Serum mir-30e and mir-223 as novel noninvasive biomarkers for hepatocellular carcinoma

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Background Liver cancer is the sixth most common cancer that accounts for 7% of all cancers. Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide with ~600,000 new cases per year, and it is the second leading cause of cancer-related deaths alone.

Aim The aim of this work was to study the clinical utility of miR-30e and miR-223 as early novel biomarkers for HCC in chronic viral hepatitis patients.

Patients and methods In this study, we tested two serum microRNAs (miRNAs), 223 and 30e, that can be used as potential biomarkers to diagnose HCC. A total of 55 patients were divided into three groups. Group I included 20 newly diagnosed patients with HCC on top of chronic hepatitis C virus infection. The HCC group included 11 male patients and nine female patients with ages ranging from 55 to 65 years. Liver masses discovered on surveillance by abdominal ultrasound were further investigated by triphasic abdominal computed tomographic scan. Group II included 20 patients of chronic hepatitis C virus with no HCC (10 male patients and 10 female patients), with ages ranging from 53 to 63 years. Group III included 15 apparently healthy participants as a control group (five male individuals and 10 female individuals), with ages ranging from 55 to 61 years and who were selected from relatives and friends. The samples were analyzed by quantitative real time PCR to detect both miRNAs.

Result Our study revealed that both miRNAs, 30e and 223, were expressed at significantly lower levels in the sera of patients with HCC compared with healthy participants.

Conclusion Expression levels of miR-30e and miR-223 were reduced in HCC sera; they have potential as noninvasive biomarkers for diagnosis of HCC, with high specificity and sensitivity for miR-30e, although with moderate sensitivity and high specificity for miR-223.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer with ~600,000 new cases per year, and it is the second leading cause of cancer-related deaths worldwide [1]. The prognosis of patients with HCC is generally poor when diagnosed at an advanced stage. The 5-year survival rate is more than 70% if patients are diagnosed at an early stage [2].

According to data from GLOBOCAN 2012, wide geographic and socioeconomic differences exist among different ethnic groups of the world with 83% of the estimated 782,000 new cancer cases worldwide prevailing in less developed countries, and 50% in China alone [3].

As regards Egypt, the prevalence of HCC is high in the Nile Delta, and it is more common in male individuals, rural residents, and farmers, especially in hepatitis C virus (HCV) and hepatitis B virus (HBV) patients. In rural areas, there are other factors such as aflatoxin, cigarette smoking, occupational exposure to chemicals such as pesticides and endemic infections in the community, such as Schistosomiasis [4,5].

HCC usually develops in a liver already chronically damaged, often from cirrhosis. The etiology of liver disease, and, consequently, that of HCC, differs geographically with consequent differences in the HCC genotype. In most areas, chronic viral hepatitis due to either HBV or HCV is the main cause of HCC [6]. Hepatocellular carcinoma is a disease that is relatively insensitive to chemotherapy and/or radiotherapy. Further, given that the liver is usually compromised in the majority of persons who develop the tumor, it is associated with an extremely poor prognosis. For this reason, mortality from HCC almost equals its incidence [7].

MicroRNAs (miRNAs) are a class of endogenous small, single-stranded, noncoding RNA of ≈ 22 (18°25) nucleotides with a characteristic hairpin secondary structure. They down regulate gene

expression during various crucial cell processes such as apoptosis, differentiation, and development. They regulate gene silencing by either targeting mRNA directly into degradation or by inhibiting translation [8]. Expression profiling of miRNAs has, therefore, become extremely important for clinicians [9].

In HCC, cell proliferation is enhanced by low expression of miR-223-3p [10]. Serum miRNAs have been implicated for potential biomarkers in several diseases. Approximately 100 circulating miRNAs have been identified as biomarkers for different diseases, and the number is growing. Growing evidence indicates that their deregulation plays an important role in cancer onset and progression [8,11].

Aim

The aim of the work was to study the clinical utility of miR-30e and miR-223 as early novel biomarkers for HCC in chronic viral hepatitis patients.

Patients and methods

Our study was approved by the Researches Ethics Committee at Faculty of Medicine, Al Azhar University, and oral informed consent was obtained from all participants.

