Circulating miRNA-122, miRNA-199a, and miRNA-16 as Biomarkers for Early Detection of Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C Virus Infection

Nevine E. EL-Abd, Nahla A. Fawzy, Suzan M. EL-Sheikh & Mohamed E. Soliman

Molecular Diagnosis & Therapy

ISSN 1177-1062
Volume 19
Number 4

Mol Diagn Ther (2015) 19:213-220
DOI 10.1007/s40291-015-0148-1

Springer

Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com”.

Springer

Author's personal copy

Mol Diagn Ther (2015) 19:213–220
DOI 10.1007/s40291-015-0148-1

CrossMark

ORIGINAL RESEARCH ARTICLE

Circulating miRNA-122, miRNA-199a, and miRNA-16 as Biomarkers for Early Detection of Hepatocellular Carcinoma in Egyptian Patients with Chronic Hepatitis C Virus Infection

Nevine E. EL-Abd[1] · Nahla A. Fawzy[1] · Suzan M. EL-Sheikh[1] · Mohamed E. Soliman[1]

Published online: 2 July 2015
© Springer International Publishing Switzerland 2015

Abstract
Background Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world. Having a very poor prognosis, it currently ranks as the third most common cause of cancer-related deaths. MiRNAs are a set of small, single-stranded, non-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level. Several miRNAs were found to be frequently deregulated in HCC.
Objective To investigate whether miRNA-122, miRNA-199a, and miRNA-16 are altered in sera of hepatitis C virus (HCV)-induced HCC patients compared with chronic HCV patients without HCC, and to assess their diagnostic value to differentiate between HCC and chronic HCV in order to develop a non-invasive diagnostic and prognostic tool for HCC.
Methods We analysed the expression of mature miRNA-122, miRNA-199a, and miRNA-16 in serum by a single-plex TaqMan two-step stem loop quantitative real-time reverse-transcription PCR (qRT-PCR) in 40 newly diagnosed HCC patients and 40 chronic HCV liver cirrhosis patients, as well as 20 apparently healthy individuals as a control group, using RNU48 as a normalisation control.
Results Serum miR-16 was significantly lower in HCC than in HCV patients ($P = 0.033$). The serum level of miR-199a in chronic HCV patients was significantly lower than in healthy controls ($P = 0.001$). Receiver operating curve (ROC) analysis for serum miRNA-16 for discriminating HCC from HCV patients showed that at the cut-off value of 0.904, the sensitivity and specificity for this marker were 57.5 and 70 %, respectively. The combination of serum miR-16 with serum alpha fetoprotein (AFP) resulted in improved sensitivity to 85% and increased diagnostic accuracy to 87.5 %. Serum miR-199a and miR-16 were significantly associated with several parameters of HCC such as tumour size and number.
Conclusion The combination of serum miR-16 and serum AFP is a significant improvement on the current best practice of serum AFP for HCC in HCVpositive patients. Serum miR-199a and miR-16 could be used as potential indicators of the progress of HCC.

Key Points

MiRNA-122, miRNA-199a, and miRNA-16 as potential biomarkers of HCC in chronic HCV patients.

MiRNA-16 and 199a are associated with the progress of disease in HCC patients.

The combination of serum miR-16 and serum AFP could be used as a diagnostic tool for HCC.

1 Introduction

Hepatocellular carcinoma (HCC) is reported to be the fifth most common cancer in men and the eighth most common cancer in women, with about 560,000 cases discovered every year, more than 80 % of which occur in developing countries. Having a very poor prognosis, it currently ranks as the third most common cause of cancer-related deaths [1] and the sixth most common cancer worldwide [2]. In Egypt, HCC was reported to account for about 4.7 % of

