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Role of circulating miR-182 and miR-150 as biomarkers for cirrhosis and hepatocellular carcinoma post HCV infection in Egyptian patients

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ABSTRACT

In Egypt, liver diseases are exceptionally high, maintaining the highest prevalence of hepatitis C virus (HCV) worldwide, and increasing rates of hepatocellular carcinoma (HCC). Available diagnostic methods show poor performance in early diagnosis of HCC. Definite pathogenic factors contributing in the development of HCV are still lacking. MicroRNAs have been reported as promising biomarkers for cancers diagnosis and in virus-host interaction.

This study was conducted to detect the role of miR-182 and miR-150 as biomarkers for development of cirrhosis and malignant transformation in HCV infected patients.

The expression of miR-182 and miR-150 was evaluated using real-time quantitative PCR (qRT-PCR) in 120 subjects: 40 HCC patients, 40 hepatitis C patients (20 cirrhotic and 20 non-cirrhotic HCV genotype 4) and 40 healthy controls.

In HCC, statistically significant decrease of miR-182 and miR-150 compared to non-cirrhotic HCV patients ($p = 0.015$, $p = 0.006$ respectively) and of miR-150 compared to controls ($p = 0.039$). In cirrhotic HCV patients, significant down regulation of miR-182 and miR-150 compared to non-cirrhotic HCV ($p = 0.003$, $p = 0.024$ respectively). On the other hand, significant upregulation of miR-182 was observed in non-cirrhotic HCV compared to controls ($p = 0.036$). Alpha-fetoprotein (AFP) showed sensitivity 15% for HCC diagnosis at the cut-off value of 400 ng/ml, while combining AFP with miR-182 and miR-150, resulted in improving sensitivity to (90%) and diagnostic accuracy to (80%).

miR-182 and miR-150 can be used as non invasive biomarkers for HCC and combination of these miRNAs and AFP markedly improve the diagnosis of HCC. Both miR-182 and miR-150 can also be used as predictive markers for detection of cirrhosis progression in HCV infected patients.

1. Introduction

Egypt holds an exceptional position in the prevalence of hepatitis and liver cancer worldwide (Kumar et al., 2015; Mangoud et al., 2004). Liver cancer represents 23.8% of the total malignancies (Ibrahim et al., 2014) and HCC constitutes 70.48% of liver tumors among Egyptians (Gomaa et al., 2014).The incidence of HCC is highest where the endemic high prevalence of hepatitis C and hepatitis B (Ryerson et al., 2016). Hepatitis C virus (HCV) is an independent risk factor for chronic hepatitis, liver cirrhosis, HCC and a major reason for liver transplantation (Mohd Hanafiah et al., 2013). Egypt has the highest HCV prevalence in the world estimated 14.7% (El-Zanaty and Way, 2009).

Serum alpha-fetoprotein (AFP) has been used as HCC biomarker. However, 40% of early stages HCC may be missed by AFP alone and it may remain normal in 15–30% of the patients with advanced HCC, also it may be elevated in some benign liver diseases e.g. hepatitis and cirrhosis (Abdalla and Haj-Ahmad, 2012).

Early diagnosis of HCC is very important to improve the prognosis and the outcome of HCC by early treatment, so sensitive and specific

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biomarkers are essential for early detection and for screening (Morise et al., 2014). Identification of pathogenic factors of HCV plays an important role in better understanding and successful eradication of the disease (Roberts et al., 2011).

miRNAs are naturally occurring, small non-coding RNA, consisting of about 21–25 nucleotides in length, and are important in RNA silencing and post-transcriptional regulation of gene expression (Bartel, 2004). miRNAs are involved in many physiological events such as differentiation, proliferation, and apoptosis, and play a critical role in carcinogenesis (Miska, 2005). miRNAs show high stability in the circulation which makes them perfect noninvasive biomarkers, especially for detection of presymptomatic diseases and early stage cancers (Su et al., 2009; Petrelli et al., 2014).

In HCC, many miRNAs are deregulated: they may act as oncogenes or as tumor suppressors; either by directly or indirectly controlling key proteins involved in cancer-associated pathways (Lujambio and Lowe, 2012). The patterns of most miRNAs expressed in HCC constantly change during disease progression, such as from acute hepatitis, to chronic fibrotic disorders and to cirrhosis (Zhao et al., 2012). Targeting miRNAs is used as therapeutic tool in many diseases (Bai et al., 2009).

In HCV infection, several miRNAs are key players in virus-host interactions; regulating virus replication and pathogenesis (Roberts et al., 2011; Cheng et al., 2012) and, there is an association between different genotypes of HCV genome and miRNAs (Mukherjee et al., 2014). HCV modulates the expression of miRNA leading to hepatocyte growth towards tumorigenesis by regulating various signaling pathways (Giordano and Columbano, 2013).

miR-182 and miR-150 act as tumor suppressors or oncogenes in various cancers. miR-182 is an important regulator of various physiological processes, such as mammalian circadian rhythm, T cell development and DNA repair and it is abnormally expressed in tumors such as, colorectal carcinoma (Liu et al., 2013), breast cancer (Lei et al., 2014), bladder cancer (Chen et al., 2016). miR-150 plays an important role in hematopoietic and immune cells, and controls B and T cell differentiation. It is downregulated in epithelial ovarian cancer (Jin et al., 2014) and overexpressed in lung cancer (Cao et al., 2014).

