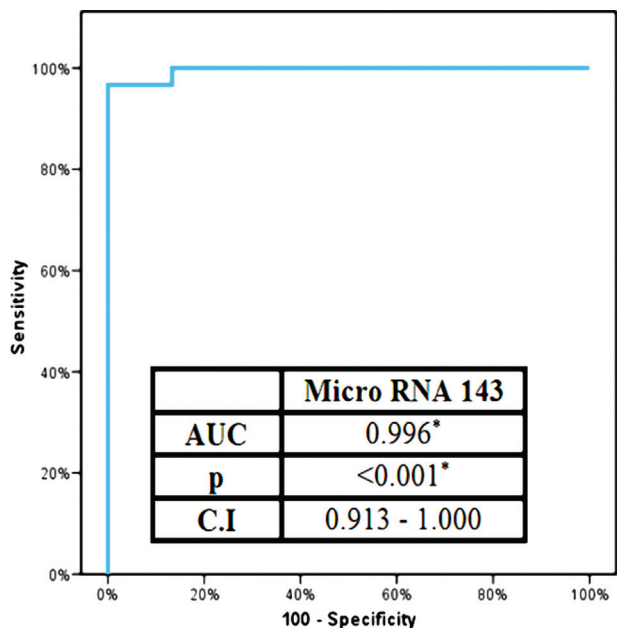
## Discussion

This study showed significantly higher levels of serum miR-143 in cirrhotic patients without HCC than in healthy participants. Similar to our study Murakami

*et al.* [14] have found miR-143 to be downregulated in chronic hepatitis and upregulated in liver cirrhosis. This result may be explained by release from damaged hepatocytes which might be the major source of hepatocyte-derived miRNAs; so, the release of miRNAs upon damage might be higher than in patients with healthy liver tissue [15]. In comparison with liver-specific miR-122, Trebica *et al.* [16] reported significant decrease of miR-122 expression in cirrhosis compared with normal controls. The finding was explained by the abundance of miR-122 in hepatocytes and its presence in much lower levels in the circulation than in healthy participants. With hepatocyte injury, miR-122 is released in the circulation and serum levels rise, with eventual loss of hepatocyte and development of fibrosis, hence, the circulating miR-122 levels drop again.

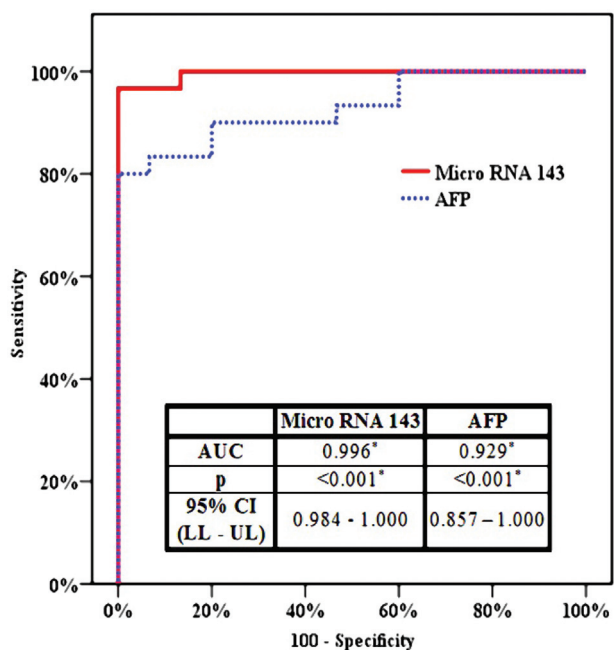
In this study, serum miR-143 was shown to be significantly higher in patients with early HCC (BCLC stage A) than cirrhotic patients without HCC ( $P<0.001$ ). As miRNAs play a key role in diverse biological processes, including development, cell proliferation, differentiation, and apoptosis,

Figure 2



ROC curve for miR-143 to differentiate HCC from liver cirrhosis. HCC, hepatocellular carcinoma; miR-143, microRNA 143; ROC, receiver-operating characteristic.