✉ Nevine E. EL-Abd
nevineelabd@yahoo.com; nevinee2013@gmail.com

1 Kasr Al-Ainy School of Medicine, Cairo University, Cairo, Egypt

Author's personal copy

chronic liver diseases (CLD) with a remarkable increase in this proportion from 4.0 to 7.2 % over the last decade [3]. The serum level of alpha fetoprotein (AFP) has been widely used as a marker for HCC for many years, although AFP is not elevated in all patients with HCC and its sensitivity and specificity are not satisfactory [4]. The American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) guidelines recommend surveillance with abdominal ultrasound every 6 months in high-risk patients [5]. However, imaging methods are limited by their high cost, and the diagnostic accuracy depends on both tumor size and vascularity [6]. MicroRNAs (miRNAs are a set of small, single-stranded, non-coding RNA molecules (18–22 nucleotides) that negatively regulate gene expression at the post-transcriptional level by translational inhibition or degradation of target mRNA, depending on the degree of complementary base pairing [7]. MiRNA-122 is a liver-specific, multifunctional RNA that controls liver homeostasis and interacts with many targets involved in lipid and cholesterol bio-synthesis, bilirubin and iron metabolism, and oxidative stress-response pathways, and is deregulated in most hepatic diseases, including HCV and HBV infections as well as alcohol and drug-induced liver injury, HCC, and non-alcoholic fatty liver disease (NAFLD). Furthermore, circulating miR-122 has been detected as a sensitive and early marker for alcohol- and chemical-induced liver injury [8, 9]. MiRNAs play a role in the most critical biological events such as proliferation, differentiation, metabolism, hematopoiesis, cell cycle, and apoptosis [10]. Accumulating evidence suggests that alterations of miRNAs expression may play a role in the development of human cancers, as they may function as regulatory molecules and act as oncogenes or tumor suppressor genes [11]. Circulating miRNAs have been considered as promising non-invasive biomarkers for cancer diagnosis and prognosis. In addition, circulating miRNAs can withstand repetitive freezing and thawing cycles, making them attractive as potential biomarkers for diverse human diseases [12].

Extensive research has been performed on miR-199a revealing its diverse expression patterns and functions in different cancer types. It can be down-regulated as a potential tumor suppressor in some cases, or may be up-regulated as an oncogene in others. Such dramatic differences in transcription may be due to its complicated expression control mechanisms, and its involvement in different cellular roles might be due to the diverse nature of its downstream targets. The miR-199 family members including miR-199a-5p, miR-199a-3p, and miR-199b are the most down-regulated miRNAs in HCV-induced HCC compared to a normal liver, post-hepatitis cirrhosis, and liver failure [13].

MiRNA-16 was shown to be implicated in the induction of apoptosis by targeting the BCL-2 gene, and it has shown to be involved in cell-cycle regulation in several tumor cell lines, e.g. regulating multiple cell-cycle genes, including CDK6 (cyclin-dependent kinase 6), CDC27 (cell-division cycle 27), and CARD10 (caspase recruitment domain 10). These findings suggest that the target of miR-16 act in concert, rather than individually, to regulate cell cycle and apoptosis. Altered expression of miR-16 has been observed in many cancers, including lung, pancreas, ovaries, and HCC [14, 15].

2 Aim of the Work

Our aim was to investigate whether the levels of miRNA-122, miRNA-199a, and miRNA-16 are altered in sera of HCV-induced HCC patients compared to those with chronic HCV liver cirrhosis, and to detect their diagnostic and prognostic value in HCC.

3 Subjects and Methods

This study was conducted on 100 Egyptian adults including 40 (27 males and 13 females) newly diagnosed HCC patients with HCV liver cirrhosis with a mean age of 56.5 ± 5.7 years, 40 patients (23 males and 17 females) with chronic HCV with liver cirrhosis without HCC, with a mean age of 56.4 ± 7.7 years, and 20 (12 males and 8 females) apparently healthy subjects as a control group, with a mean age of 32.9 ± 2.2 years. Patients with HCC and chronic HCV were recruited from the Gastroenterology and Hepatology Department, Cairo University.

All patients and controls were subjected to full history taking. HCC patients were diagnosed by triphasic abdominal CT scan. Data of all subjects were obtained from medical records and personal interviews. All laboratory tests were assayed in the Chemical Pathology Unit, Cairo University Hospital.

The study was approved by Cairo University Hospital research ethics committee and has been performed in accordance with the ethical standards of the Declaration of Helsinki. An informed consent was obtained from all individual participants included in the study.

3.1 Clinical Chemistry analysis

Blood samples were collected and investigated for serum total and direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), total protein, albumin, alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT) on the automated BECKMAN COULTER

Author's personal copy

AU680 autoanalyzer (Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821, USA)

3.2 Serum AFP Detection

Sera from chronic HCV and HCC patients were used for estimation of serum level of AFP by solid phase two sequential chemiluminescent immunometric assay using immulite 2000 system analyzer, the kits were supplied by Siemens (Siemens Healthcare Diagnostics, United States, cat#L2KAP2), values up to 10 ng/mL were considered normal.