Accordingly, we aimed to detect the variations in serum levels of miR-182 and miR-150 in Egyptian patients following hepatitis C infection, progression to Cirrhosis and HCC, and their correlation with the clinicopathological features.

2. Materials and methods

2.1. Ethical approval

Approval of the study protocol was obtained from the ethical committee at the faculty of Medicine, Cairo University (according to the WMA Declaration of Helsinki). Informed consent was obtained directly from each subject before data collection and after explanation of the study objectives.

2.2. Patients and samples

This study was conducted on 120 Egyptian adults who were divided into three groups; Group I: included 40 patients with HCC, post HCV infection)26 males and 14 females(with mean age of 58.0 ± 8.6 years. Diagnosis was done according to European Association for the study of the liver (EASL) guidelines (Llovet et al., 2012). Group II: included 40 patients with HCV infection who were further subdivided into 2 groups according to presence of cirrhosis: 20 patients with cirrhotic liver (15 males and 5 females) with mean age of 57.2 ± 9.7 years and 20 patients with non-cirrhotic liver (13 males and 7 females) with mean age of 52.5 ± 9.04 years. All HCC and HCV patients were recruited from the Tropical Medicine Department and Cairo University Center for Hepatic fibrosis (CUC-HF), Cairo University. Group III: included 40 age and sex matched healthy subjects as a control group, with normal liver biochemistry, no history of liver disease or alcohol abuse and no viral hepatitis (34 males and 6 females) with mean age of 57.5 ± 10.0 years. Patients with chronic HBV infection, patients who received antiviral therapy for HCV infection or any loco-regional therapy for HCC were excluded.

All patients and control were subjected to careful history taking. Data were obtained from medical records and direct interviews. Group I and II patients were subjected to ultrasound to document the presence of cirrhosis and hepatic focal lesion(s). Only patients with hepatic focal lesion(s) underwent Triphasic abdominal CT for the diagnosis of HCC.

2.3. Laboratory investigations

Blood samples were collected and investigated for complete blood picture, coagulation profile, liver function tests, kidney function tests, hepatitis C and B markers and serum alpha fetoprotein.

2.4. Analysis of miRNA gene expression

2.4.1. 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 recentrifuged at 15,000 rpm for 15 min to precipitate cell debris and the supernatants were stored at −80 °C until RNA extraction. RNA for miRNA expression profiling was isolated from the serum using the miRNeasy Mini Kit (Qiagen, cat. no. 217,004). The concentration and purity of RNA were determined by measuring the absorbance at 260 and 280 nm using NanoDrop 1000 A Spectrophotometer (NanoDrop Technologies, Waltham, MA). These samples were used for determination of expression level of mature miR-182 and miR-150 with Syn-cel-miR-39 miScript miRNA Mimic (cat. no. MSY0000010) was used as reference gene for normalization.

2.4.2. TaqMan_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), 3 µl stem-loop RT primer and 7 µl RT Reaction Master Mix using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Sequences of mature miRNAs and Cel-mir-39 were identified using the miRbase (http://www.mirbase.org) and shown in Table 1.

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, No 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

Table 1
Target miRNA and control Sequences to be Studied and Amplified by qRT-PCR.

Target MiRNA	MiRbase No.	Mature sequence
MiR-182	MIMAT0000259	UUUGGCAAUGGUAGAACUCACACU
MiR-150	MIMAT0000451	UCUCCCAACCCUUGUACCAGUG
Cel-mir-39	MIMAT0000010	UCACCGGGUGUAAAUCAGCUUG

TaqMan® MicroRNA RT primer 5× (Applied Biosystems, USA, part No. 4427975).
TaqMan®MicroRNA assays 20× (Applied Biosystems, USA, part No.4427975).
Gene symbol: hsa-miR-182 and hsa-miR-150.
miRBase ID: hsa-miR-182-5p and hsa-miR-150-5p.
TaqMan®Cel-mir-39 Control Assay 20× (Applied Biosystems, USA, part No.4427975).
Gene symbol: Cel-mir-39.
miRBase ID: Cel-mir-39-3p.

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 the control were identified using the miRbase (http://www.mirbase.org). Cel mir-39 was used as reference genes (Syn-cel-miR-39 miScript miRNA Mimic. cat. no. MSY0000010). Relative expression of miRNA was calculated using the comparative cycle threshold (Ct) method. Fold change of each candidate miRNA within each group was then calculated using the equation $2^{-\Delta\Delta CT}$

2.5. Statistical methods

Statistical analysis was done using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Quantitative data were tested for normality using Kolmogorov-Smirnov test and Shapiro-Wilk test. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using ANOVA for normally distributed numeric data or Kruskal-Wallis test (non-parametric ANOVA)then post-Hoc "Schefe test" was used for pair-wise comparisonbased on Kruskal-Wallis distribution for not normally distributed data. Spearman-rho method was used to test correlation between numerical variables. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values. For multiple comparisons, p-value was corrected using Bonferroni method. All tests were two-tailed. A p-value < 0.05 was considered significant.