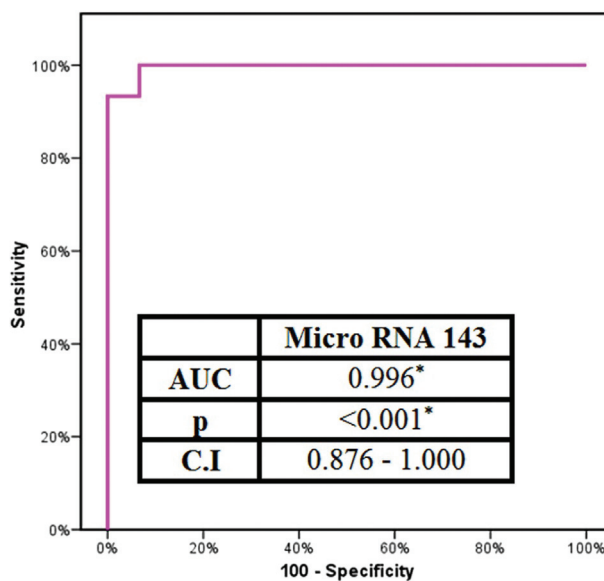
Figure 4



ROC curve for miR-143 and AFP to differentiate HCC from liver cirrhosis. AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; miR-143, microRNA 143; ROC, receiver-operating characteristic.

altered miRNA expression is likely to contribute to human disease, including cancer. The underlying mechanisms of miRNAs deregulation are largely uncertain [17]. In parallel with our results, Zhu *et al.* [18] reported that elevated serum levels of miR17HG protein (a protein encoded by miR-

Figure 3



ROC curve for miR-143 to differentiate liver cirrhosis from healthy participants. miR-143, microRNA 143; ROC, receiver-operating characteristic.

17-92 cluster host gene) in HCC tissues may suggest that the miR-17-92 cluster is activated early in HCC, even at the precancerous cirrhosis stage.

Taken together, these data suggest that the miR-143 is activated in early HCV-related HCC and may play a role in hepatocarcinogenesis.

In the current work, we found that serum miR-143 levels positively correlate with BCLC staging as HCC stage D patients have a higher level of serum miR-143 than in stages A and B patients and HCC stage B patients have a higher serum level of miR-143 than stage A patients. In this regard, Zhang *et al.* [19] reported similar results showing that miR-143 is upregulated in HBV-HCC and promotes cancer cell invasion and migration.

In the present study, serum miR-143 levels were significantly higher in HCC patients than in the healthy group and cirrhotic patients without HCC. ROC curve analysis using miR-143 serum expression level for discriminating cirrhotic patients with HCC from cirrhotic patients without HCC showed that sensitivity and specificity were 96.67 and 100%, respectively at a cutoff level of more than 3.26 and the AUC was 0.996 indicating good discriminative power and was statistically significant ( $P \leq 0.001$ ). This may suggest that serum miR-143 can be used as a biomarker for the diagnosis of HCV-related HCC. Zhang *et al.* [19] demonstrated that miR-143 might have a role in the regulation of target genes in HCC

where microvesicles packaged with miRNAs are released mainly from the tumor cells. They also reported that the levels of miR-143 were dramatically overexpressed in metastatic HBV-related HCC of both transgenic mice and HCC patients and this overexpression is transcribed by nuclear factor kappa B that favors liver tumor cell invasive and metastatic behavior and they found that local liver metastasis and distant lung metastasis are significantly inhibited by blocking miR-143 and fibronectin-type III domain containing 3B.