3.3 Analysis of MiRNA Gene Expression

3.3.1 Serum Preparation and miRNA Extraction

Two milliliters of blood were collected into a plain vacutainer tube and centrifuged 3000 rpm for 15 min at room temperature. The supernatant was transferred to Eppendorf tubes. These samples were re-centrifuged at 15,000 rpm for 15 min to precipitate cell debris and the supernatants were stored at −20 °C until RNA extraction.

RNA was isolated from 400 µL serum using mirVana™ PARIS™ isolation kit (Ambion, Part Number AM1556) following the enrichment procedure for small RNAs purification according to the manufacturer's protocol [16].

The concentration and purity of RNA was determined by measuring the absorbance at 260 and 280 nm using NanoDrop 1000A Spectrophotometer (NanoDrop Technologies, Waltham, MA). RNA concentration was displayed in ng/µL. The ratio of A260 to A280 value is a measure of RNA purity; pure RNA used in this study had a ratio of between 1.8–2.1.

3.4 TaqMan® Two-Step Stem-Loop Reverse Transcription and Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR)

Reverse transcription (RT) was performed in a 15 µL reaction volume using 5 µL of RNA (10 ng per reaction), stem-loop RT primer and TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The tube was incubated on ice for 5 min and loaded into the thermal cycler using the following conditions: 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min.

For real-time PCR, 1.33 µL RT products were mixed with 10 µL TaqMan® Universal PCR Master Mix II (2×), No AmpErase UNG, 1 µL TaqMan MicroRNA Assay and 7.67 µL nuclease free water in a final volume of 20 µL according to manufacturer instructions. All reactions were run on StepOne real time PCR system (Applied Biosystems, USA) using the following conditions: 95 °C for 10 min, 45 cycles of 95 °C for 1 s and 60 °C for 60 s. Sequences of mature miRNAs and endogenous controls were identified using the miRbase (http://www.mirbase.org). Two endogenous controls were used as reference genes, RNU48, was expressed at high levels in serum and relatively invariant across large numbers of samples whereas, RNU6B exhibited extremely low or undetectable expression (Ct values >37) in both normal and patient sera so it was excluded from the study.

Relative expression of miRNA was calculated using the comparative cycle threshold (Ct) method. ΔC_T was calculated by subtracting the Ct values of RNU48 from the Ct values of the selected miRNA. $\Delta\Delta C_t$ was then calculated by subtracting mean ΔC_T of the control samples from ΔC_T of tested samples. Fold change of each candidate miRNA within each group was then calculated using the equation $2^{-\Delta\Delta CT}$ [17].

3.5 Statistical Methods

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean ± standard deviation (SD) for parametric data or median (minimum–maximum) for non-parametric data as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables. For non-normally distributed quantitative data, comparison between two groups was performed using the Mann–Whitney test (non-parametric *t* test). Comparison between three groups used the Kruskal–Wallis test (non-parametric ANOVA) then post-hoc was used for pair-wise comparison based on Kruskal–Wallis distribution.

To evaluate the diagnostic value of serum miRNAs in HCC, receiver operating characteristic (ROC) curve analysis was performed with prediction of the best possible cut-off values in which sensitivity was plotted on the *Y* axis and 1-specificity on the *X* axis.

4 Results

4.1 Study Subjects

The routine laboratory parameters and the clinical data of the studied groups are summarized in Table 1.

4.2 Serum Level of miRNAs and AFP

According to median values of fold change (FC), serum level of miR-16 was significantly lower in HCC patients compared to HCV patients ($P = 0.033$), whereas no significant difference was observed for miR-122 and miR-

Author's personal copy

Table 1 Routine laboratory parameters and clinical data of the studied groups

Variables	HCC ($n = 40$)	HCV ($n = 40$)	Control ($n = 20$)	P value
ALT (U/L)	55 (12–240)[a]	40 (6–111)[a]	13 (5–46)[b]	**<0.001**
AST (U/L)	61 (15–433)[a]	63.5 (14–457)[a]	21 (11–35)[b]	**<0.001**
T. Bil (mg/dl)	1.6 (0.4–18.1)[a]	1.9 (0.3–18.4)[a]	0.5 (0.2–1.0)[b]	**<0.001**
D. Bil (mg/dl)	0.65 (0.1–12)[a]	0.8 (0.1–15.1)[a]	0.1 (0.01–0.2)[b]	**<0.001**
T. Ptn (g/dl)	6.5 (2.1–8.3)[a]	6.3 (4.5–8)[a]	7.7 (6.7–8.0)[b]	**<0.001**
Albumin (g/dl)	2.8 (1.3–4.5)[a]	2.5 (1.2–4.5)[b]	4.7 (3.8–5.1)[c]	**<0.001**
ALP (U/L)	144 (29–453)[a]	101.5 (29–1631)[b]	73 (33–118)[c]	**<0.001**
GGT (U/L)	63.5 (15–354)[a]	44 (9–472)[a]	17.5 (9–32)[b]	**<0.001**
AFP (ng/mL)	23.7 (1.8–22,465)[a]	1.9 (0.7–9)[b]	–	**<0.001**