3. Results

3.1. The laboratory and the clinical data

The groups of the current study describe chronic HCV infection and its sequelae (non-cirrhotic HCV, cirrhotic HCV and HCC). The clinical and laboratory data of the studied groups are summarized in Table 2.

3.2. Serum level of miRNAs

The Median Values of Fold Changes of miRNAs among the Studied Groups are shown in Fig. 1.

A. Role of serum levels of miR-182 and miR-150 in detecting HCV infection and cirrhosis

Comparisons between non-cirrhotic HCV patients, cirrhotic HCV patients and control group were done to detect if the studied miRNAs can differentiate different HCV groups from each other and from the control group. Serum level of miR-182 and miR-150 were statistically significantly higher in non-cirrhotic HCV group than cirrhotic HCV group *(P = 0.003, P = 0.024 respectively)*. On comparing cirrhotic HCV group with the control group, serum levels of miR-182 and miR-150 showed no statistically significant difference between both groups *(p = 0.198, P = 0.121 respectively)*. miR-182 was found to be significantly higher in the non-cirrhotic HCV group when compared to the control group *(p = 0.036)*, but miR-150 showed no statistically significant different *(p = 0.288)*.

B. Role of serum levels of miR-182 and miR-150 in detecting HCC

To investigate the role of the serum levels of miR-182 and miR-150 in detection of HCC; their levels in HCC group were compared to non-cirrhotic, cirrhotic HCV patients and the control group. By comparing HCC group with cirrhotic HCV group, no statistically significant difference was detected regarding serum level of miR-182 and miR-150 *(p = 0.335, P = 0.894 respectively)*. Comparing HCC with non-cirrhotic HCV group revealed that serum level of miR-182 and miR-150 were statistically significantly higher in non-cirrhotic HCV group than HCC

Table 2
The laboratory and the clinical data of the studied groups.

Variables	HCC (n = 40)	HCV Cirrhotic (n = 20)	HCV non-cirrhotic (n = 20)
Hb(g/dl)	12.1(8.8-16.2)[a]	10.7(6.4-14.0)[b]	13.2(11.4-16.1)[c]
TLC(10^3/cmm)	4.8(2.0-13.7)[a]	6.2(2.8-22.0)[b]	5.9(4.0-14.5)[ab]
Plt(10^3/cmm)	125.0(45-360)[a]	153.0(50-354)[a]	199.5(130-336)[b]
PT(sec)	14.4(11.5-21.3)[a]	17.0(11.9-44.7)[b]	12.8(10.7-13.6)[c]
PC(%)	80.0(40-100)[a]	61.0(16-92)[b]	89.0(73-99)[c]
INR	1.2(1.0-2.0)[a]	1.4(1.0-4.9)[b]	1.0(0.8-1.3)[c]
ALT (U/L)	41.5(11.0-105.0)[a]	33.5(7.0-57.0)[a]	14.5(10.0-138)[b]
AST (U/L)	66.0(14.0-145)[a]	50.0(17.0-122)[b]	22.5(16.0-91)[c]
T.Bil(mg/dl)	1.10(0.12-4.30)[ab]	1.20(0.20-17.90)[a]	0.85(0.28-1.50)[b]
Alb (g/dl)	3.2(2.2-4.8)[a]	2.7(1.2-4.5)[b]	4.5(3.5-5.3)[c]
Creat.(mg/dl)	0.90(0.40-6.60)[a]	1.00(0.60-3.58)[a]	0.81(0.60-1.00)[b]
AFP(ng/mL)	75.3(3.5-87545)[a]	2.2(0.7-6.0)[b]	1.9(0.7-8.0)[b]
Child Pugh Class			
A	23(57.5%)a	7(35.0%)a	
B & C	17(42.5%)a	13(65.0%)a	
Ascites			
Absent	26(65.0%)a	7(35.0%)b	20(100.0%)c
Present	14(35.0%)a	13(65.0%)b	0(0.0%)c
Variables		**HCC (n = 40)**	
Number of tumors			
Single		25(62.5%)	
Multiple		15(37.5%)	
Size of main tumor			
< 3 cm		10(25%)	
≥ 3 cm		30(75%)	

All data are presented as median (min.-max.).
Groups bearing same initials are not significantly different from each other at P value = 0.05.
Groups bearing different initials are significantly different from each other at P value = 0.05.
P-value < 0.01 is moderately significant, P-value < 0.001 is highly significant.
No child pugh score is done for non-cirrhotic patients.