Patients were divided into three groups:

Group I included 20 newly diagnosed patients with HCC on top of chronic HCV infection. They comprised 11 male patients and nine female patients with ages ranging from 55 to 65 years. Liver masses discovered on surveillance by abdominal ultrasound were further investigated by triphasic abdominal computed tomographic scan.
Group II included 20 patients of chronic HCV with no HCC (10 male patients and 10 female patients), with ages ranging from 53 to 63 years.
Group III included 15 age-matched and sex-matched apparently healthy participants as a control group (five male individuals and 10 female individuals), with ages ranging from 55 to 61 years, selected from relatives and friends.

The HCC patients are diagnosed according to the guidelines formulated by the European Association for the Study of Liver for HCC diagnosis and management.

All participants in this study were subjected to the following:

(1) Full history and clinical examination.
(2) Groups I and II patients were subjected to ultrasound to document the presence of hepatic focal lesion(s). Number, size, site, and pattern of focal lesion(s) were commented upon if present. Only patients with hepatic focal lesion(s) underwent triphasic abdominal computed tomography.
(3) Laboratory investigations:

Four milliliters of blood was collected in a serum vaccutainer tube, left for 10 min to clot and then centrifuged at 3000 rpm for 5 min; the separated serum was used for determination of routine laboratory investigations [alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, and total protein and albumin. Blood sugar, blood urea nitrogen (BUN), creatinine in addition to determining alpha fetoprotein (AFP) and detecting HCV antibody].

Two milliliters of blood was collected in an EDTA-containing tube to perform glycated hemoglobin (HbA1c%).

Two milliliters of blood was collected in a Na citrate-containing tube to perform prothrombin time (PT).

Methods of determination

(1) Liver function tests:
Liver function tests were carried out in the Clinical Pathology Unit, Al-Zahraa Hospital, on the automated INTEGRA400 PLUS analyzer. Routine tests carried out were as follows: ALT by kinetic with L-alanine and 2-oxoglutarate and AST kinetic with L-aspartate and 2-oxoglutarate. Albumin by Bromocresol green dye-binding method. Total bilirubin (Diazo method) and direct bilirubin (colorimetric assay). Total protein by Biuret method. PT using full automated coagulation analyzer (Stago, France).
(2) Kidney function test: creatinine by Jaffé method. BUN. First we measured the urea by the kinetic method with urease and then calculated nitrogen by dividing urea by 2.14.
(3) Glucose-colorimetric assay was carried out by the hexokinase method.
(4) HbA1c%: hemoglobin analysis was carried out by high-performance liquid chromatography D-10 (Bio-Rad, France)

(5) AFP was analyzed by solid-phase two-site sequential chemiluminescent immunometric assay using immulite 2000 system analyzer; the kits were supplied by Siemens (cat#L2KAP2; Siemens Healthcare Diagnostics, USA); values up to 10 ng/ml were considered normal.
(6) Hepatitis C marker: for HCV antibody was performed using VITROS ECiQ Immunodiagnostic System in the Clinical Pathology Unit, Al-Zahraa Hospital.
(7) Analysis of miRNA expression:
    (a) RNA extraction.
    (b) Reverse transcription.
    (c) Detection.

Two milliliters of blood were collected on a serum vaccutainer tube and centrifuged 3000 rpm for 15 min at room temperature. The supernatant was transferred to Eppendorf tubes. These samples were recentrifuged at 15 000 rpm for 15 min to precipitate cell debris, and the supernatants were stored at −20°C until RNA extraction. These samples were used for determination of expression level of mature miR-223 and miR-30e.

Assay procedure

I-RNA extraction:

I-RNA extraction was carried out by miRNeasy Mini Kit (cat. No 217004) CliniLab Inst.

Kit contents

Preparations per kit

(1) QIAamp spin columns (clear).
(2) Collection tubes (1.5 ml).
(3) Collection tubes (2 ml).
(4) Qiazole lysis reagent 50 ml.
(5) Buffer RWT 45 ml.
(6) Buffer RPE 45 ml.
(7) RNase-free water 10 ml.

Preparation of reagent

Buffer RPE and RWT working solutions are prepared by adding four volumes of ethanol (96–100%) to obtain a working solution.

