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Original article

Possible diagnostic role of microRNA-122 in chronic HCV infection and hepatocellular carcinoma

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ABSTRACT

Background: Infection with hepatitis C virus frequently progresses to cirrhosis and liver cell cancer. **Objectives:** The objective of this study was testing the usefulness of miR-122 as a marker for diagnosis of cirrhosis in chronic infection with hepatitis C virus (CHC) and as a diagnostic tool for early detection of hepatocellular cancer. **Methods:** This study included 118 patients; the first group included eighty-eight patients with chronic hepatitis C (CHC) and HCV related cirrhosis, the second group included thirty patients with HCC on to of chronic HCV infection, and the third one included twenty controls. Quantification of the viral RNA by real-time-PCR. MicroRNA-122 expression level was measured by RT-PCR. **Results:** The mean serum levels of miR-122 were much higher in CHC, compensated cirrhosis and decompensated cirrhosis patients' than in controls, while they were less in HCC patients than control group (p = 0.0001). Serum miR-122 revealed gradual decrease in levels with progression of fibrosis stage, with more significant decrease in late fibrosis stages including F3 and F4 (p=0.01). **Conclusion:** The mean levels of serum miR-122 decreased in patients of HCC thus can differentiate HCC from CHC and liver cirrhosis. MicroRNA -122 had high efficiency compared to other noninvasive indices in prediction of HCV, and progression towards HCC.

Introduction

Hepatitis C virus (HCV) infection usually leads to cirrhosis and liver cell cancer, and is considered an important health threat in Egypt [1, 2]. According to the Global Hepatitis report of WHO 2017, the Eastern Mediterranean region possesses the highest infection rate with HCV worldwide, with six countries have more than 50% of the total infections, Egypt is one of them [3].

In Egypt, HCV is the frequent predisposing factor for liver cirrhosis (LC) and hepatocellular cancer (HCC) [4]. The most frequent genotype responsible for 92.5% of infections is genotype 4, then genotype 1 (3.6%) [5]. Hepatitis C virus infection in Egypt is attributed to glass syringes re-usage in mass parenteral anti-schistosomiasis campaigns in the period from 1950s to 1980s that accounted for extensive HCV infection [5].

Micro-RNAs (miRNAs) are minute RNA-sequences of about 20-25 nucleotides length, and they are non-coding sequences that prevent expression of genes by regulating synthesis of

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protiens and the stability of mRNA [6, 7]. Micro-RNA-122 is a unique miRNA of hepatocytes. The expression of miR-122 increases in the liver through the embryonic development. There are more than 60 thousand copies in each cell of adult liver. There is growing evidence about the role of miR-122 in carcinogenesis, invasion, and spread of hepatocellular carcinomas HCCs [8, 9]. Dis-regulation of miR-122 is associated to the aggressive behavior of some kinds of HCC in patients of HCV [10, 11].

On the other hand, miR-122 was postulated to have tumor-suppressing effect, and its down-regulation could promote carcinogenesis. miR-122 when added to HCC cells could alleviate its malignant chriteria [7]. Many studies revealed that miR-122 have tumor suppressor activity through arresting the cell cycle and starting apoptosis in cancer cells [11-13].

It is interesting to use miR-122 as a marker for diagnosis as they are more stable and accurately measurable in various specimens by RT- PCR. Its assessment non-invasively makes it an interesting marker for variation in HCV replication and a potential biomarkers for cancer screening [14,15].

This study was planned to quantify and test the difference in the levels of miR-122 in patients of CHC, patients with cirrhosis, and HCC. To test if the serum levels of miR-122 may be useful as a diagnostic marker of liver cell injury in patients with chronic HCV infection and whether it can be used as a prognostic tool for the early detection of HCC.

Patients and methods

The study is cross-sectional and was conducted in Faculty of Medicine in Sohag University by the cooperation of Gastroenterology department, Central Research Laboratory, and the department of Microbiology and Immunology, through the duration from January 2020 to January 2022.

This study involved one hundred and eighteen patients attended the outpatient clinic or admitted to inpatient Department of Internal Medicine Department of Sohag University Hospitals and twenty apparently healthy controls.

Study population was categorized into three groups

I. Group I: Eighty-eight non-HCC patients were subdivided into:

- Twenty-six patients with CHC.
- Sixty-two patients with HCV related cirrhosis: Twenty eight patients with compensated cirrhosis and Thirty four patients with decompensated cirrhosis.

II. Group II: Thirty patients with HCC complicating HCV infection

III. Group III (control group): Twenty apparently healthy volunteers.

Inclusion criteria

All patients were diagnosed as CHC (presence of anti-HCV antibodies and for detectable level of HCV-RNA by RT-PCR). The level of viremia was classified into three levels by definition: low viremia was defined by the presence of less than 2×10^5 IU/mL, moderate viremia defined by $>2 \times 10^5$ viral RNA copies or 1×10^6 IU/mL, and highy viremia by $>1 \times 10^6$ IU/mL [16].