(P = 0.015, P = 0.006 respectively). miR-150 was found to be statistically significantly higher in the control group than HCC group *(p = 0.039)*, but miR-182 showed no statistically significant difference *(p = 0.694)*.

C.Correlation between miR-182 and miR-150 in HCC, cirrhotic HCV and non-cirrhotic HCV patients

Statistically significant positive correlation was found between the two miRNAs in the HCV non-cirrhotic group (*p = 0.001, r = 0.689*) (Fig. 2).

D. Diagnostic Performance of miRNA

Receiver operating curve (ROC) analysis was performed to evaluate the usefulness of serum miR-182 and miR-150 as potential diagnostic markers to discriminate between HCC and non-cirrhotic HCV. Serum miR-182 at the cut-off value of 2.39248, showed sensitivity of 72.5% and specificity of 65% with areas under curve (AUC) was 0.675 *(P = 0.015*, 95% CI 0.537- 0.813), and serum miR-150 at the cut-off value of 1.00005, showed sensitivity of 67.5% and specificity of 70% with AUC was 0.704 (P = 0.006, 95% CI 0.571- 0.838) (Fig. 3A). Thus, miR-182 and miR-150 can be used as markers for detection of malignant transformation in HCC when compared with non-cirrhotic HCV group.

ROC curve analysis was performed to evaluate the usefulness of serum miR-150 as a potential diagnostic marker to discriminate

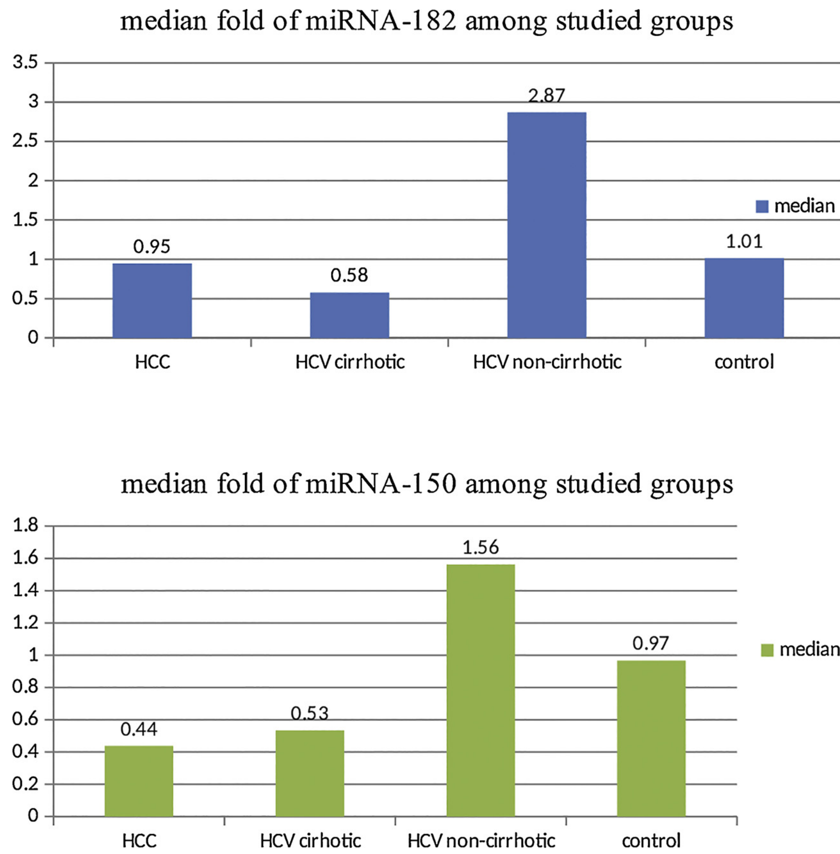

Fig. 1. The Median Values of Fold Changes of miRNAs among the Studied Groups.

between HCC and the control. At the cut-off value of 0.67453, serum miR-150 showed sensitivity of 60% and specificity of 70% with areas under curve (AUC) was 0.638 *(P = 0.039*, 95% CI 0.512- 0.763) (Fig. 3B), showing that miR-150 can be used as a diagnostic marker for differentiating HCC from the healthy control.

To discriminate between non-cirrhotic and cirrhotic HCV, serum miR-182 at the cut-off value of 1.59916, showed sensitivity of 80% and specificity of 70% with AUC was 0.760 *(P = 0.003*, 95% CI 0.603-0.917). Serum miR-150 at the cut-off value of 0.98652, showed sensitivity of 65% and specificity of 70% with AUC was 0.711 (*P = 0.024*, 95% CI 0.551- 0.871) (Fig. 4A). This proves that miR-182 and miR-150 can detect fibrosis progression and cirrhosis when comparing non-cirrhotic and cirrhotic HCV patients.

Regarding non-cirrhotic HCV and the control, ROC curve analysis showed at the cut-off value of 2.49480, serum miR-182 showed sensitivity of 75% and specificity of 60% with areas under curve (AUC) was 0.689 *(P = 0.036*, 95% CI 0.540- 0.837) (Fig. 4B). This Proves that miR-182 can be used as circulatory biomarkers in HCV infection as it differentiates HCV non-cirrhotic from the control.