Child Pugh class no. (%)	HCC ($n = 40$) (%)	HCV ($n = 40$)	P value
A	19 (47.5)	11 (27.5 %)	
B	10 (25)	18 (45 %)	0.11
C	11 (27.5)	11 (27.5 %)	
Ascites			
Absent	12 (30)	9 (22.5 %)	
Mild to moderate	18 (45)	24 (60 %)	0.404
Severe to refractory	10 (25)	7 (17.5 %)	
Hepatic encephalopathy			
Absent	19(47.5)	25(62.5 %)	
Mild to moderate (grade I–II)	20 (50)	15 (37.5 %)	0.178
Severe (grade III–IV)	1 (2.5)	0	

BCLC stage	HCC ($n = 40$) (%)
0	3 (7.5)
A	15 (37.5)
B	8 (20)
C	5 (12.5)
D	9 (22.5)

Quantitative data are presented as median (min–max.)

Qualitative data are presented as number (%)

Groups bearing different initials are significantly different from each other at $P < 0.05$

Bold values are intended to highlight a statistical significant difference in the results among the studied groups

199a between the two groups. The median level of serum AFP showed a significant higher value in HCC group versus HCV group (P value < 0.001) (Table 2).

On comparing the median values of FC of the studied miRNAs between HCV patients and the control group, we found that their serum levels were lower in HCV patients than in controls (Table 3). Interestingly, the level of miR-199a in patients with HCV was significantly lower than that in healthy controls ($P = 0.001$). Median values of FC of the studied miRNAs between HCC and the controls, showed no statistically significant difference between either of the two groups. In the HCC group, serum miR-16

Table 2 Comparison of the median values of fold change of the studied MiRNAs and AFP as markers for differentiating HCC Group from hepatitis C virus Group

Variables	HCC ($n = 40$)	HCV ($n = 40$)	P value
miR-122	0.62 (0.03–32.79)	0.72 (0.02–21.89)	0.317
miR-199a	0.59 (0.04–5.6)	0.37 (0.03–8.02)	0.281
miR-16	0.71 (0.00–17)	0.99 (0.09–14.46)	**0.033**
AFP (ng/ml)	23.7 (1.8–22,465)	1.9 (0.7–9.0)	**<0.001**

All data are presented as median (min–max.)

Bold values are intended to highlight a statistical significant difference in the results among the studied groups

Author's personal copy

Table 3 Comparison of the median values of fold change of the studied MiRNAs as markers for differentiating hepatitis C virus group from control group

Variables	HCV ($n = 40$)	Control ($n = 20$)	P value
miR-122	0.72 (0.02–21.89)	1.163 (0.02–21.89)	0.424
miR-199a	0.37 (0.03–8.02)	1.4897 (0.437–4.814)	**0.001**
miR-16	0.99 (0.09–14.46)	1.048 (0.549–3.457)	0.447

All data are presented as median (min–max.)

Bold value is intended to highlight a statistical significant difference in the results among the studied groups

showed a statistically significant positive correlation with serum direct bilirubin ($r = 0.311$, $P = 0.041$). Moreover, serum miR-199a showed a statistically significant positive correlation with main tumor size ($r = 0.4$, $P = 0.011$) (Table 4). In the HCC group, serum miR-122 showed a statistically significant positive correlation with serum miR-199a ($r = 0.493$, $P = 0.001$). Also, serum miR-16 showed a statistically significant negative correlation with serum AFP ($r = -0.323$, $P = 0.042$). However, no statistical significant correlation was found among the studied miRNAs and serum AFP in the HCV group.

ROC curve analysis of serum miR-16 (Fig. 1) revealed that at a cut-off value of 0.904, serum miR-16 could differentiate HCC from HCV patients with a sensitivity of 57.5 % and a specificity of 70 %; area under curve (AUC) 0.638 ($P = 0.033$, 95 % CI 0.515–0.762), As for AFP, ROC curve analysis showed that at a cut-off value of 400 ng/mL, the sensitivity and specificity for AFP were 25 and 100 %, respectively; however, on lowering the cut-off of AFP to 10 ng/mL, the sensitivity increased to 70 % and the specificity remained 100 %; the AUC was 0.944 ($P = 0.001$, 95 % CI 0.898–0.990) (Fig. 2).