Diagnosis of HCV cirrhosis using the clinical criteria, laboratory tests, and ultrasonography. HCC was diagnosed and confirmed by magnetic resonance imaging (MRI) according to the criteria of the European Association of the Liver diseases [15]. Child-Puph grading the severity of CHC, and for HCC staging, Barcelona cancer liver clinic guidelines (BCLC) were used [17].

Exclusion criteria

Coinfection with HBV, any associated malignancies other than HCC, schistosoma, HIV, diabetes, autoimmune diseases, immunosuppression, and organ trans-plantation,

Ethical considerations

A written informed consent was obtained from each study participant. The study obtained an approval from the committee of scientific research ethics.

Methods

Specimen: blood samples (5 Ml) were withdrawn in plain tube following aseptic conditions from all participants in this study. centrifugation at 4000 rpm for 10 minutes was done. The supernatant was carefully collected and transferred into a clean 1.7 ml eppendorf tubes; (clear sera were collected within 1 h after receiving whole blood) and stored frozen at -80°C till the time of the assay.

I. Estimation of serum miRNA-122 expression by RT-PCR with reverse transcrition

1. **RNA extraction** from serum samples performed by using the miRNA easy Mini Kit (QIAGEN, Germany) for extraction and purification of RNA following the instructions of the manufacturer. The concentration of isolated RNA and its purity

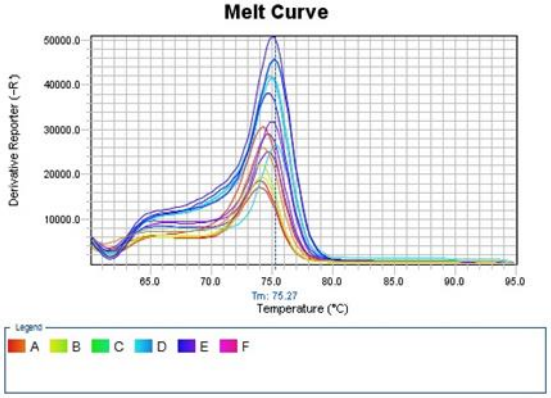
were assessed by NANODROP (Quawell Q5000 micro-volume UV-Vis Spectrophotometer, USA).

2. **Compementary DNA (cDNA) synthesis, amplification and detection (reverse transcription)** RNA obtained after the extraction step was subjected to reverse transcription to obtain the compementary DNA (cDNA) using the miScript II RT Kit (QIAGEN, GmBH, Germany) according to the manufacturer's instructions. The cDNAs were then immediately freezed and stored at – 20°C till its use.
3. **RT-PCR assay for miRNA-122 quantification** Real-time PCR quantification was performed with a RT- PCR cycler (Applied Biosystems™, Singapore) using the miScript SYBR Green master mix kit (Qiagen,Valencia, CA, USA) according to the protocol of the kit. The reference or housekeeping gene primer miRNA SNORD 68 (Qiagen) as an endogenous control was used and served as a stable reference normalization control. The thermal cycling was programmed as follows: initial denaturation at 95◦C for 15min, then 40 cycles of: 94◦C for 15s, 55◦C for 30s, and finally 70◦C for 34s. Melting curve was analysed after the end of cycling to ensure that the amplification was specific and yielded the target RNA. The temperature was gradually elevated (from 65°C to 95°C) with monitoring the intensity of fluorescence signal **(Figure 1).**

II. Calculation of PCR results:

The cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold in real-time PCR. Expression of miRNAs will be reported as ΔCt value. The ΔCt will be calculated by subtracting the Ct values of miRNA SNORD68 from the Ct values of the target miRNAs. There is an inverse correlation between ΔCt and miRNA expression level, and the lower ΔCt values will be associated with increased miRNA. The resultant normalized ΔCt values were used in calculating relative expression values by using $2^{-\Delta(Ct)}$, and these values are directly related to the miRNA expression levels. The $2^{-\Delta\Delta(Ct)}$ method will be used to determine relative-quantitative levels of individual miRNAs.

Figure 1. Melt curve of quantitative PCR for miR-122.



Results

The study involved 118 patients (72 males and 46 females) and 20 healthy volunteers as a control group. Patients were categorized into 3 groups; chronic hepatitis with compensated or decompensated cirrhosis, and HCC. The socio-demographic data of the study groups expressed in **(Table 1)**.

Twenty six patients with chronic hepatitis (mean age 66.57±7.08 years) were included in the study; twenty eight patients with compensated cirrhosis (mean age 53.29±8.26 years), thirty four patients with decompensated cirrhosis (mean age 60.82±8.19 years), and thirty patients with HCC

Interpretation of the laboratory data showed that the synthetic liver capacity; mean prothrombin concentration and albumin level decrease with a statistical significant difference (p=0.0001, p<0.0001, respectively) with deterioration of liver diseases in the studied groups **(Table 2).**

According to Child-Pugh classification, all CHC and most compensated cirrhosis (71.43%) patients were Child A, while all HCC patients were Child B and C (53.33%, 46.67%, respectively). Furthermore, Child C was more prevaent in HCC patients (p <0.0001). According to determined fibrosis score, most CHC patients were F0 (30.77%) and F1 (38.46%). On the other hand, all cirrhotic and HCC patients were F4 (p <0.0001). Clinical evaluation and abdominal ultra-sonographic examination of patients revealed that hepatomegaly, splenomegaly, portal vein dilatation, and marked ascites were significantly more common in HCC patients compared to other groups. In addition, varices, jaundice, and hepatic encephalopathy were also more frequent in HCC group **(Table 3).**