E. Analysis of markers combinations

The capability for differential diagnosis of HCC and non-cirrhotic HCV was analyzed by combinations of serum miR-182, miR-150 and AFP as shown in Table 3.

4. Discussion

In our study, median fold of miR-182, was significantly higher in non-cirrhotic HCV patients than the control (P = 0.036), this suggests that HCV infection has a great impact on miR-182 expression and suggesting the possible role of miRNA in HCV pathogenesis and

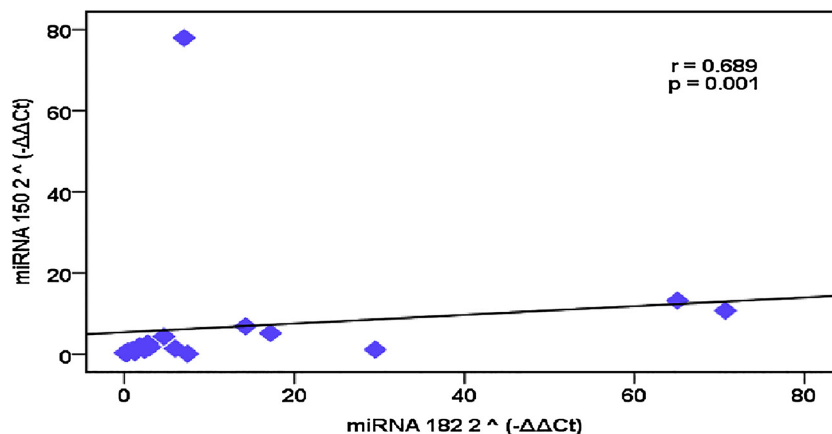

Fig. 2. Positive correlation between the studied miRNAs in the HCV non-cirrhotic group.