To date, only few studies have reported the association between miR-143 and HCC. In agreement with our data, Zhang *et al.* [20] reported that the expression of miR-143 in serum was significantly upregulated in patients with HCC and serum miR-143 can be used as potential biomarkers for the diagnosis of HCC. On the other hand, Shen *et al.* [21] demonstrated that a number of miRNAs have not only diagnostic but also prognostic and therapeutic implications in HCC.

Contrary to our results Liu *et al.* [22] reported that miR-143 was underexpressed in hepatoma and so upregulating its expression level could inhibit its potential target gene toll-like receptor 2 and nuclear factor- $\kappa$ B in the downstream pathway, leading to the inhibition of hepatoma proliferation. Wang *et al.* [23] reported that the expression of miR-143 was significantly lower in HCC tissues than in paired adjacent normal tissues.

Since HCC is a complex disease and has an unpredictable behavior in the regulation of noncoding RNAs, the discrepancy between the results of our study and other studies may be due to the multiple risk factors that have been implicated in the development of HCC [24–26]. Also, the large ethnic and geographic variability in the incidence of HCC among the different populations may lead to etiology-related differences in miRNA expression. This may have contributed to the difference between our study and others. Moreover, there are different HCV genotypes other than genotype 4, which represents more than 90% of the cases in Egypt. In our study, we measured miR-143 in serum sample while other contradicting studies detected miR-143 in the tissue sample. So, the different sampling may provide another possible reason for the variation in results. Compared with miR-122 which is downregulated in primary HCC tissues, serum miR-122 is upregulated in HBV patients with HCC, possibly due to miR-122 release from tissues into circulation [27]. However, Mamdouh *et al.* [28]

reported that serum miR-143 is upregulated in HCV-related HCC and can be used as a diagnostic and prognostic marker and may promote malignant progression of HCC in agreement with our results.

ROC curve analysis for serum miR-143 and AFP in discriminating cirrhotic HCC group A patients from non-HCC group B revealed a significant difference between AUC of miR-143 and AFP ( $P < 0.001$ ), with a sensitivity and specificity of 96.67 and 100% for miR-143 and 80 and 100% for AFP, respectively, AUC=0.996 for miR-143 and 0.929 for AFP at a cutoff level of more than 3.26 for miR-143 and more than 72 for AFP. This result may indicate that miRNA-143 has a better diagnostic performance than AFP in the detection of HCC. In agreement with this, Lin *et al.* [29] identified a set of seven miRNAs including miR-143 that were upregulated in the serum of patients with HBV-related HCC compared with patients with chronic HBV. Using a training cohort and two independent validation cohorts, they developed and validated a seven-miRNA panel including miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505. This panel was more sensitive than AFP and could detect small AFP-negative HCC samples, providing hope for earlier detection of HCC.

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## Conclusion

The serum level of miR-143 is significantly higher in cirrhotic patients with early HCC than in cirrhotic patients without HCC, suggesting a possible role in hepatocarcinogenesis. Serum levels of miR-143 showed significant increase in HCV-related cirrhosis and HCC in comparison with non-HCC cirrhotic patients and healthy participants with high sensitivity and specificity in detecting HCC. This suggests its value as a potential biomarker for early diagnosis of HCV-related HCC.

Ahmed M. El-Gohary provided the study concept and design, study supervision, and approval of the final revision of the manuscript. Ahmed E. Zeid: analysis and interpretation of data, statistical analysis, manuscript drafting, and revision and approval of the final revision of the manuscript. Mohamed E. Ibrahim: performed imaging work of participants of the study, analysis and interpretation of data, manuscript revision and approval of the final revision of the manuscript. Fatma I. Dewedar: performed the laboratory work of participants of the study, analysis and interpretation of data, statistical analysis, manuscript writing, and revision and approval

of the final revision of the manuscript. Essam A. Elzoheiry: acquisition and assembly of data, analysis and interpretation of data, statistical analysis, drafting and revision of the manuscript, and approval of the final revision of the manuscript.

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#### Conflicts of interest

There are no conflicts of interest.

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