Regarding the associated clinic-pathological features of HCC group; HCC patients have variable AFP values (mean±SD, 594.17±433.25 ng/ml). Only 6.67% of HCC patients had AFP <20 ng/ml, and 33.33% had AFP from 20 to 400 ng/ml, while most patients (60%) had AFP>400 ng/ml during diagnosis. Staging of HCC patients revealed that most patients were stage A then B (40%, 26.67%, respectively), while the minority were stage D (6.67%). CT-imaging revealed that, hepatic focal lesion was solitary in more than half (60%) of HCC patients, and 40% of the lesions were >5 cm size in the biggest diameters. 5.80% of patients had portal vein (PV) thrombosis.

- **Serum level of miR-122 in studied groups**

The mean serum levels of miR-122 were significantly increased CHC, compensated cirrhosis and decompensated cirrhosis patients' compared to the control group, while it was less in HCC patients than in controls (p = 0.0001).

The elevation in serum levels of miR-122 in CHC patients compared to both controls and HCC groups was of statistical significance (p = 0.0001 for each), while there was insignificant increase of the mean serum miR-122 in CHC patients compared to both compensated cirrhosis and decompensated cirrhosis groups (p = 0.30 and p = 0.15, respectively), while the comparison between compensated and decompensated cirrhosis patients revealed that mean serum miR-122 level lower in patients with hepatic de-compensation with a statistically significant difference (p = 0.0001). Hepatocellular cancer (HCC) patients have lower serum miR-122 than CHC, decompensated cirrhosis patients, and controls (p = 0.0001for each) **(Table 4).**

- **Correlation between miR-122 expression level and different laboratory parameters in non-HCC group**

We correlated the level of miR-122 with surrogate parameters routinely used to evaluate chronic hepatitis C. The results are displayed in **table (5).** Regarding parameters of liver cell damage AST, ALT, and ALP; there was statistically significant positive correlation between Serum miR-122 expression level and AST (p=0.002), and ALT (p=0.0006)

The correlation between the levels of serum miR-122 and serum albumin level, prothrombin concentration, and the serum bilirubin. was positive with a statistical significance.

- **Correlation between miR-122 expression level and different laboratory parameters in HCC group**

In HCC patients, a negative correlation between miR-122 and total bilirubin, direct bilirubin, ALP, INR, and AFP was detected, while negative correlations with AST and ALT didn't reach the statistical significance. On the other hand, significant positive correlations with albumin and PC were detected **(Table 6).**

- **Relation of serum miR-122 levels from one side with grade of fibrosis and Child-Pugh classes from the other side**

Regarding its relation to liver fibrosis grades, circulating serum miR-122 revealed gradually decreased levels with progression of fibrosis stage, with more significant decrease in advanced fibrosis stages; F3 and F4 (p=0.01). Statistically-significant increase of serum miR-122 levels in F0 compared to F4 (p=0.02).

Analysis of sera from non-HCC and HCC patients with different Child-Pugh classes revealed no significant differences of the mean serum miR-122 levels between different Child classes (p = 0.36, p = 0.68, respectively). However, there was a trend toward lower mean serum levels with increased Child score **(Table 7).**

- **Relation of serum miR-122 with the level of viraemia and the staging of HCC**

The relation between the serum levels of miR-122 and serum HCV RNA was investigated. miR-122 exression was higher in patients with high viremia than in those with moderate and low viremia, although insignificant (p = 0.87). It was observed that there is no significant differences of the serum miR-122 level in different BCLC stages (P = 0.12), although there was a trend toward lower mean levels with advanced HCC stage, but didn't reach statistical significance **(Figure 2).**

- **Receiver Operator Characteristic (ROC) curves**

To evaluate the diagnostic performance of miR-122, ROC were established to discriminate groups; CHC patients from controls, cirrhotics from non-cirrhotic, and HCC from non-HCC patients. Our results disclosed that miR-122 could predict HCV with sensitivity 76.9%, specificity 100%, accuracy 88.5%, and cut off value >5.7 (RQ) (p < 0.0001). However, miR-122 had lower sensitivity (64.5%) and specificity (53.8) in discriminating cirrhotic from non-cirrhotic cases, with diagnostic accuracy 59.15% and cut off value ≤19.4 (RQ) (p=0.15)

Furthermore, miR-122 could predict HCC with sensitivity 93.3%, specificity 100%, accuracy 96.65%, and cut off value ≤2.1 (RQ) (p<0.0001)., while, AFP could predict HCC with sensitivity 86.7%, specificity 93.2%, accuracy 89.95%, and cut off value >33 (ng/dl) (p < 0.0001). Thus, both miR-122 and AFP showed high diagnostic performance in distinguishing HCC patients from non-HCC patients (p<0.0001 for each). However, the sensitivity, the specificity, and the diagnostic accuracy of miR-122 was higher, although there was insignificant difference between the AUC values of miR-122 and AFP (difference between areas = 0.022, p = 0.12 **(Figure 3).**

Table 1. Socio-demographic characteristics of the study groups.