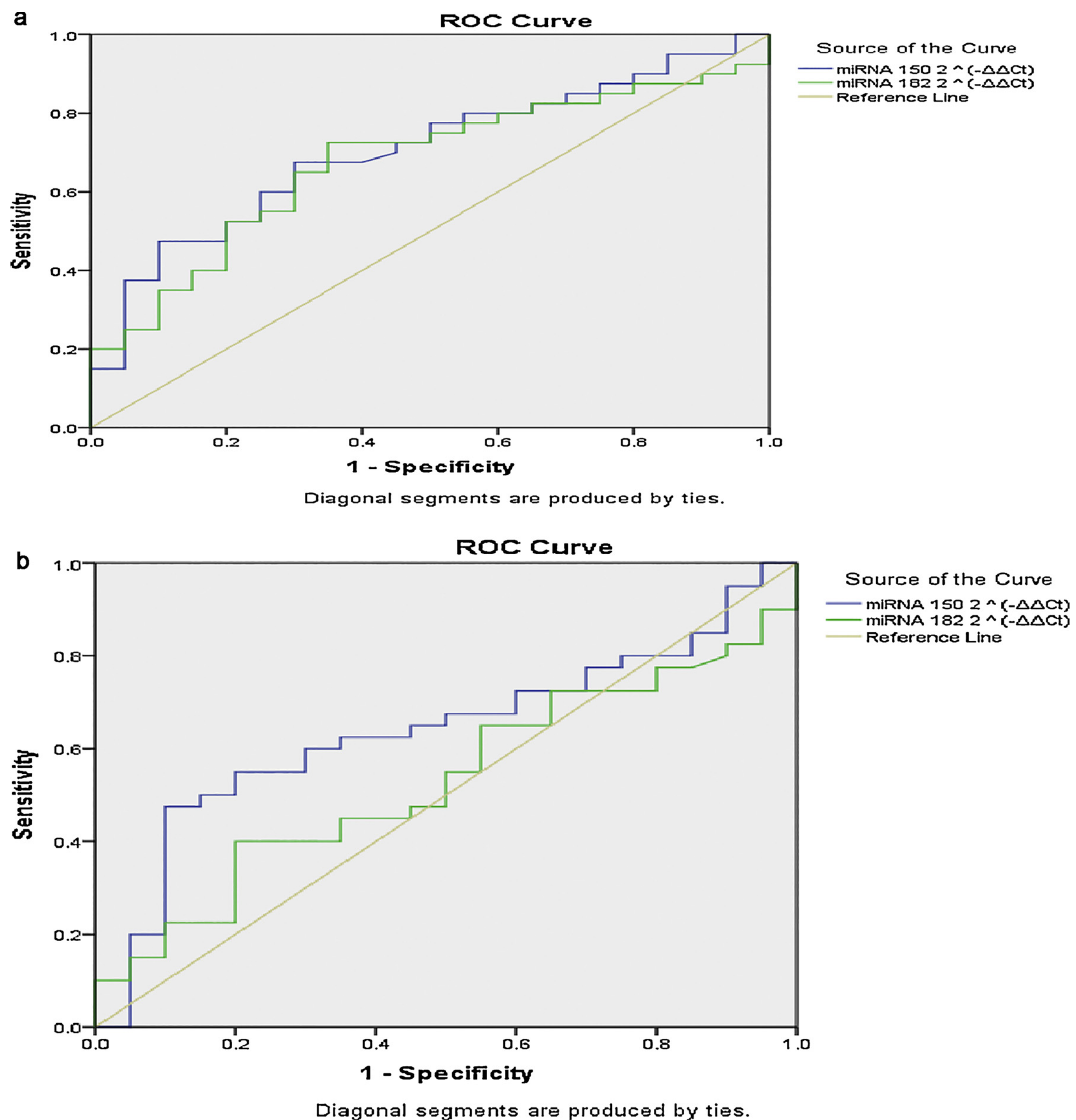

Fig. 3. A: ROC curve analysis showing AUC of the studied miRNAs in HCC group versus HCV non-cirrhotic group. **B:** ROC curve analysis showing AUC of the studied miRNAs in HCC group versus control group.

replication. Thus, miR-182 can be used as circulatory biomarker in HCV infection. One of the major challenges in HCV research is the detection of early stage liver disease which will allow for rapid intervention and improved outcome of antiviral treatment. Shrivastava et al. found increased expression of miR-20a and miR-92a in sera specific to HCV associated liver disease but not in sera of patients with non-HCV related liver disease (Shrivastava et al., 2013). van der Meer et al. found higher levels of miR-122 and miR-192 in sera from patients with chronic hepatitis C as compared to sera from healthy controls (van der Meer et al., 2013). Serum levels of miR-134, miR-320c and miR-483-5p were found by Shwetha et al. significantly upregulated in HCV infected patients (Shwetha et al., 2013).

As the disease progresses from non-cirrhotic to cirrhotic HCV stage, a significant reduction in miR-182 can be observed ($P = 0.003$), which suggests the role of miR-182 in detecting fibrosis progression and cirrhosis. This is in accordance with a study done by Riazalhosseini et al., they found that miR-182 was down-regulated in patients with chronic hepatitis B who progressed to cirrhosis as compared to those without progression (Riazalhosseini et al., 2017). In contrast Van Keuren-Jensen et al. found that miR-182 was significantly upregulated in liver tissue from chronic HCV patients with advanced fibrosis (stage F3 and F4), compared to liver biopsies from patients with early fibrosis (stages F1 and F2) (Van Keuren-Jensen et al., 2016).

Comparing cirrhotic HCV group with HCC group revealed a second rise in miR-182, however it didn't reach a statistical significance, but might support its oncogenic role. miR-182 was statistically significantly higher in non-cirrhotic HCV group compared to HCC group ($P = 0.015$). This shows that miR-182 can be used as marker for detection of malignant transformation.

Chen et al., studied serum miRNA-182 in HCC patients and compared it with benign liver diseases and healthy controls. They found that the level of serum miR-182 was significantly increased in HCC patients compared with benign liver diseases and healthy controls and there was no statistical difference of serum miR-182 level between the benign and control group. However, discrepancy with our study is mainly due to emergence of HBV as the main risk factor for HCC and absence of cirrhosis in almost half of cases (Chen et al., 2015).