Furthermore, by combining markers, a positive result of at least one marker was considered to indicate HCC; the results of this study showed that the combining of serum miR-16 with serum AFP resulted in significantly improved sensitivity (85 %) and increased diagnostic accuracy (87.5 %) (logistic regression analysis; $P < 0.001$) when compared with either of them alone (Table 5). The association of the studied miRNAs with the prognostic factors in HCC patients (Table 6); revealed that the median value of serum miR-16 was significantly lower in HCC patients with multiple tumors than those having single tumor (P value of 0.032). Whereas, median value of serum miR-199a was significantly higher in HCC patients with multiple tumors than those having a single tumor (P value of 0.042) and was significantly higher in HCC patients with tumor size $\geq$3 cm than those having tumor size <3 cm ($P = 0.048$). Nevertheless, the three studied MiRNAs were not significantly different among different Barcelona clinic liver cancer (BCLC) stages of HCC patients.

5 Discussion

MiRNAs may have clinical relevance as pathological markers for early diagnosis, classification, and prognostic stratification of HCC patients. Furthermore, anti-miRNA oligonucleotides could be used to modulate HCC functions, indicating a therapeutic potential of miRNAs as targeted molecular therapy for the treatment of HCC patients [8]. In this study, we have characterized the role of miR-122, miR-199a, and miR-16 as diagnostic and prognostic markers for HCC patients compared to chronic HCV patients with liver cirrhosis. We found that the median

Table 4 Correlation analysis between the studied MiRNAs and clinical parameters in the hepatocellular carcinoma group

	miR-122		miR-199a		miR-16	
	r	P value	r	P value	r	P value
Age	0.022	0.892	−0.092	0.57	−0.179	0.268
ALT (U/L)	0.017	0.919	0.171	0.291	−0.109	0.502
AST (U/L)	0.006	0.97	0.232	0.149	−0.009	0.954
ALT/AST	0.043	0.794	−0.09	0.58	0.056	0.734
T. Bil (mg/dl)	−0.017	0.917	0.087	0.595	0.294	0.065
D. Bil (mg/dl)	0.006	0.971	0.103	0.527	0.311	**0.041**
T. Ptn (g/dl)	−0.064	0.694	−0.026	0.871	−0.124	0.446
Albumin (g/dl)	−0.118	0.47	−0.18	0.265	0.178	0.14
ALP (U/L)	0.014	0.932	0.247	0.125	−0.095	0.56
GGT (U/L)	0.13	0.425	0.257	0.109	0.033	0.841
Main tumor size	0.209	0.195	0.493	**0.011**	−0.203	0.209

r = Correlation coefficient, $r < 0.3$: no correlation, $r = 0.3$ to <0.5: weak correlation, $r = 0.5$: fair correlation, r = >0.5 to 0.75: good correlation, $r > 0.75$: very good correlation

Bold values are intended to highlight a statistical significant difference in the results among the studied groups

Author's personal copy

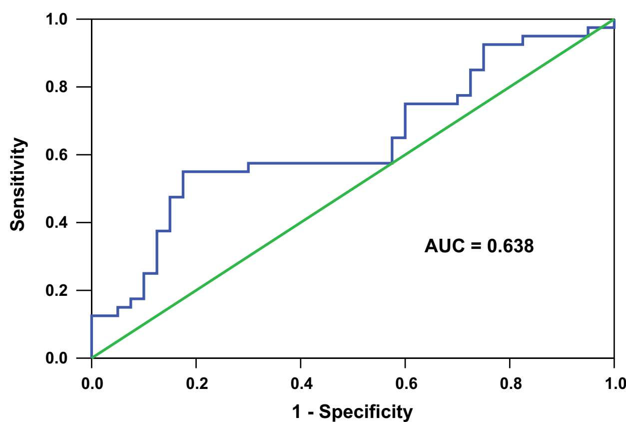

Fig. 1 Receiver operating curve analysis of serum miR-16 for discriminating hepatocellular carcinoma group from hepatitis C virus group