Variable	CHC (N=26)	Compensated cirrhosis (N=28)	Decompensated cirrhosis (N=34)	HCC (N=30)	Healthy controls (N=20)	*P* value
Age (years)						
Mean ± SD	42.69±14.24	53.29±8.26	60.82±8.19	66.57±7.08	53.5±14.99	<0.000*
Median (IQR)	45 (35:54)	53.5 (48:58)	60 (56:67)	67 (61:72)	57.5 (40:63)	
Age groups						
<40 years	10 (38.46%)	2 (7.14%)	0	0	4 (20.00%)	
40-60 years	14 (53.85%)	20 (71.43%)	18 (52.94%)	6 (20.00%)	8 (40.00%)	<0.0001*.
>60 years	2 (7.69%)	6 (21.43%)	16 (47.06%)	24 (80.00%)	8 (40.00%)	
Gender						
Female	14 (53.85%)	12 (42.86%)	12 (35.29%)	8 (26.67%)	6 (30.00%)	0.25
Male	12 (46.15%)	16 (57.14%)	22 (64.71%)	22 (73.33%)	14 (70.00%)	
Residence						
Rural	16 (61.54%)	18 (64.29%)	22 (64.71%)	20 (66.67%)	14 (70.00%)	0.98
Urban	10 (38.46%)	10 (35.71%)	12 (35.29%)	10 (33.33%)	6 (30.00%)	
Smoking						
No	18 (69.23%)	18 (64.29%)	22 (64.71%)	14 (46.67%)	12 (60.00%)	0.85
Yes	6 (23.08%)	8 (28.57%)	10 (29.41%)	12 (40.00%)	6 (30.00%)	
Ex-smoker	2 (7.69%)	2 (7.14%)	2 (5.88%)	4 (13.33%)	2 (10.00%)	
Hypertension						
No	20 (76.92%)	20 (71.43%)	24 (70.59%)	22 (73.33%)	16 (80.00%)	0.94
Yes	6 (23.08%)	8 (28.57%)	10 (29.41%)	8 (26.67%)	4 (20.00%)	

* *P* value that express a highly significant statistical difference

Table 2. Laboratory data of the studied groups.

Variable	CHC (N=26)	Compensated cirrhosis (N=28)	Decompensated cirrhosis (N=34)	HCC (N=30)	Healthy controls (N=20)	*P* value
Albumin (g/dl)						
Mean ± SD	4.45±0.64	3.75±0.94	2.58±0.60	2.31±0.48	4.32±0.64	<0.0001 *
Median (IQR)	4.2 (4:4.8)	3.75 (3:4.5)	2.7 (2.2:2.9)	2.5 (1.8:2.8)	4.25 (3.8:4.8)	
Total bilirubin (mg/dl)						
Mean ± SD	0.81±0.27	1.05±0.46	2.84±1.36	3.26±1.36	0.77±0.25	0.0001 *
Median (IQR)	0.7 (0.6:0.9)	0.9 (0.7:1.3)	2.1 (2:3.2)	2.9 (2.1:4.5)	0.75 (0.6:0.9)	
Direct bilirubin (mg/dl)						
Mean ± SD	0.29±0.10	0.46±0.37	1.09±0.51	1.37±0.73	0.27±0.13	0.0001 *
Median (IQR)	0.3 (0.2:0.4)	0.38 (0.2:0.5)	0.9 (0.8:1.4)	1.1 (1:1.9)	0.27 (0.2:0.4)	
AST (IU/ml)						
Mean ± SD	52.08±17.14	64.07±27.44	86.59±47.92	133.47±80.42	23.5±9.13	0.0001 *
Median (IQR)	47 (38:65)	60 (40:84)	76 (53:98)	98 (74:165)	21.5 (16:30)	
ALT (IU/ml)						
Mean ± SD	44.38±17.53	55.43±23.54	83.88±49.95	149.88±80.66	20.6±9.16	0.0001 *
Median (IQR)	48 (29:56)	56 (36:73)	74 (38:113)	116 (89:193)	20.5 (12:27)	
ALP (IU/ml)						
Mean ± SD	99.08±46.67	104.5±47.07	135.76±65.29	193.13±78.37	87.6±26.99	0.0001 *
Median (IQR)	87 (63:134)	96.5 (69:145)	123 (83:172)	187 (147:230)	82 (65:114)	
PC (%)						
Mean ± SD	97.88±2.89	90.74±12.00	82.72±15.99	74.52±16.23	98.6±1.72	0.0001 *
Median (IQR)	99 (97.3:100)	97.5 (80:100)	88.3 (68.5:96)	75 (60:92)	99.5 (97:100)	
INR						
Mean ± SD	1.12±0.13	1.09±0.19	1.25±0.21	1.48±0.20	1.02±0.10	0.0001 #
Median (IQR)	1.1 (1:1.2)	1 (0.98:1.28)	1.23 (1.04:1.4)	1.5 (1.34:1.67)	1.01 (0.98:1.07)	
Hb (g/dl)						
Mean ± SD	13.51±2.33	12.25±2.35	11.25±1.84	10.79±1.53	12.99±1.33	<0.0001 *
Median (IQR)	13.5 (12.5:14.8)	11.7 (10.5:14.1)	11.5 (10:12.5)	10.5 (9.63:12.5)	13.2 (11.8:13.9)	
Platelets (thousand/mm3)						
Mean ± SD	235±69.54	191.5±86.42	129±48.48	103.8±49.42	230.6±75.06	0.0001*
Median (IQR)	231 (198:267)	177 (111:243)	113 (90:164)	90 (73:130)	210.5 (171:250)	
WBCs						
Mean ± SD	7.48±2.27	6.64±2.51	8.60±4.83	7.27±2.86	7.09±2.09	0.35 #
Median (IQR)	7.3 (6.2:8.1)	6.45 (4.8:7.9)	7.5 (5.62:10.2)	7.3 (5.1:9.5)	7.15 (5.5:8.86)	
Creatinine (mg/dl)						
Mean ± SD	0.77±0.22	0.88±0.23	1.18±0.61	1.44±0.77	0.84±0.17	0.0001 *
Median (IQR)	0.78 (0.6:0.9)	0.86 (0.75:1)	0.94 (0.85:1.5)	1.2 (0.8:1.8)	0.87 (0.72:1)	
Urea (mg/dl)						
Mean ± SD	12.77±3.69	18.35±8.28	49.71±25.28	56.6±33.53	17.0±8.63	0.0001 *
Median (IQR)	12 (10:15)	15.5 (11:27)	43 (30:75)	48 (25:80)	14 (10:22)	
AFP (ng/ml)						
Mean ± SD	4.83±2.90	11.44±6.28	14.6±8.83	594.17±433.25	6.34±4.55	0.0001 *
Median (IQR)	3.7 (2.8:6.7)	9.5 (7.4:15)	13 (8:18)	590 (220:830)	5.7 (2.4:9.7)	

