

The Significance of Circulating Micro RNA-122 as a Non Invasive Diagnostic Marker of Liver Injury in Egyptian Chronic Hepatitis C virus Infected and Cirrhotic Patients with and without Hepatocellular Carcinoma

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Abstract Background: Micro RNAs (miRNAs) are small RNA molecules that have the ability to regulate gene expression by targeting mRNA degradation or translational repression. miRNAs regulate HCV life cycle either by supporting viral replication or by inhibiting interferon signaling pathway. **Purpose:** This work was carried out to evaluate the expression level of (miRNA-122) as a non invasive diagnostic marker of liver injury in Egyptian chronic hepatitis C virus infected and cirrhotic patients with and without hepatocellular carcinoma (HCC). **Patients and Methods:** This study was conducted on 35 Chronic HCV infected and cirrhotic patients. Patients divided into two groups: Group I comprised 20 patients with cirrhosis, group II comprised 15 patients with HCC. Both groups were compared to 15 (age and sex matched) healthy persons (group III). Patients and control were subjected to detailed history taking, clinical examination, abdominal ultrasonography, CT and Liver biopsy when needed for HCC cases. Laboratory investigation including complete blood picture, Liver function tests, and viral hepatitis markers, serum AFP and Expression levels of Micro RNA-122 in whole blood using Real Time quantitative polymerase chain reaction method (RT-PCR) were determined. **Results:** Mean expression level of miRNA-122 was significantly higher in both patient groups (I and II) as compared to control group (III) ($p < 0.001$) for each, Patients with HCC showed non significant higher expression level of miRNA-122 as compared to cases with cirrhosis alone. Furthermore, expression levels of Micro RNA-122 was positively correlated with ALT, AST, and Alkaline phosphatase in both patient groups and negatively correlated with prothrombin concentration in both patients groups. While Micro RNA-122 not correlated with total bilirubin, Serum Albumin, and platelets count, or AFP serum level. **Conclusion:** The study concluded that there was increased expression of miRNA-122 in Egyptian chronic hepatitis C virus (CHCV) infected and cirrhotic patients with and without HCC. Micro RNA 122 can be used as novel biomarkers for liver injury but not specifically for HCC. We recommend further studies on large scale to evaluate the diagnostic, prognostic value of miRNA 122 and to evaluate anti-miRNA 122 as a therapeutic option for hepatic C virus infection and its related complications.

Keywords Micro RNA-122, Chronic HCV, Liver cirrhosis and hepatocellular carcinoma

1. Introduction

Worldwide, HCV is a major etiologic agent of chronic hepatitis that progressively can lead to cirrhosis, hepatocellular carcinoma (HCC) and liver failure[1]. Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease (CLD) in Egypt[2]. Hepatocellular carcinoma

(HCC) is the commonest primary liver cancer with increasing incidence to become the 5th commonest malignancy worldwide and the third leading cause of cancer-related death.[3]. In Egypt, between 1993 and 2002 there was an almost twofold increase in HCC among chronic liver patients, HCC was reported to account for about 4.7% of chronic liver disease patients[4].

The most common condition associated with hepatocarcinogenesis is cirrhosis which develops 20 to 40 years of chronic liver disease. Major causes of cirrhosis in patients with HCC include hepatitis B, hepatitis C, alcoholic liver disease and possibly nonalcoholic steatohepatitis. It has

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been reported that not only increased cellular proliferation but also, diminished cell death play important roles in hepatocarcinogenesis and tumor progression in HCC[5].

In recent years microRNAs (miRNAs), a family of short (average of 20~25 nucleotide long), naturally occurring, small antisense non-coding RNAs have emerged as important post-transcriptional regulators of gene expression. There may be thousands of miRNA genes in the human genome, transcribed by RNA polymerase as long primary miRNA molecules, then processed in the nucleus forming pre-miRNAs[6]. These pre-miRNAs get transported from the nucleus to the cytoplasm for further processing. miRNAs are predicted to control the activity of more than 60% of all protein-coding genes[7]. It has been estimated that miRNAs regulate up to 30% of human genes. Like mRNAs, some miRNAs also show restricted tissue distribution; for example, miRNA-122 is highly enriched in liver, whereas miR-124 is preferentially expressed in neurological tissues[8]. study shows that changes in the spectrum of cellular miRNAs correlate with various pathological disease including inflammation, diabetes, and several types of cancers[8&9].