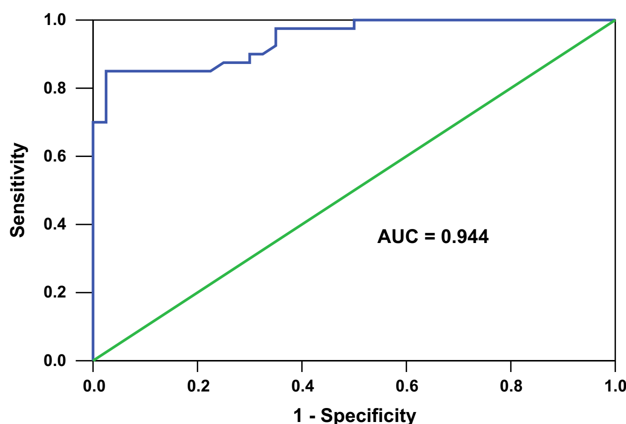

Fig. 2 Receiver operating curve analysis of serum alpha fetoprotein for discriminating hepatocellular carcinoma group from hepatitis C virus group

level of serum AFP was significantly higher in HCC versus HCV patients (P value < 0.001). Similarly, a former study suggested the same ($P < 0.001$) [18]. Another study found a significantly higher value of serum AFP in HCC than in patients with CLD ($P = 0.01$) [19]. Serum miRNAs (miR-122, miR-199a, and miR-16) were found to be down-regulated in HCC patients compared to the controls. Although not reaching a statistically significant difference, there was a trend towards lower serum values in HCC versus the control group, suggesting their possible role in the pathogenesis of HCC.

MiR-16 is one of the most prominent miRNAs implicated in cell-cycle regulation and induction of apoptosis. In this study, we demonstrated that miR-16 was significantly lower in HCC than in HCV patients ($P = 0.033$). Moreover, it was significantly lower in HCC patients with multiple tumors than those with a single tumor at a P value of 0.032. At the cut-off value of 0.904, serum miR-16 was able to discriminate between HCC and HCV groups with a sensitivity of 57.5 %, specificity of 70 %, and diagnostic accuracy of 63.8 %. By combining serum miR-16 with AFP, we observed a significant improvement in the sensitivity (85 %) with increased diagnostic accuracy to 87.5 % when compared with either of them alone, which comes in agreement with a recently published study by Qu et al. who reported that the combination of serum miR-16 with serum AFP resulted in a significantly improved sensitivity (87.6 %) [19]. We also observed that serum miR-16 showed a statistically significant positive correlation only with serum direct bilirubin ($r = 0.311$, $P = 0.041$).

Our finding of decreased circulating levels of miR-16 in HCC patients was not surprising, since several studies have reported the potential role of miR-16 to differentiate patients with HCC from those without [19, 20]. Ge et al concluded that the expression of serum miR-16 was found to be down-regulated in the patients with a tumor size more than 5 cm in diameter [20], which means that it could be considered as the potential prognostic marker to estimate the tumor size.

Decreased miR-199a expression in HCC has been repeatedly reported [21, 22]. In the present study, we demonstrated that the median value of serum miR-199a in HCV patients was significantly lower than healthy controls ($P = 0.001$); however, it showed a non-significant difference when comparing its level in HCC versus HCV and control groups. Moreover, the median value of serum miR-199a was significantly lower in HCC patients with a single

Table 5 Comparison of receiver operating curves for serum AFP, MiR-16, and combination of both in hepatocellular carcinoma versus hepatitis C virus group

Variable	Cut off	Sensitivity (%)	Specificity (%)	PVP (%)	PVN (%)	DA (%)
AFP	10 ng/ml	70	100	100	76.9	85
AFP	400 ng/ml	25	100	90	43.3	55
miR-16	0.904	57.5	70	65.7	62.2	63.8
AFP + miR-16	One positive of two	85	90	89.5	85.7	87.5

PVP predictive value of positive, *PVN* predictive value of negative, *DA* diagnostic accuracy

Author's personal copy

Table 6 Comparison of median values of the studied MiRNAs and different prognostic factors in the hepatocellular carcinoma (HCC) group