*****P value that express a highly significant statistical difference.

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, PC: prothrombin concentration, INR: international normalization ratio, Hb: hemoglobin, WBCs: white blood cells, and AFP: alpha-fetoprotein.

Table 3. Clinico-pathological data of patients.

Variable	CHC (N=26)	Compensated cirrhosis (N=28)	Decompensated cirrhosis (N=34)	HCC (N=30)	*P* value
Child-Pugh classification					
A	26 (100%)	20 (71.43%)	2 (5.88%)	0	<0.0001 *
B	0	8 (28.57%)	18 (52.94%)	16 (53.33%)	
C	0	0	14 (41.18%)	14 (46.67%)	
Fibrosis score					
F0	8 (30.77%)	0	0	0	
F1	10 (38.46%)	0	0	0	<0.0001 *
F2	4 (15.38%)	0	0	0	
F3	4 (15.38%)	0	0	0	
F4	0	28 (100%)	34 (100%)	30 (100%)	
Liver size					
Average	22 (84.62%)	20 (71.43%)	14 (41.18%)	10 (33.33%)	<0.0001 *
Enlarged	4 (15.38%)	4 (14.29%)	12 (35.29%)	16 (53.33%)	
Shrunken	0	4 (14.29%)	8 (23.53%)	4 (13.33%)	
Spleen size					
Average	26 (100%)	24 (85.71%)	24 (70.59%)	18 (60%)	0.002 *
Enlarged	0	4 (14.29%)	10 (29.41%)	12 (40.00%)	
Ascites					
Absent	26 (100%)	28 (100%)	0	0	
Mild	0	0	8 (23.53%)	6 (20.00%)	<0.0001 *
Moderate	0	0	16 (47.06%)	12 (40.00%)	
Marked	0	0	10 (29.41%)	12 (40.00%)	
Portal vein					
Not dilated	26 (100%)	24 (85.71%)	26 (76.47%)	16 (53.33%)	<0.0001 *
Dilated	0	4 (14.29%)	8 (23.53%)	14 (46.67%)	
Varices					
Absent	26 (100%)	24 (85.71%)	24 (70.59%)	18 (60.00%)	0.002 *
Present	0	4 (14.29%)	10 (29.41%)	12 (40.00%)	
Jaundice					
Absent	26 (100%)	28 (100%)	22 (64.71%)	18 (60.00%)	<0.0001 *
Present	0	0	12 (35.29%)	12 (40.00%)	
Encephalopathy					
Absent	26 (100%)	28 (100%)	24 (70.59%)	18 (60.00%)	<0.0001 *
Present	0	0	10 (29.41%)	12 (40.00%)	

* *P* value that express a significant statistical difference

Table 4. Mean serum levels of miR-122 in different study groups.