Recent studies have identified several miRNAs as key players in virus-host interactions, during HCV infection it has been demonstrated that a liver-specific miRNA, miR-122, binds to the 5' nontranslational region of hepatitis C virus (HCV) genomic RNA and is essential for HCV replication in human hepatoma-derived Huh7 cells[10]. This observation is further supported by a recent report showing that HCV (JFH1 strain) infection of Huh7 cells requires the functional miRNA biogenesis pathway[11]. Several studies demonstrated that miR-122 is required for HCV replication in infected cells[10&11].

The use of non-invasive biomarkers for the diagnosis of diseases has become a rapidly growing area of clinical research. Micro RNA expression has been studied at cellular level and in tissues in patients with HCC.

Aim of the study: This work was carried out to evaluate the expression level micro RNA-122 as a non invasive diagnostic marker of liver injury in Egyptian chronic hepatitis C virus infected and cirrhotic patients with and without HCC.

2. Patient and Methods

The study included Thirty five patients with chronic hepatitis C virus (CHCV) infected and cirrhotic patients (23 males and 12 females, age range 41- 68 years). Attending the outpatient and inpatient clinic of the Department of Internal Medicine - Sayed Galal University Hospital, and outpatient and inpatient clinic of Tropical and Internal Medicine Departments of Al Zahraa University Hospital, Cairo, Egypt, from January 2013 till June 2013.

According to the results of abdominal ultrasonography, spiral CT scan, liver biopsy and laboratory investigation including alpha fetoprotein, the patients were classified into two groups as follows:

Group I: included twenty patients with post HCV liver cirrhosis they were 13 males and 7 females and their ages ranged between 41 and 64 years old with a mean $\pm$ SD (53.5 $\pm$ 5.9 years).

Group II: included 15 chronic hepatitis C virus (CHCV) infected and cirrhotic patients with proved HCC. They were (10 males and 5 females), their ages ranged between 45 and 68 years with a mean $\pm$ SD (55.5 $\pm$ 6.5 years).

Exclusion criteria: The patients with known history of autoimmune diseases, Diabetes mellitus, chronic inflammatory disease and a history of cancer other than hepatocellular carcinoma, as well as patients with chronic liver disease not HCV related were excluded.

In addition fifteen healthy volunteers were included into the study as control group (Group III) they were 10 males and 5 females their ages ranged between 40 and 57 years with a mean $\pm$ SD (48.5 $\pm$ 7.1years).

This study was performed after obtaining informed consent from all participating subjects.

All patients and control were subjected to the following:

1- Through history taking and complete clinical examination with stress on jaundice, hepatomegaly, ascites, splenomegaly, lower limb oedema, and encephalopathy.

2- Abdominal ultrasound. To assess the echo pattern and size of the liver and the presence of periportal fibrosis, the presence of ascites, the size of spleen or any other abnormalities .

3- Abdominal spiral CT scanning and or MRI were performed for patients as well as liver biopsy and histopathological examination when indicated.

Laboratory investigation:

* Liver function tests were measured using COBAS® INTEGRA 400 plus Autoanalyzer (Roche-Germany) using Kits supplied from Roche Diagnostics GmbH, Mannheim, Germany (as routine follow up).

* Prothrombin concentration was done by Thrombol –S Kit using Fibrintimer (Dade Behring-Germany).

* Complete blood cell counts were measured by Sysmex K-21 automatic cell counter.

* Serum HBsAg were done using an ELISA Kit (Murex HBsAg Version 3) provided by DiaSorin S.P.A. UK branch Ref 9F80-01. HCV antibody was done using an ELISA Kit (ORTHO HCV 3) provided by Ortho Clinical Diagnostics, NJ.USA. HCV infection was confirmed by the detection of HCV-RNA using a qualitative reverse transcription –polymerase chain reaction (PCR).