Clinical parameters of HCC (*n* = 40)	Median values of miRNAs			*P* value
Child Pugh Score	A	B	C	
miR-122	0.29 (0.03–5.73)	0.57 (0.3–7.45)	0.62 (0.06–32.7)	0.669
miR-199a	0.4 (0.05–5.47)	0.87 (0.23–5.6)	0.43 (0.04–2.76)	0.265
miR-16	0.9 (0.00–3.3)	0.93 (.00–17)	0.59 (0.43–4.96)	0.599
Clinical parameters of HCC (*n*= 40)	Median values of miRNAs			*P* value
Ascites	No	Mild to mod	Sever	
miR-122	0.28 (0.035.73)	0.56 (0.03–7.45)	0.85 (0.07–32.79)	0.337
miR-199a	0.81 (0.07–5.47)	0.87 (0.04–5.6)	0.37 (0.07–2.85)	0.639
miR-16	0.31 (0.00–2.02)[a]	1.23 (0.48–17)[b]	0.68 (0.33–7.56)[a,b]	**0.026**
Clinical parameters of HCC (*n* = 40)	Median values of miRNAs			*P* value
Number of tumor	Single	Multiple		
miR-122	0.5(0.03–7.45)	0.63(0.06–32.79)		0.533
miR-199a	0.4(0.04–5.6)	1.14(0.09–5.47)		**0.042**
miR-16	1.12(0.04–13.96)	0.55(0.00–17)		**0.032**
Clinical parameters of HCC (*n* = 40)	Median values of miRNAs			*P* value
Size of main tumor	<3 cm	≥3 cm		
miR-122	0.35(0.03–7.45)	0.63(0.03–32.79)		0.856
miR-199a	0.2 (0.05–5.6)	0.82 (0.04–5.47)		**0.048**
miR-16	1.08 (0.04–17)	0.64 (0.00–13.96)		0.254

All data are presented as median (min–max.)

Groups bearing different initials are significantly different from each other at $P < 0.05$

Bold values are intended to highlight a statistical significant difference in the results among the studied groups

tumor than those with multiple tumors ($P = 0.042$) and was significantly lower in HCC cases with a tumor size <3 cm than those with a tumor size ≥3 cm ($P = 0.048$). Furthermore, the median value of serum miR-199a was significantly lower in HCC patients with patent portal vein than those with portal vein thrombosis ($P = 0.011$). Another important finding is that serum miR-199a showed a statistically significant positive correlation with main tumor size ($r = 0.4$, $P = 0.011$). Nevertheless, the three studied miRNAs were not significantly different among different BCLC staging of HCC patients. Other studies investigated the deregulation of miR-199a in HCC have been also reported [9, 19, 23, 24]. MiR-122 is one of the miRNAs that has received much attention in HCC as it is abundantly expressed in the liver and is deregulated in most hepatic diseases, including HCC. Similar to what was previously reported by Koberle et al. [18], the current study showed that serum miR-122 did not differ between patients with and without HCC. Although the median value of serum miR-122 did not reach statistical significance, there was a trend toward a lower serum level in the HCC group as compared to the control group. However, several studies have found that the level of serum miR-122 was significantly elevated in patients with HCC compared with the control group [1, 7].

The discrepancy between the results of our study and other studies may be attributed to the large ethnic and geographic variability in the incidence of HCC among the different populations. Moreover, there are different HCV genotypes other than genotype 4, which represents over 90 % of the cases in Egypt. Further, additional risk factors for HCC other than HCV were identified, such as HBV and alcohol-induced HCC and the fact that several miRNAs are unique to certain virus-related HCC [25].

In conclusion, we suggest that serum miR-16 might serve as a non-invasive diagnostic marker of HCC, and the combination of serum miR-16 and AFP could be a more useful diagnostic tool for HCC than either alone. Moreover, serum miR-199a and miR-16 were significantly associated with several parameters of HCC, indicating that these miRNAs could be considered as potential indicators to estimate the tumor number and size in HCC patients.

Author's personal copy

Acknowledgments This study was funded by Cairo University and there is no conflict of interest among the authors of this research; Nevine E. EL-Abd, Nahla A. Fawzy, Suzan M. EL-Sheikh and Mohamed E. Soliman. This research was funded by Cairo University as the only party and there is no conflict of interest among the authors of this study.

Authors contributions Nevine E. EL-Abd (corresponding author): contributed in laboratory methodology, statistical analysis of data and writing the paper. Nahla A. Fawzy: contributed in analysis of results and writing the paper. Suzan M. EL-Sheikh: contributed in laboratory methodology, analysis of data and writing the paper. Mohamed E. Soliman: contributed in subjects selection (patients and controls) and all the clinical and radiological assessment of patients and clinical staging of HCC. The guarantor for the overall content is: Suzan M. EL-Sheikh