Procedure

(1) 1000 µm Qiazole lysis reagent was added to 200 µm of the sample to disrupt and homogenize the sample.
(2) The homogenate was incubated at room temperature (15–25°C) for 5 min.
(3) A total volume of 200 µm chloroform was added, and the tube was capped securely and then shaken vigorously for 15 s.
(4) Incubation at room temperature for 2 to 3 min.
(5) Centrifugation for 15 min at 12 000*g* at 4°C.
(6) The upper aqueous phase was transferred to a new collection tube. About six hundred micron was taken and avoid transferring any interphase then 900 µm of 100% ethanol was added, and mixed thoroughly by pipetting.
(7) Up to 750 µm sample was pipetted, including any precipitate, into RNeasy mini column in a 2 ml collection tube. The lid was closed and the sample was centrifuged at more than 8000*g* for 15 s at room temperature. The flow-through was discarded.
(8) Repeat step 7 using the remainder of the sample.
(9) A total volume of 700 µm buffer RWT was added to the RNeasy mini column; the lid was closed and the mixture centrifuged for 15 s at more than 8000*g*; the flow through was discarded.
(10) A total volume of 500 µm buffer RPE was pipetted on to the RNeasy mini column; the lid was closed, and the mixture was centrifuged for 15 s at more than 8000*g*; the flow through was discarded.
(11) A total volume of 500 µm buffer RPE was added to the RNeasy mini column; the lid was closed, and the mixture was centrifuged for 2 min more than 8000*g*.
(12) The RNeasy mini column was transferred to a new 1.5 ml collection tube and 30 to 50 µm RNAse-free water was pipeted directly into the RNease mini column membrane, the lid was closed and the mixture centrifuged for 1 min at more than 8000*g* to elute.
(13) Total RNA in a volume of 1 µl was evaluated for concentration and purity using spectrophotometer Nano Drop (ND-1000, Thermo, USA), and 75 ng was used in reverse transcription reaction.

Reverse transcription

The kit used here is miScript II RT kit (cat. nos. 218160, 218161; CliniLab Inst).

Kit component

5x miScript Hi spec buffer (specific to miRNA).

Reverse transcriptase.

Nucleic mix.

Procedures

(1) Template RNA and 10x miScript Nucleics Mix were thawed and 5x miScript Hi spec buffer at room temperature (15 to 25°C). Each solution was mixed by flicking the tubes and centrifuged briefly to collect residual liquid from the sides of the tubes and then stored on ice.
(2) The reverse transcription master mix was prepared on ice, mixed and then stored on ice. The reverse transcription master mix contains all components required for first strand cDNA synthesis except the template.
(3) Template RNA was added to each tube containing reverse transcription master mix, gently mixed, briefly centrifuged, and then placed on ice.
(4) Incubation for 60 min at 37°C.
(5) Incubation for 5 min at 95°C to inactivate miScript reverse transcriptase then mixed and placed on ice.

Detection

Detection was carried out by real time quantitative by using miScript SYBR Green PCR Kit (200).

Kit content

(1) Cyber green.
(2) Universal primer.
(3) RNase-free water.

Procedure

(1) RNase-free water was dispensed into the individual wells of the PCR plate according to Tables 1 and 2.
(2) Template cDNA was dispensed into the individual wells of rotor disc.
(3) The reaction mix was mixed thoroughly and dispensed in appropriate volumes into the wells containing template cDNA.
(4) Carefully and tightly the rotor disc was sealed with caps, film, or rotor disc heat-sealing film.
(5) The real-time cycler was programmed according to Table 3.
(6) The plates were placed in the real time cycler and the cycling program was started.
(7) Fluorescence measurements were made in every cycle. The cycling conditions used were as follows: PCR initial active step at 95°C for 15 min, followed by 40 cycles, which included denaturation at 94°C for 15 s, annealing at 55°C for 30 s then extension at 70°C for 30 s.
(8) Melting curve analysis was performed after the thermal profile to ensure specificity in the amplification. The temperature increased very slowly (from 65 to 95°C) with monitoring of fluorescence signal. Melting curve analysis resulted in the detection of a single sharp peak for each target.

Cycling conditions

Calculation of quantitative-PCR results

The ΔC_T was calculated by subtracting the C_T values of SPIK IN 63 from the C_T values of the target miRNAs. The resulting normalized ΔC_T values were used in manually calculating relative expression values of each miRNA by $2^{-\Delta\Delta CT}$ method.

Finally, they were processed into relative expression (fold-change of expression). Fold regulation represents fold-change results in a biologically meaningful way. Fold-change values more than 1 indicate a positive or an upregulation, and the fold regulation is equal to the fold change. Fold-change values less than 1 indicate a negative or downregulation, and the fold regulation is the negative inverse of the fold change.

Statistical analysis

Data were analyzed using statistical program for the social science, version 20.0. Quantitative data were expressed as median interquartile range. Qualitative data were expressed as frequency and percentage.