* Serum AFP was measured using COBAS 411 by Kit supplied from Roche Diagnostic. GmbH. Ref N 04481798

miRNA-122 isolation and real –time quantitative RT-PCR:

miRNA Isolation and Sample Preparation

For sample preparation, the EDTA blood samples were preserved at -80°C prior to miRNA isolation, and miRNA was extracted from the samples by High Pure miRNA isolation kit Ref N 05080576001 (Roche Diagnostics GmbH

Manheim, Germany), following the 1-column protocol for the isolation of total RNA containing small RNA.

75 µl of whole EDTA blood + 156 µl Binding Buffer+ 100 µl Binding enhancer were dispensed in High Pure filter tube and then spin at 13000 rpm for one minute, then the flow through discarded. 250 µl of wash buffer was dispensed then the tube was spin for 30 seconds at 13000 rpm. The flow through discarded. 150 µl of wash buffer was dispensed then the tube was spin for 30 seconds at 13000 rpm. The flow through discarded then the tube was spin for 1 minutes at 13000 rpm. Collection tube discarded and the filter tube was placed in fresh 1.5 ml micro centrifuge tube. 50 µl of Elution buffer was add on the center of the filter, incubated for 1 minute at 25°C then the tube was spin for 1 minutes at 13000 rpm, giving purified total RNA including small RNA.

Real-Time Relative expression Assay

The expression levels of miRNA-122 and its house keeping gene glyceraldehyde-3- phosphate dehydrogenase (GAPDH) were measured by quantitative RT-PCR.

Real-time assay was performed with SYBR Green detection mix from Roche Molecular Diagnostics (USA) using Roche Light Cyclor 2.0.

The working master mix was prepared following the manufacturer instructions by adding 4.4 µL PCR grade H₂O, 4.0 µL 5X RT-Buffer, 0.2 µL Specific Primer for mir-122 & GAPDH, 0.4 µL DNTPs Mix, 0.4 µL AMV Reverse Transcriptase & 0.6 µL RNase Inhibitor per reaction. Finally, 10 µl of working master mix and 5 µl of the sample were dispensed in each capillary and were run on Roche Light Cyclor 2.0.

Thermal profile used was as follows: **Reverse transcription** 1 cycle 55°C for 10 min; **Polymerase activation** one cycle of 95°C for 10 min; **Cycling** 45 cycles of 95°C for 10 sec, 61°C for 10 sec and 72°C for 15 sec; **Melting curve analysis** 95°C for 0 sec, 65°C for 15 sec then 95°C for 0 sec; followed by a single **Cooling cycle** 40°C for 30 sec.

The selected primer forward for miRNA 122 gene Bank reference sequence number (1448488) was the following sequence

Forward: 5'GACAAGCCTGGCTACTGTGTT-3'.

The selected Reverses: primer for miRNA 122 gene Bank reference sequence number (1448489) was the following sequence

Reverses: 5'-GTGGCCCATCTTGTCTTC-3'.

Glyceraldehyde-3- phosphate dehydrogenase (**GAPDH**) was used as input control. The primer sequence of **GAPDH** was as follow:

Forward: 5' CGCTCTCTGCTCCTCCTGTT ' 3

Reverse: 5' CCATGGTGTCTGAGCGATGT ' 3

Real-time data were analyzed with LC software, version 4.0 (Roche Molecular Diagnostics, USA). The expression levels of miRNA 122 in tested samples were expressed in the form of CT (cycle threshold) level then Normalized copy number (Relative quantitation) was calculated using the $\Delta\Delta C_t$ equation[12], with the use of GAPDH as reference (housekeeping control) genes. miRNA expression levels are expressed in copy number/ml.

Statistical analysis:

Data was analyzed by Microsoft Office XP (excel) and SPSS computer program version 15.0, IBM Corporation, New York, USA. Parametric data was expressed as mean $\pm$ SD, and non-parametric data was expressed as number and percentage of total, the analysis of data was done to test statistical significant difference for quantitative data using Student ' t test. Chi square (X² test) was used for qualitative data. Correlation coefficients (r) were calculated using the Pearson's correlation analysis. P value was significant at P<0.05 level.