Regarding miR-150, the pattern was interpretative, the highest level

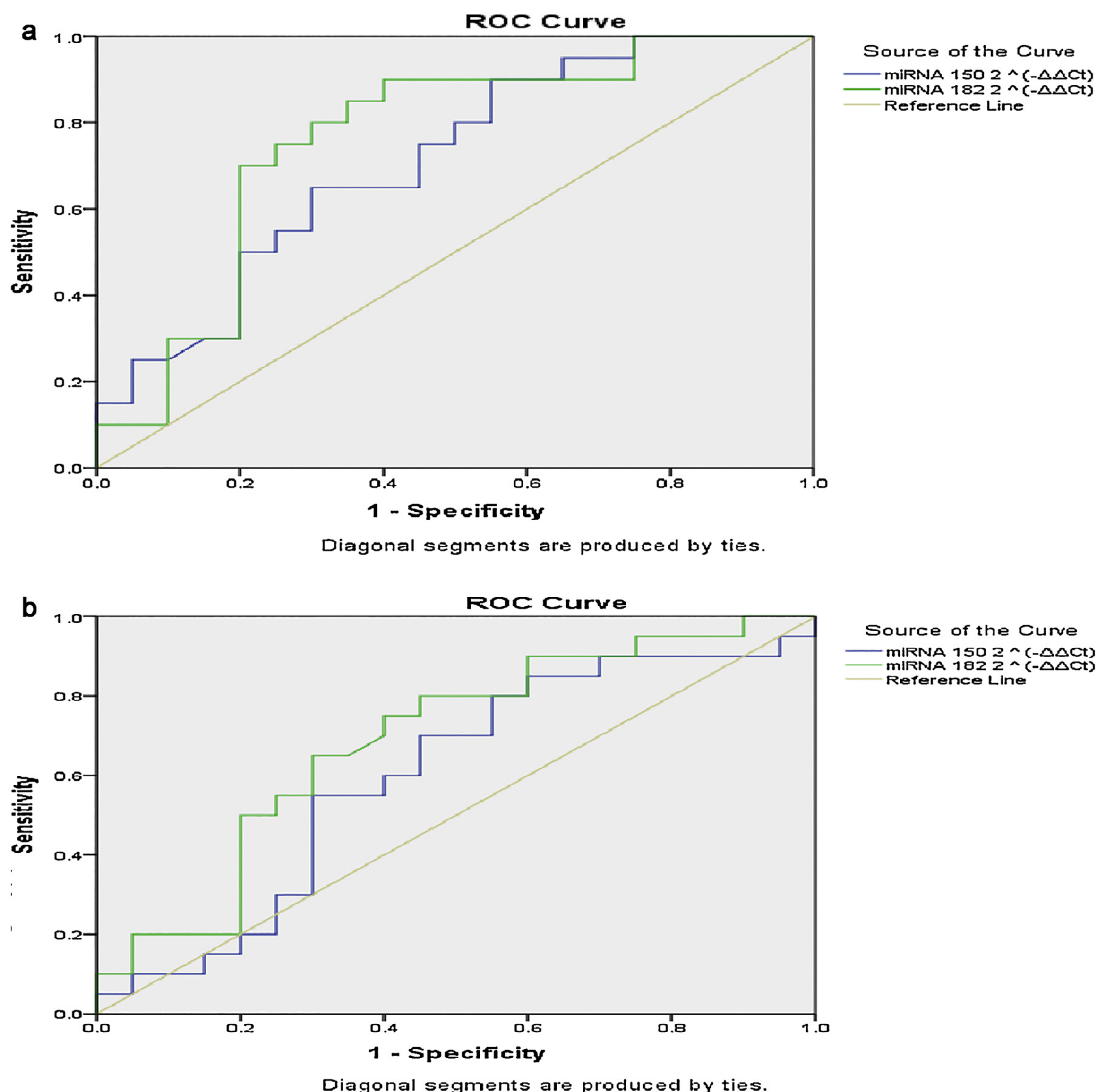

Fig. 4. A: ROC curve analysis showing AUC of the studied miRNAs in HCV cirrhotic group versus HCV non-cirrhotic group. **B:** ROC curve analysis showing AUC of the studied miRNAs in HCV non-cirrhotic group versus control group.

Table 3
Diagnostic accuracy of miR-182, miR-150 and AFP between HCC group and HCV non cirrhotic group.

Variable	Cut off	Sensitivity	Specificity	PPV	NPV	DA
AFP	400 ng/ml	15.0%	100%	100%	37.0%	40.3%
MiR-182	2.39248	72.5%	65%	80.6%	54.2%	70.0%
miR-150	1.0005	67.5%	70%	81.8%	51.9%	68.3%
AFP400 + miR-182	One positive of two	77.5%	65.0%	81.6%	59.1%	73.4%
AFP400 + miR-150	One positive of two	70.0%	70.0%	82.4%	53.8%	70.0%
AFP400 + miR-182 + miR-150	One positive of three	90.0%	75.0%	81.8%	60.0%	80.0%

PPV: Positive Predictive Value, NPV: Negative Predictive Value, DA: Diagnostic Accuracy.

was detected in non-cirrhotic HCV group (1.562) followed by the cirrhotic HCV group (0.534) and the lowest level in HCC group (0.437). No statistically significant difference was found between the HCV non-cirrhotic and control groups ($p = 0.288$), this proves that HCV infection has no effect on miR-150 expression, unlike miR-182.

As the patient passes from the stage of non-cirrhotic to the cirrhotic HCV stage statistically significant reduction in miR-150 can be detected ($P = 0.024$). This is similar to miR-182 which was found down-regulated with cirrhosis. The reduced amount of both miR-182 and miR-150 in cirrhotic patients can be due to the decrease in the number of hepatocytes during fibrosis progression and the increase in the numbers of lymphocytes, Kupffer cells and hematopoietic stem cells, this may explain the reduced amount of these miRNAs with hepatocyte loss at advanced stage of fibrosis. Same explanation was given by Estrabaud et al. who found reduced levels of miR-122 in both serum and liver samples from patients with chronic hepatitis C with advanced fibrosis (Estrabaud et al., 2014).

Although the expression of miR-150 decreases when the patient passes from cirrhotic HCV stage to the malignant stage, there was no statistically significant differences between cirrhotic HCV and HCC group ($p = 0.894$). This can be strong evidence that cirrhotic HCV

patients are in a premalignant stage that needs close observation and follow up for early detection of malignant transformation

Comparison of HCC with the control and non-cirrhotic HCV group revealed statistically significant down regulation of miR-150 in HCC (P = 0.039, P = 0.006 respectively). Similar results were found by Yu et al. who studied the expression of circulating miR-150 in hepatitis B virus-related HCC (Yu et al., 2015) and Shi et al. who analyzed the miR-150 expression in HCC tissues versus the adjacent non tumorous tissues. These results showed that miRNA-150 was decreased in the HCC tissues (Shi et al., 2015).