Results

Analysis of the results between the patient groups (I, II) and control group revealed

There was a high significant increase in serum ALT, AST, total bilirubin, direct bilirubin, and AFP in two

Table 1 Content of reverse transcription kit

Components	Volume/reaction
5x miScript Hi spec buffer	4 µm
10x miScript nucleics mix	2 µm
miScript reverse transcription	2 µm
Templete RNA added in step 3	12 µm
Total volume	20 µm

Table 2 Content of detection kit

Components	Volume/reaction
Syber green	10 µm
Universal primer	2 µm
Assay primer	2 µm
RNase-free water	4 µm
Templet cDNA added at step 2	2 µm
Total volume	20 µm

Table 3 Program of cycling

Steps	Time	Temperature	Additional comment
Initial activation step	15 min	95°C	HotStar Taq DNA polymerase is activated by this heating step
3 steps cycling			
Denaturation	15 s	94°C	Perform florence data collection
Annealing	30 s	55°C	
Extension	30 s	70°C	
Cycle number	40 cycle		Cycle number depends on the amount of template cDNA and abundance of the target

Table 4 Statistical comparison between all groups with regard to laboratory investigations using the Kruskall–Wallis test

Laboratory data	Group I	Group II	Group III	*H* test	*P* value
ALT (U/L)					
Median (IQR)	47 (82.5)	26 (9.25)	20 (6)	14.742	<0.001
Percentile (25th–75th)	26.5–109	20.25–29.5	17–23		
AST (U/L)					
Median (IQR)	66.5 (131.5)	40 (18)	17 (4)	30.752	<0.001
Percentile (25th–75th)	48–179.5	35.5–53.5	16–20		
Total bilirubin (mg/dl)					
Median (IQR)	3.1 (5.75)	1 (2.8)	0.3 (0.2)	26.698	<0.001
Percentile (25th–75th)	1.15–6.9	0.7–3.5	0.2–0.4		
Direct bilirubin (mg/dl)					
Median (IQR)	1.55 (4.775)	0.3 (1.95)	0.16 (0.13)	21.029	<0.001
Percentile (25th–75th)	0.325–5.1	0.225–2.175	0.09–0.22		
AFP (U/L)					
Median (IQR)	83 (896.78)	15 (16.25)	1.5 (1)	36.901	<0.001
Percentile (25th–75th)	30.73–927.5	8.25–24.5	4.5		
PT%					
Median (IQR)	17.9 (4.08)	15.8 (2.725)	12.6 (1.1)	36.834	<0.001
Percentile (25th–75th)	16.23–20.30	14.125–16.85	12.2–13.3		
TP (g/dl)					
Median (IQR)	6.2 (0.85)	6.9 (1.2)	7.2 (0.6)	16.928	<0.001
Percentile (25th–75th)	5.8–6.65	6.3–7.5	6.7–7.3		
Albumin (g/dl)					
Median (IQR)	2.1 (0.6)	2.95 (1.225)	4.4 (0.4)	37.821	<0.001
Percentile (25th–75th)	1.9–2.5	2.3–3.525	4.3–4.7		
Glucose (mg/dl)	99.5(15.5)	93.5(17)	89(12)	1.926	0.382
Median (IQR)	89.50–105	84.75–101.7	88–100		
Percentile (25th–75th)					
HbA1c%					
Median (IQR)	5.35 (1.4)	5.6 (2.15)	5.4 (0.2)	3.080	0.214
Percentile (25th–75th)	4.6–6	4.625–6.775	5.4–5.6		
BUN					
Median (IQR)	14(26.15)	14.5(28.45)	12(4.4)	1.945	0.378
Percentile (25th–75th)	10.1–36.25	8.55–37	11–15.4		
Creatinine (mg/dl)					
Median (IQR)	1(0.78)	0.95(0.7)	0.7(0.4)	4.798	0.091
Percentile (25th–75th)	0.60–1.38	0.6–1.3	0.5–0.9		

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; HbA1c, glycated hemoglobin; IQR, interquartile range; PT, prothrombin time; TP, total protein.

patients groups, as compared with controls, in addition to prolongation of PT. There was a high significant decrease in serum total protein and albumin. There was a nonsignificanct difference in serum glucose and HbA1c%, BUN, and serum creatinine, as shown in Table 4.

Profiling of serum miRNA levels in the patient groups (I and II) and control group (III) was carried out. We observed that the expression level of serum miRNA-30e and miRNA-223 level revealed high significant downregulation in groups I and II compared with the control group, as shown in Table 5.