3. Results

Results obtained are presented in tables 1-4 and Figure 1.

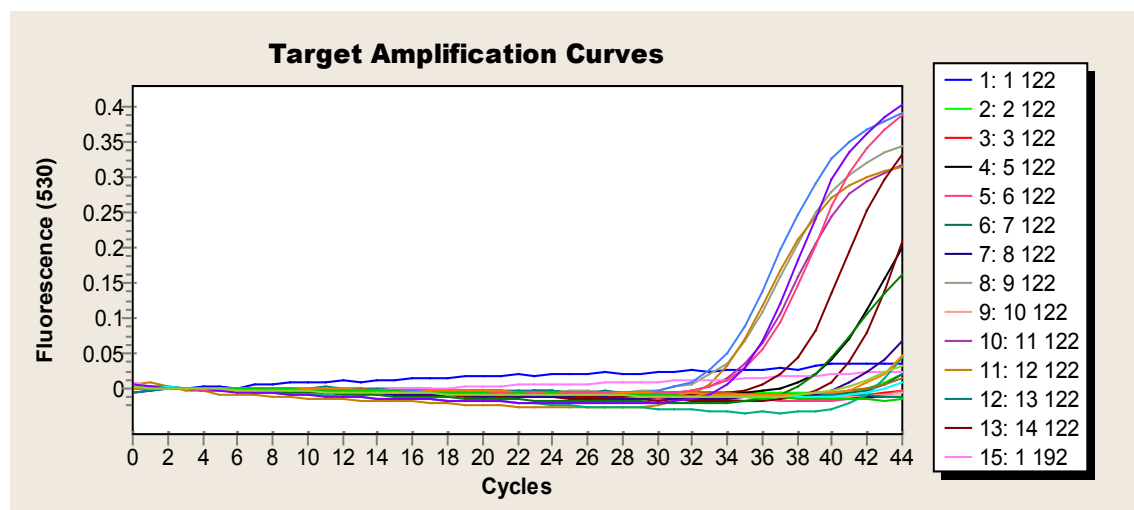


Figure (1). Showing Micro RNA-122 expression by RT-PCR in studied groups

Table (1) Shows demographic data, and clinical characteristics of studied patients, hepatomegaly, splenomegaly, jaundice, melena and hematemesis were not significantly different among both groups ($p>0.05$). While ascites was significantly increased in HCC cases.

A highly significant increase in AST, ALT, ALP, and total bilirubin were detected in both patients group compared to controls. While, serum albumin, prothrombin concentration and platelets were significantly decreased (Table 2).

AFP serum levels were significantly increased in all patients groups as compared to control group, its levels in HCC patients was significantly higher than in cirrhotic

patients without HCC. Mean expression level of miRNA-122 was significantly higher in both patient groups (I and II) as compared to control group (III) ($p<0.001$) for each, Patients with HCC showed non significant higher expression level of miRNA-122 as compared to cases with cirrhosis alone (Table 3).

The data from all groups (I and II) showed that Micro RNA-122 expression levels were positively correlated with AST, ALT, and ALP and negatively correlated with prothrombin concentration. While Micro RNA-122 not correlated with total bilirubin, Serum Albumin, and platelets count or AFP serum level.

Table 1. Demographic and clinical data of the studied patients groups (CHCV)

Variables	Cirrhosis alone (G1) N=20	HCC on top of cirrhosis G (II) N=15	P-value
Gender:			
(Male N & %)	13 (65%)	10(66.6%)	>0.05
(Female N & %)	7 (35%)	5 (33.3%)	>0.05
Age (Mean $\pm$ SD)	53.5 $\pm$ 5.9	55.5 $\pm$ 6.5	>0.05
Clinical Data			
Jaundice	9 (45%)	8(53.3%)	>0.05
Hepatomegaly	15(75%)	12(80%)	>0.05
Splenomegaly	12 (60%)	10 (66.6%)	>0.05
Ascites	4(20%)	7(46.6%)	<0.05
Melena	7 (35%)	5(33.3%)	>0.05
Hematemesis	5 (25%)	4(26.6%)	>0.05