Down regulation of miR-150 in HCC patients supported its role as tumor suppressor and its possible role in the pathogenesis of HCC. Several studies have illustrated that miR-150 can function as a tumor suppressor or oncogene in various cancers. Similar to our findings, Feng et al. found that miR-150 expression was downregulated in colorectal cancer compared to paired non-cancerous tissue and acted as a tumor suppressor (Feng et al., 2014). On the contrary, a study done by Cao et al. found that miR-150 was overexpressed in lung cancer (Cao et al., 2014).

AFP at a cut-off value of 400 ng/ml, showed sensitivity and specificity for HCC 15% and 100%, respectively. Our results showed that the combination assay can markedly increase the differential diagnostic value of non-cirrhotic HCV and HCC better than serum AFP alone, reaching sensitivity 90% and diagnostic accuracy 80%. Same result was found by Chen et al., who found that combination assay of serum miR-182 and AFP can markedly increase differential diagnostic value of benign and malignant liver diseases, especially better than serum AFP alone (Chen et al., 2015).

Elemeery et al. aimed to investigate the prospective importance of certain serum miRNAs as early biomarkers for the diagnosis of HCV-related HCC in Egyptian population. Serum miRNA profiles were studied from mild fibrosis progression to HCC. This study concluded that miRNAs have a strong power as putative diagnostic and prognostic biomarkers for HCV-induced HCC. Moreover, some of these miRNAs could be considered as early biomarkers for tracking the progress of liver fibrosis to HCC. (Elemeery et al., 2017).

In our study, analysis of the potential associations of serum miR-182 and miR-150 levels with various clinic-pathological characteristics of HCC was done. miR-182 was found to have tendency to be statistically significantly higher in HCC patients with tumor size $\geq$ 3 cm than those having tumor size < 3 cm *(P = 0.072)* and miR-182 was also higher in HCC patients with ascites but was not statistically significant. miR-150 was statistically significantly higher in patients with child Pugh score A than those having score B&C *(P = 0.012),*also it was found higher in HCC patients with small tumor mass(< 3 cm) and those without ascites but was not statistically significant. These result revealed that miR-150 usually associated with better presentation and prognosis than miR-182.

Similarly, Wang et al. found that miR-182 was associated with bad prognosis as miR-182 contributed to metastasis of hepatocellular carcinoma (Wang et al., 2012). Li et al. found that miR-150 in HCC was usually associated with good prognosis as it was decreased in metastatic cancer tissues compared with pair primary tissues (Li et al., 2014).

No statistically significant association was found between the studied miRNAs and age, sex and number of tumors. No statistically significant correlation was found between the studied miRNAs and laboratory parameters (hemoglobin, total leucocytic count, platelet count, PT, PC, INR, AST, ALT, total bilirubin, albumin, creatinine, AFP). In concordance to this study, Chen et al. found that the level of serum miR-182 was positively correlated with tumor size (P = 0.013) and found no significant correlation with age and sex (p > 0.05). However, contrary to our results they found positive correlation with serum AFP (P = 0.001) (Chen et al., 2015). Regarding miRNA-150, findings of Yu et al. were matching with what we found, there was no significant association of miR-150 with clinical features such as sex, age, AFP levels and tumor size *(P > 0.05)* (Yu et al., 2015).

In addition, associations and correlations of the studied miRNAs with the clinical and laboratory characteristics of cirrhotic HCV and non-cirrhotic HCV patients were done, there were no significance apart from the presence of ascites in HCV cirrhotic group. We found that the median value of serum miR-150 has tendency to be statistically significantly higher in patients without ascites than those having ascitis (P = 0.056)

We uniquely analysed the correlation between miR-182 and miR-150 in HCC, cirrhotic HCV and non-cirrhotic HCV patients.

The discrepancy between the results of this study and other studies may be attributed to the large ethnic and geographic variability in the incidence of HCC among the different populations, the different HCV genotypes other than genotype 4 which represents over 90% of cases in Egypt and additional risk factors for HCC other than HCV including HBV and Alcohol induced HCC.

5. Conclusions

miR-182 and miR-150 can be used as non invasive biomarkers for HCC and combination of these miRNAs with AFP markedly improved the diagnostic accuracy of HCC. They also can be used as predictive markers for detection of cirrhosis progression in HCV infected patients. miR-182 may be useful as circulatory marker for HCV infection.

Thus, it is necessary to reproduce studies in ethnically diverse populations, with different HCV genotypes and additional risk factors for HCC to evaluate the real importance of these miRNAs in HCC, the reduced sample size might be a limitation of our study so further studies, with larger sample sizes are necessary.

Conflicts of interest

All authors have no actual or potential conflicts of interest.

Disclosure

All authors have no conflict of interest.

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