Table 5 Statistical comparison between groups according to miR-30e and miR-223 using the Kruskall–Wallis test

	Group I	Group II	Group III	*H* test	*P* value
RQ of serum miR-30e					
Median (IQR)	−10.38 (58.36)	−3.06 (1.75)	1.20 (0.06)	37.252	<0.001
Percentile (25th–75th)	−61.16–2.80	−3.88–2.13	1.20–1.26		
RQ of serum miR-223					
Median (IQR)	−8.14 (40.74)	−3.98 (6.00)	1.01 (0.07)	34.255	<0.001
Percentile (25th–75th)	−43.90–3.16	−7.57–1.57	0.99–1.06		

Data expressed as median [interquartile range (IQR)].

Table 6 Statistical comparison between groups I and II according to miR-30e and miR-223

	Group I	Group II	*z* test	*P* value
RQ of serum miR-30e				
Median (IQR)	−10.38 (58.36)	−3.06 (1.75)	−3.071	0.002
Percentile (25th–75th)	−61.16–2.80	−3.88–2.13		
RQ of serum miR-223				
Median (IQR)	−8.14 (40.74)	−3.98 (6.00)	−1.975	0.048
Percentile (25th–75th)	−43.90–3.16	−7.57–1.57		

Data expressed as median [interquartile range (IQR)].

Table 7 Diagnostic performance of RQ of serum miR-30e, RQ of serum miR-223, and combination in the discrimination between the positive group (hepatitis C virus and hepatocellular carcinoma) and the control group

Marker	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
RQ of serum miR-30e	−1.83	95	93	97.4	87.5	94.5
RQ of serum miR-223	−1.22	80	93	97.4	63	84.7
Multi-ROC: miR-30e at −1.83						
RQ of serum miR-223	−0.42	97.5	100	100	93.8	97.5

AUC, area under the curve; NPV, negative predictive value; ROC, receiver operating characteristic; PPV, positive predictive value.

Analysis of the results between the two patient groups (I, II)

There was a high significant increase in serum ALT, AST, total bilirubin, and AFP in group I, as compared with group II, in addition to prolongation of PT. High significant decrease in serum total protein and albumin was observed. There was a nonsignificant difference in serum glucose, HbA1c%, BUN, and serum creatinine.

We also observed that the expression level of serum miRNA-30e and miRNA-223 revealed a high significant downregulation in group I compared with group II, as shown in Table 6.

The expression levels of miR-30e and miR-223 in these serum samples were measured, and receiver operating characteristic analysis was performed on individual miRNAs. RQ of serum miR-30e was −1.83, with a sensitivity of 95%, specificity of 93%, positive predictive value of 97.4%, negative predictive value of 87.5% and with diagnostic accuracy of 94.5%. RQ of serum miR-223 was −1.22, with a sensitivity of 80%, specificity of 93%, positive predictive value of 97.4%, negative predictive value of 63% and with diagnostic accuracy of 84.7%. Multi-receiver operating characteristic was carried out and revealed at cut off a value of −0.42. Sensitivity of 97.5%, specificity of 100%, positive predictive value of 100%, negative predictive value of 93.8% with diagnostic accuracy of 97.5% was observed, as shown in Table 7.

Three models were determined by multiregression test and showed that model 2 was the most important and useful one for choosing the best biomarkers for testing HCC (biomarkers are total bilirubin, direct bilirubin, and miRNA 30e). Model 2 has more *F* ratio than model 3; hence, model 2 was considered the best biomarker for HCC testing.

Discussion

HCC is one of the most common malignant tumors, with poor prognosis and is a major cause of death worldwide [12]. Serum miRNAs have been implicated as potential biomarkers in several diseases and represent a promising wide area of research for clinical diagnostics. Growing evidence indicates that their deregulation plays an important role in cancer onset and progression. In this study, we found two