References

1. Qi J, Wang J, Katamaya H, Sen S, Liu SM. Circulating micro-RNAs (cmiRNAs) as novel potential biomarkers for hepatocellular carcinoma. Neoplasma. 2013;60(2):35–142.
2. Scaggiante B, Kazemi M, Pozzato G, Dapas B, Farra R, Grassi M, Zanconati F, Grassi G. Novel hepatocellular carcinoma molecules with prognostic and therapeutic potentials. World J. Gastroenterol. 2014;20(5):1268–88.
3. Hamdy H, Mahmoud E, Fouad R. Impact of hepatocellular carcinoma on health related quality of life in Egyptian patients: a single centre study. J Egypt Soc Parasitol. 2013;43(1):183–94.
4. Forner A, Vilana R, Ayuso C, et al. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology. 2008;47:97–104.
5. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLOS Med. 2014;11(4):1–18.
6. Zhang B, Pan X, Cobb GP, Anderson TA. MicroRNAs as oncogenes and tumor suppressors. Dev Biol. 2007;302(1):1–12.
7. Xu J, Wu C, Che X, Wang L, Yu D, Zhang T, Huang L, Li H, Tan W, Wang C, Linet D. Circulating MicroRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. Mol Carcinog. 2011;50:136–42.
8. Zhao X, Zhen Y, Li G, Li D, Zhao Y, Wu Y, Robson S, He L, Xu Y, Miao R, Zhao HT. The role and clinical implications of microRNAs in hepatocellular carcinoma. Sci China Life Sci. 2012;55(10):906–19.
9. Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG. Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. Cancer Res. 2007;67:6092–9.
10. Ceccarelli S, Panera N, Gnani D, Nobili V. Dual role of Micro-RNAs in NAFLD. Int J Mol Sci. 2013;14:8437–55.
11. Saito Y, Friedman JM, Chihara Y, Egger G, Chuang JC, Liang G. Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells. Biochem Biophys Res Commun. 2009;13;379(3):726–3.
12. Alevizos I, Illei GG. MicroRNAs as biomarkers in rheumatic diseases. Nat Rev Rheumatol. 2010;6:391–8.
13. Ezzat H, Lotfy AM, Alalfy MN, El-Taher SM, Mokhtar A, Mohamed SA, El-Senosy FM. The significance of circulating MicroRNA-122as a non invasive diagnostic marker of liver injury in Egyptian chronic hepatitis C virus infected and cirrhotic patients with and without hepatocellular carcinoma. Clin Med Diagnost. 2014;4(1):1–8.
14. Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M, Lim L. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. Mol Cell Biol. 2007;27:2240–52.
15. Chen RW, Bemis LT, Amato CM, Myint H, Tran H, Birks DK, Eckhardt SG, Robinson WA. Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma. Blood. 2008;112:822–9.
16. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova- Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA. 2008;105(30):10513–18.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. Methods. 2001;25:402–8.
18. Koberle V, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, Welker MW, Elhendawy M, Zeuzem S, Piiper A, Waidmann O. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer. 2013;49:3442–9.
19. Qu KZ, Zhang K, Li H, Afdhal NH, Albitar M. Circulating microRNAs as biomarkers for hepatocellular carcinoma. J Clin Gastroenterol. 2011;45:355–60.
20. Ge W, Yu DC, Li QG, Chen X, Zhang CY, Ding YT. Expression of serum miR-16, let-7f, and miR-21 in patients with hepatocellular carcinoma and their clinical significances. Clin Lab. 2014;60(3):427–34.
21. Varnholt H, Drebber U, Schulze F, Wedemeyer I, Schirmacher P, Dienes HP, Odenthal M. MicroRNA gene expression profile of hepatitis C virus-associated hepatocellular carcinoma. Hepatology. 2008;47:1223–32.
22. Jiang J, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmittgen TD. Association of microRNA expression in hepatocellular carcinomas with hepatitis infection, Cirrhosis and patient survival. Clin Cancer Res. 2008;14:419–27.
23. Shen Q, Cicinnati VR, Zhang X, Iacob S, Weber F, Sotiropoulos GC, Radtke A, Lu M, Paul A, Gerken G, Beckebaum S. Role of microRNA-199a-5p and discoidin domain receptor 1 in human hepatocellular carcinoma invasion. Mol Cancer. 2010;9(227):1–12.
24. Borel F, Han R, Visser A, Petry H, van Deventer SJ, Jansen PL. Adenosine triphosphate-binding cassette transporter genes up-regulation in untreated hepatocellular carcinoma is mediated by cellular microRNAs. Hepatology. 2012;55:821–32.
25. Ura S, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. Hepatology. 2009;49:1098–112.