$P>0.05$ is not statistically significant, $P<0.05$ is statistically significant

Table 2. Comparison of laboratory tests in all studied groups

Parameter	Cirrhosis alone N=20 Mean $\pm$ SD	HCC on top of cirrhosis N=15 Mean $\pm$ SD	Control group N=15 Mean $\pm$ SD	P-value
AST (U/L)	85 $\pm$ 36	92 $\pm$ 39	19 $\pm$ 12	P1 <0.01 P2 <0.01 P3 >0.05
ALT (U/L)	89 $\pm$ 38	97 $\pm$ 40	22 $\pm$ 15	P1 <0.01 P2 <0.01 P3 >0.05
ALP (U/L)	152 $\pm$ 34	198 $\pm$ 42	59 $\pm$ 23	P1 <0.01 P2 <0.01 P3 <0.05
Serum Albumin (gm/dl)	3 $\pm$ 0.51	2.8 $\pm$ 0.45	4.2 $\pm$ 0.38	P1 <0.05 P2 <0.05 P3 >0.05
T.Bilirubin (mg/dl)	2.9 $\pm$ 1.6	3.2 $\pm$ 1.51	0.69 $\pm$ 0.21	P1 <0.01 P2 <0.01 P3 >0.05
Prothrombin Conc (%)	68 $\pm$ 12.2	62 $\pm$ 11.8	92.5 $\pm$ 3.2	P1 <0.01 P2 <0.01 P3 >0.05
Platelets Count (X10 ³ /ul)	140 $\pm$ 38	132 $\pm$ 33	212 $\pm$ 49	P1 <0.01 P2 <0.01 P3 >0.05

P1= between cirrhosis and control,
P2= between HCC and control,
P3= between cirrhosis and HCC patients

Table (3). AFP, Micro RNA-122 expression in studied groups

Parameter	Cirrhosis alone N=20 Mean±SD	HCC on top of cirrhosis N=15 Mean±SD	Control group N=15 Mean±SD	P-value
AFP (ng/ml)	42±28	295±78.5	3.8± 1.8	P1<0.01 P2<0.001 P3<0.001
Micro RNA-122 Copies/ml	790±208	850±240	45±17	P1<0.001 P2<0.001 P3>0.05

P1= between cirrhosis and control,
P2= between HCC and control
P3= between cirrhosis and HCC patients

Table (4). Correlation between Micro RNA-122 expression levels and studied parameter

Variable	Mico RNA-122			
	Cirrhosis alone (N=20)		HCC on top of cirrhosis (N=15)	
	R	P value	R	P value
AST	0.49	<0.05	0.51	<0.05
ALT	0.56	<0.05	0.57	<0.05
ALP	0.43	<0.05	0.46	<0.05
Serum Albumin	-0.25	>0.05	-0.27	<0.05
Total Bilirubin	0.26	>0.05	0.29	>0.05
Prothrombin Conc	-0.42	<0.05	-0.45	<0.05
Platelet count	-0.27	>0.05	-0.29	>0.05
AFP	0.21	>0.05	0.28	>0.05

P>0.05 is not significant, P<0.05 is significant

4. Discussion

Chronic HCV infection causes normally quiescent hepatocytes to divide repeatedly, leading to fibrosis, cirrhosis and occasionally progression to hepatocellular carcinoma (HCC)[13]. Liver cirrhosis is the most common complication of chronic HCV infection. Once cirrhosis is established, the risk of developing HCC is increased[14].