Model 1							
Item	Regression coefficient	*T*	*P*	Significance	*F* ratio	*P*	Significance
Constant	1.539	1.352	0.188	NS			
Age	0.016	1.605	0.121	NS			
Glucose	0.002	1.122	0.272	NS			
HbA1c	−0.12	−1.086	0.288	NS			
Albumin	−0.078	−0.48	0.635	NS			
Total bilirubin	0.561	2.421	0.023	S			
Direct bilirubin	−0.607	−2.342	0.027	S			
TP	−0.147	−1.118	0.274	NS			
Urea	0.003	0.316	0.755	NS			
Creatinine	−0.048	−0.155	0.878	NS			
BUN	−0.023	−1.186	0.247	NS			
PT	0.015	0.429	0.672	NS			
AST	0	−0.137	0.892	NS			
ALT	0.00009044	0.032	0.975	NS			
AFP	0.0000132	0.89	0.382	NS			
30e.t1	−0.006	−2.078	0.048	S			
223.t2	−0.004	−0.86	0.398	NS			
					3.589	0.002	HS
Model 2							
Item	Regression coefficient	*T*	*P*	Significance	*F* ratio	*P*	Significance
Constant	0.626	4.692	0	HS			
Total bilirubin	0.507	3.583	0.001	HS			
Direct bilirubin	−0.539	−3.25	0.002	HS			
30e.t1	−0.009	−3.539	0.001	HS			
					12.138	0	HS
Model 3							
Item	Regression coefficient	*T*	*P*	Significance	*F* ratio	*P*	Significance
Constant	0.883	7.456	0	HS			
Total bilirubin	0.05	2.946	0.005	HS			
30e.t1	−0.01	−3.682	0.001	HS			
					10.619	0	HS

serum miRNAs, miR-30e and miR-223, were present at lower levels in HCC patient sera compared with healthy controls. The role of miR-30e and miR-223 in the development of HCC is poorly understood, but it was found that miRNA-223 is responsible for regulating cytochrome B5. Cytochrome B5 is a hemoprotein that transfers electrons to several enzymes to fulfill functions in fatty acid desaturation [13]. Downregulation of miR-223 can activate cell proliferation via insulin-like growth factor 1 receptor and helps to maintain cholesterol homeostasis. The overexpression of miR-223 in HCC increases the sensitivity of anticancer drugs in HCC cell lines [14]. Junjie *et al.* [15] found that miRNA-30e is downregulated in HCC, and they suggested that miR-30e has a critical role in the suppression of miR-30e via the mechanism of miRNA-mediated JAK1 expression in cancer cells that might be a good prognostic marker for survival of HCC patients. Laura *et al.* [16] found that both miR-223 and miR-30e are downregulated in HCC. Rui and colleagues reported that miR-223 showed a higher level of downregulation in HCC tissues, compared with that in healthy controls ($P<0.001$). This was carried out by studying 371 patients with HCC (252 men and 119 women) and 50 normal controls; they found that there was a significant difference in the expression of miR-223 between the HCC tissues (9.112±1.436) and healthy controls (10.300±0.811; $P<0.001$) [17]. Mao and colleagues found downregulation of miR-30e in HepG2 and HuH7 cells and reported that miR-30e significantly inhibited the proliferation, migration, and invasion of HepG2 and HuH7 cells, and promoted cell apoptosis, but did not influence the cell cycle, and they also suggested that miR-30e has a critical role in the suppression of HCC and presents a novel mechanism of miRNA-mediated JAK1 expression in cancer cells that might be a good prognostic marker for survival of HCC patients [18].

Bhattacharya and colleagues (who studied 70 participants, including 14 HCV-infected patients with HCC, 14 HBV-infected patients with HCC,

11 nonviral-associated patients with HCC, 17 chronic liver disease patients, and 14 healthy volunteers) found that the expression level of both of miR-30e and miR-223 was statistically significantly downregulated in HCC patients than in the chronic liver disease patients (mainly HCV) and that serum levels of miR-30e and miR-223 were significantly lower in HCC patients than in the control group [19].

The study of Hala and colleagues on 180 Egyptian patients, who were divided into three groups of healthy controls (group I), CHC patients (group II), and hepatitis C patients with HCC (group III); found that serum ALT, AST, total bilirubin, and PT were statistically significantly higher in the HCC group than in the HCV group ($P<0.001$), and the serum albumin was statistically significantly lower in the HCC group than in the HCV group ($P<0.001$). They found that the serum AFP level was statistically significantly higher in the HCC group than in the HCV group [20].

Conclusions

Expression levels of miR-30e and miR-223 were reduced in HCC sera; they have potential as noninvasive biomarkers for the diagnosis of HCC, with high specificity and sensitivity for miR-30e, although with moderate sensitivity and high specificity for miR-223.

Recommendation

Future studies on a large population for the use of miRNA-30e and miR-223 as a prognostic predictor of cancer outcome, target of therapy and monitoring treatment response of HCC could be needed to fully understand the function of these miRNAs in the liver.

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Conflicts of interest

There are no conflicts of interest.

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