Variable	CHC (N=26)	Compensated cirrhosis (N=28)	Decompensated cirrhosis (N=34)	HCC (N=30)	Healthy controls (N=20)	*P* value
Mean ± SD	42.6±60.41	24.93±32.67	17.93±16.50	1.06±1.18	2.6±1.53	0.0001#
Median (IQR)	19.7 (6.3:47.3)	8.85 (4.3:31.4)	11.8 (4.5:29.8)	0.74 (0.07:1.3)	2.35 (1.2:3.6)	

P1 = 0.30, P2 = 0.15, P3 = 0.0001, P4 = 0.0001, P5 = 0.93, P6 = 0.0001, P7 = 0.0001, P8 = 0.0001, P9 = 0.0001, P10=0.0001; .P 1 compared CHC vs. compensated cirrhosis, P2 compared CHC vs. decompensated cirrhosis, P3 compared CHC vs. HCC, P4 compared cirrhosis vs. decompensated cirrhosis, P5 compared compensated cirrhosis vs. HCC, P6 compared decompensated vs. compensated cirrhosis vs. HCC, P7 compared CHC vs. control, P8 compared compensated cirrhosis vs. control, P9 compared decompensated cirrhosis vs. control, and P10 compared HCC vs. control.

Kruskal Wallis test is used with Mann-Whitney test for pairwise comparison

Table 5. Correlation between miR-122 expression level and different laboratory parameters in non-HCC group.

Correlations		
Laboratory data	**Serum MIR-122**	
	R	***P*-value**
Albumin	-0.27	0.18
Total bilirubin	0.55	0.004*
Direct bilirubin	0.43	0.03*
AST	0.57	0.002*
ALT	0.62	0.0006*
ALP	0.32	0.11
PC	-0.40	0.04
INR	0.36	0.045
Hb	-0.13	0.47
Platelets	-0.24	0.20
WBCs	0.12	0.56
Creatinine	-0.42	0.03*
Urea	-0.27	0.18
AFP	0.37	0.06

* *p*value that express a significant statistical difference

Table 6. Correlation between miR-122 expression level and different laboratory parameters in HCC group.

Laboratory data	**Serum MIR-122**	
	R	***p*-value**
Albumin	0.28	0.0008*
Total bilirubin	-0.20	0.02*
Direct bilirubin	-0.19	0.03*
AST	-0.07	0.42
ALT	-0.15	0.08
ALP	-0.19	0.03*
PC	0.23	0.008*
INR	-0.26	0.002*
Hb	0.59	0.001*
Platelets	0.16	0.36
WBCs	0.07	0.44
Creatinine	-0.24	0.005*
AFP	-0.36	<0.0001*

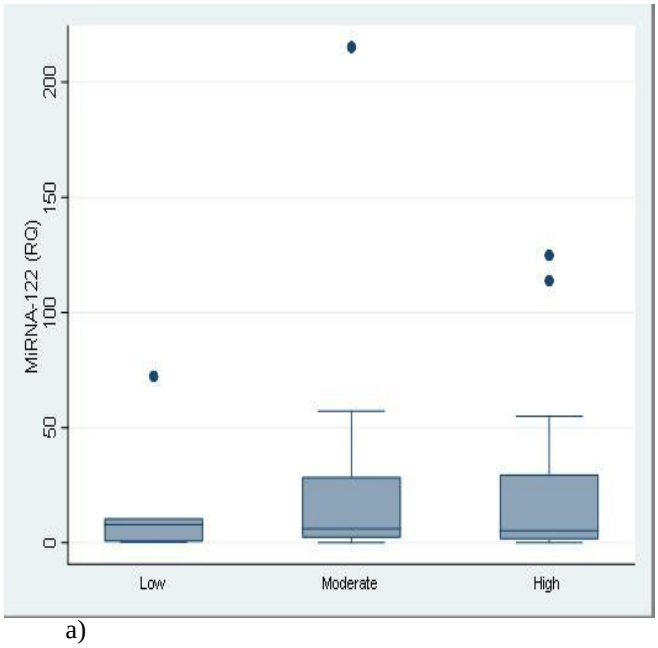
* *P* value that express a significant statistical difference

Table 7. Relation of serum miR-122 with grade of fibrosis and Child-Pugh in all patients.