AFP is a serum glycoprotein that was first recognized as a marker for HCC more than 40 years ago and has been described to detect preclinical HCC. The fetal yolk sac and fetal liver generate high levels of AFP, which decline to < 10 ng/dl within 300 days of birth[15]. Our results showed that AFP serum levels were significantly increased in all patients groups as compared to control group, its levels in HCC patients was significantly higher than in cirrhotic patients without HCC. This finding is in agreement with **yang et al., 2005**[16] and **Scholz et al.**,[17]. A study from Thailand found that HCC patients with AFP >400 ng/ml tend to have greater size, bilobar involvement, portal vein thrombosis and decreased survival[18]. Liver diseases other than HCC are accompanied by high levels of AFP. In addition, a significant proportion of HCC patients did not have an elevated AFP. Non-tumors liver cells also abundantly express AFP mRNA. AFP represents liver cell-specific, not tumor specific markers[19].

Nowadays, the search for non-invasive biomarkers for the diagnosis of diseases has become a rapidly growing area of clinical research. Micro RNA expression has been studied at cellular level and in tissues in patients with HCC.

MicroRNAs (miRNAs) are small non-coding RNAs that inhibit messenger RNAs (mRNAs) by binding to their 3' untranslated regions (UTRs). miRNA are predicted to repress up to 1/2 of all human genes post-transcriptionally through translational arrest and/or mRNA degradation[7]. miRNAs regulate diverse functions, including cell proliferation and apoptosis, and they are dysregulated in cancers, including HCC[20] Micro RNAs are important mediators of HBV and HCV infection as well as liver disease progression, and therefore could be potential therapeutic target molecules[21].

Various microRNAs are now being investigated in hepatitis C virus infection with the most popular one being Micro RNA-122, Micro RNA-122 is the most abundant micro RNA in the liver[22] where it has many important biological roles, such as in fatty acid metabolism[23], and circadian rhythms under normal conditions[24]. Micro RNA-122 was found to interact with HCV RNA enhancing its replication[25]. It was suggested that miR-122 triggers HCV replication by post-transcriptional repression of heme oxygenase enzyme[26]. Blockage of miRNA -122 has been considered as a therapeutic approach against chronic hepatitis C[27]. Prior studies investigating miRNA -122 expressions in liver cancers have produced conflicting results.

In the current study we investigated the expression levels of circulatory micro RNA- 122 in whole blood which may be useful tools as a non invasive diagnostic marker of liver injury in Egyptian chronic hepatitis C virus infected and cirrhotic patients with and without HCC.

The current study revealed a significant increase in circulating Micro RNA-122 expression level in patient with liver cirrhosis as compared to control group. This finding is in agreement with **Cermelli et al., 2011**[28] who reported that miRNA-122 and miRNA-34a levels positively correlated with disease severity and stated that serum levels of miRNA-34a and miRNA-122 may represent novel, noninvasive biomarkers for diagnosis of histological disease severity in patients with CHC or NAFLD. **Bihrer et al., 2011**[29] reported that sera from patients with CHC contained higher levels of miRNA -122 than sera from healthy controls. They add that the serum level of miRNA -122 strongly correlates with serum ALT activity and with necroinflammatory activity in patients with CHC and elevated ALT levels, but not with fibrosis stage and functional capacity of the liver.

Moreover, in our study Micro RNA-122 expression level were further elevated in patients with HCC. This finding is in agreement of **Varnholt et al., 2008**[30] who examined the microRNA expression profiles in a large set of 52 human primary liver tumors consisting of premalignant dysplastic liver nodules and hepatocellular carcinomas by quantitative real-time polymerase chain reaction. All patients were infected with hepatitis C, and most had liver cirrhosis. They stated that miRNA-122, miRNA100, and miRNA -10 were over expressed whereas miR-198 and miR-145 were up to 5-fold down-regulated in hepatic tumors compared to normal liver parenchyma. They concluded that miR-122 was strongly up-regulated in

dysplastic and malignant liver nodules in the large sample set, and they suggested that miRNA122 might down regulate target mRNA of undetermined tumor suppressor genes and thus lead to increased tumor growth.

In our study MicroRNA-122 expression did not differ significantly between patients with HCC and liver cirrhosis alone. This finding is in agreement with **Köberle et al., 2013** [31].

The data from all groups (cirrhosis and HCC) showed Micro RNA-122 expression levels were positively correlated with AST, ALT, and ALP and negatively correlated with prothrombin concentration. These results were in agreement with **Köberle et al., (2013)** who reported serum miRNA -122 correlated with clinical chemistry parameters of hepatic necroinflammation, liver function and synthetic capacity [31].