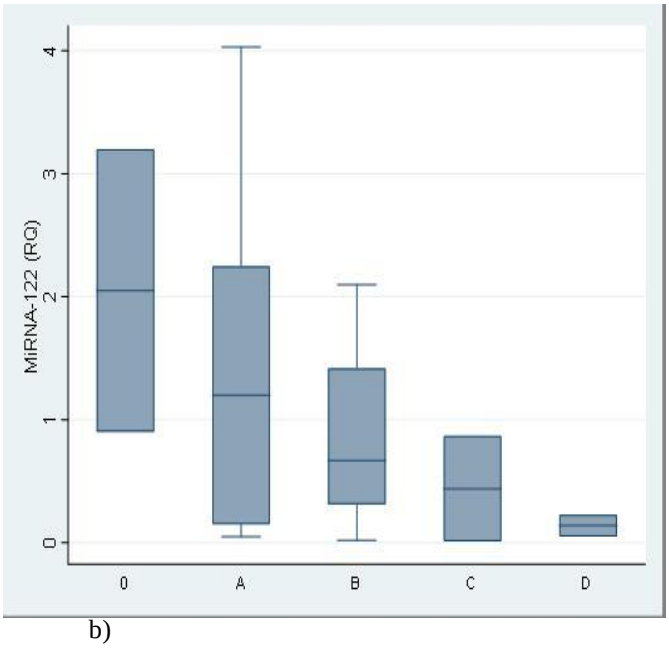
Parameter	F0	F1	F2	F3	F4	*P*-value
Serum miR-122 in all patients **Mean ± SD** **Median (IQR)**	N=8 58.95±75.24 31 (7.2:88.1)	N=10 49.14±68.57 24.15 (6.3:47.3)	N=4 21.95±20.05 18.2 (5.95:37.95)	N=4 14.2±13.88 11.3 (2.09:25.5)	N=92 14.55±22.66 4.4 (1.2:19.4)	0.01 #
P1= 0.86, P2 = 0.49, P3 = 0.17, P4 = 002, .P5 = 0.67, P6 = 0.20, P7 = 0.01, P8 = 0.38, P9 = 0.17, P10 = 0.53: P value is for comparison of all group. P1 compared F0 A vs. F1, P2 compared F0 vs. F2, P3 compared F0 vs. F3, P4 compared F0 vs. F4, P5 compared F1 vs. F2, P6 compared F1 vs. F3, P7 compared F1 vs. F4, P8 compared F2 vs. F3, P9 compared F2 vs. F4, P10 compared F3 vs. F4						
Parameter	**Child A**	**Child B**	**Child C**			***P*-value**
Serum miR-122 in non-HCC patients **Mean ± SD** **Median (IQR)**	N=48 (54.55%) 33.09±47.73 16.4 (4.75:39.35)	N=26 (29.55%) 21.32±31.33 6.5 (4.5:19.4)	N=14 (15.91%) 19.47±15.79 11.8 (3.8:38.7)			0.36
Serum miR-122 in HCC patients **Mean ± SD** **Median (IQR)**	N=0	N=16 (53.33%) 1.08±1.38 0.59 (0.15:1.05)	N=14 (46.67%) 1.02±0.94 0.87 (0.05:1.3)			0.68

Kruskal Wallis test is used with Mann-Whitney test for pairwise comparison

Figure 2. Relation of serum miR-122 with; a) the level l of viraemia and b) HCC staging.

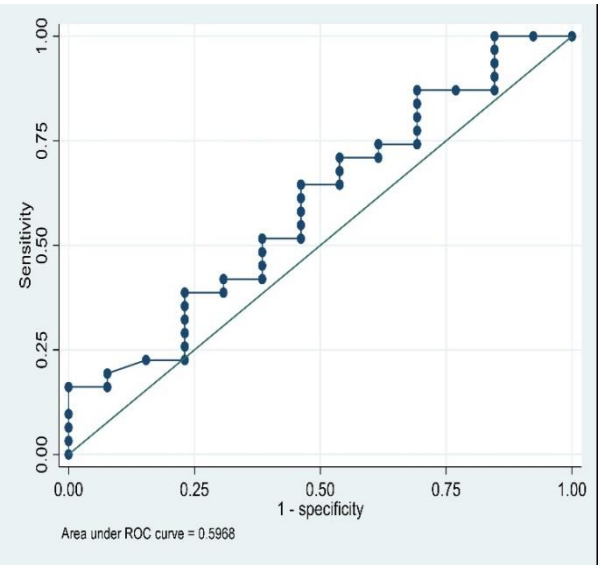


a)

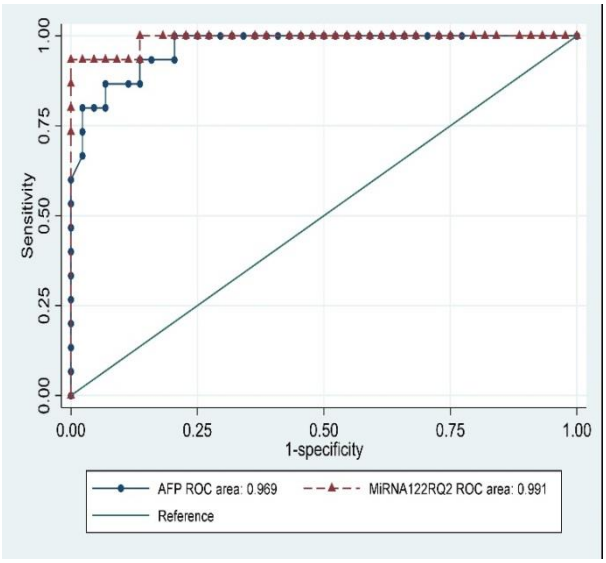


b)

Figure 3 a) Roc curve of miRNA-122 for discrimination between cirrhotic and noncirrhotic HCV patients, b) Roc curve comparison of MiRNA-122 and AFP for discrimination between HCC and non-HCC cirrhotic patients.



a)



b)

Discussion

MicroRNA-122 has recently been examined as a probable marker for various hepatic diseases [18]. As regard laboratory parameters, our study showed a highly significant increased severity of liver functions tests in HCC patients. This agrees with **Zekri et al.** [19], **Dooley et al.** [20] and **Elnakeeb et al.** [21]

Our study stated that albumin and prothrombin concentration are reduced significantly during liver disease deterioration, with more significant decrease in HCC group. This agrees with **Gopal et al.** [22], **Amr et al.** [23], **Demerdash et al.** [24], **Shaker et al.** [17], and **Elfert et al.** [25]. This may be due to suppression of the liver synthetic function with the aggravation of liver damage.