Contrary to our findings of miRNA 122 up-regulation in HCV-associated HCCs, other authors have reported a down-regulation in HCC cell lines[32] and rodent HCCs, [33] all of which had etiologies other than HCV infection. **Varnholt et al** confirmed this down-regulation in their HCC cell lines that originated in the non-HCV setting[30]. **Gramantieri et al (2007)** suggest that miRNA -122 abundance is generally reduced in HCC[34].

Micro RNA-122 positively modulates HCV infection through direct interactions with viral RNA and stimulates HCV translation[35]. It forms an oligomeric complex in which one miRNA -122 molecule binds to the 5' UTR of

HCV RNA with 3' overhanging nucleotides, masking the 5' terminal sequences of HCV genome[36]. Furthermore, specific internal nucleotides as well as 3' terminal nucleotides in miRNA-122 were absolutely required for maintaining HCV RNA abundance[18], and the sequestration of miRNA-122 in liver cells has been shown to result in a marked loss of autonomously replicating HCV RNAs.[25]

Qi et al., 2011[37] reported that miRNA -122 in serum was significantly higher in HCC patients than healthy controls .More importantly; they reported that the levels of miRNA-122 were significantly reduced in the post-operative serum samples when compared to the pre-operative samples. They stated that although serum miR-122 was also elevated in HBV patients with HCC comparing with those without HCC, the difference was at the border line suggested that serum miRNA -122 might serve as a novel and potential noninvasive biomarker for detection of HCC in healthy subjects, they add it might serve as a novel biomarker for liver injury but not specifically for detection of HCC in chronic HBV infection patients.

Hou et al., 2011 reported that miRNA -122 expressions was maintained in both HBV- and HCV-associated cancer [38], while **Coulouarn et al., 2009** reported higher miRNA-122 expression levels in HCV versus HBV-associated cancers[39].

Wang et al., 2012 found that miRNA -122 expression in liver was significantly down-regulated in patients with HBV infection compared with healthy controls, and the miRNA -122 levels were negatively correlated with intrahepatic viral load and hepatic necroinflammation, and they concluded that miR-122 down-regulates its target cyclin G1, and thus interrupts the interaction between cyclin G1 and p53 and abrogates p53-mediated inhibition of HBV replication. Loss of miR-122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G1-modulated P53 activity[40].

Li et al., 2013 reported that all HBV mRNAs harboring a miRNA-122 complementary site act as sponges to bind and sequester endogenous miRNA-122, miRNA-122 saturation and depletion by binding to the complementary sites located in HBV mRNAs. Downregulation of miR-122 during chronic HBV infection induces increased expression of its target pituitary tumor-transforming gene 1 (PTTG1) binding factor (PBF), and they demonstrated that HBV replication causes an obvious increase in PBF levels. Furthermore, they observed that the miR-122 levels were decreased and PBF was upregulated in chronic hepatitis B (CHB) and hepatocellular carcinoma (HCC)[41].

Because miRNA -122 closely interacts with the HCV genome and the miRNA -122 expression pattern in HCV-associated HCCs is directly opposed to non-HCV-infected HCC. Further studies on the role of miRNA -122 in HCCs of non-HCV etiologies are needed to fully understand the function of this unique miRNA in the liver. Those data have a potential therapeutic implication, especially because **Kru"tzfeld et al., 2005**[42] have already pioneered a specific

down-regulation of miRNA -122 in a mouse model following injection of chemically engineered anti-miR-122 oligonucleotides (so-called antagomirs).

The study concluded that increase expression of miRNA-122 in Egyptian chronic hepatitis C virus (CHCV) infected and cirrhotic patients with and without HCC. Micro RNA 122 can be used as novel biomarkers for liver injury but not specifically for HCC. We recommend further studies on large scale to evaluate the diagnostic, prognostic value of miRNA-122 and to evaluate anti-miRNA-122 as a therapeutic option for hepatitis C virus infection and its related complications.

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