In our study the mean levels of total and direct bilirubin were elevated in HCC group. This agrees with **Gopal et al.** [22], **El-Abd et al.** [26], **Motawi et al.** [16], **Shaker et al.** [16], and **Gaber et al.** [27]. Subsequently, jaundice was also significantly more common in HCC patients, in agreement with **Shiha et al.** [28, 29]. The platelets count was significantly lower in HCC patients and these results were similar to other studies [16, 22, 23, 27,30]. Thrombocytopenia may be due to splenic sequestration, and bone marrow suppression by CHC infection [31].

Sixty percent of HCC patients in our study had high AFP levels (> 400 ng/ml). Similar results stated by **Mitchell et al.** [32] and **Motawi et al**. [16, 33].

Regarding miR-122, analysis of serum miR-122 expression showed significantly increased expression level of miRNA-122 in CHC patients' sera in comparison with other groups despite the relatively small number of the control group which is considered a limitation to our study. This agrees with **Elfert et al.** [25] and **Gaber et al.** [27] who reported augmented gene expression of miR-122 in chronic HCV patients. The explanation is that miR-122 is a liver specific miRNA, and its serum level is increased with hepatocyte injury induced by HCV [28, 34].

MicroRNA-122 showed also significantly increased expression in compensated cirrhosis and decompensated cirrhosis patients' sera in comparison with the control group. In agreement with **Ghoneim et al.** [35], **Motawi et al.** [16], **Amr et al.** [23], **Demerdash et al.** [24] and **Shiha et al.** [28], while **EL-Abd at al.** [26] and **Gaber et al.** [36]. MicroRNA-122 displayed a fold decrease in expression in cirrhotic in comparison to normal controls. Comparison between compensated and decompensated cirrhosis patients revealed that serum miR-122 was significantly lower patients with liver decompensation. Our results agree with **Waidmann et al.** [37], **Kholeif et al.** [38], and

Amin et al. [39, 40]. The explanation could be the higher volume distribution due to ascites [37].

On the other hand, our findings revealed significant decrease in serum miR-122 in HCC patients compared to CHC patients and controls. These findings were consistent with previous studies of; **El-Abd et al.** [26], **Amr et al.** [23], and **El-Ahwany et al.** [41] which observed a decline in the levels of miR-122 during carcinogenesis which support its function as a tumor suppressor gene [42].

Gaber et al. [27] reported an elevation in serum miR-122 in the HCC group in comparison with the normal control groups suggesting that miR-122 might down regulate target mRNA of genes responsible for tumor suppression and thus lead to further tumor growth [40]. Hepatocyte damage in HCC is the cause of excess miR-122 in circulation [43].

In our study the levels of circulating miR-122 in patients with early fibrosis were higher than those in patients with severe fibrosis. Our results were in accordance with **Ullah et al.** [1] who found that serum and hepatic miR-122 levels decreased significantly with the fibrosis. **Wang et al.** [44] also discovered a negative correlation between miR-122 and fibrosis stage in chronic HCV infection, HCV-based HCC, and cirrhosis**.** In contrast to our results, **Demerdash et al.** [24] found decreased expression levels of miR-122 in F2 stage as compared to F3 and F4.

A trend toward lower levels of miR-122 with increased Child-Pugh score or progression of HCC stage was observed in our study, although statistical significance was not reached this finding agreed with **Amin et al.** [45] who reported negative correlation with statistical significance between serum miR-122 level and Child score. **Ahmed et al.** [46] who reported also a lower expression level of miR-122 among the Child-Pugh class C patients compared to classes A and B. This is also in agreement with **Fang et al.** [47] who reported significant increase in miR-122 in Child A compared to Child C.

Regarding the variation in miR-122 expression with the viral load, we found higher miR-122 expression in patients with high viremia, although differences between groups didn't reach significance, this finding agreed with **Malik et al.** [48] who reported positive correlation between miR-122 level and HCV-RNA load.

For evaluation of miR-122 as a potential diagnostic marker, ROC curves were constructed and revealed that miR-122 could predict HCV with sensitivity 76.9%, specificity 100%, accuracy 88.5%, and cut off value of greater than 5.7 (RQ). ROC curve analysis also showed that miR-122 showed high efficacy in distinguishing HCC patients from non-HCC patients. **El-Ahwany et al.** [41] also stated that miR-122 could predict HCC with high specificity and sensitivity, making it a suitable tumor marker.

For conclusion, serum level of the hepatocyte specific miR-122 was increased in chronic HCV infected than in cirrhotic and HCC patients, and hence it has a strong potential to serve as a novel biomarker for liver injury. Expression of miR-122 is reduced in patients with hepatic de-compensation. Serum levels of miR-122 decreased in HCC patients thus can differentiate HCC from CHC and LC. MicroRNA-122 had high efficiency in prediction of CHC, development of malignancy, and prognosis of HCC.

Competing interests

The authors state that they have no cofict of interest

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