Total microRNA-cDNA and Viremia Versus Iron Status in Chronic Hepatitis C Patients

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Abstract—The possibility that any porphyrogenic agent; including chronic hepatitis C virus (HCV) infection, can add more to iron overload or not is still questionable nowadays. Also, there is growing evidence that up-regulation of iron in the liver can mediate hepatic injury. Further, MicroRNAs are important players in HCV infection and pathogenesis. Herein, HCV was tested for its porphyrogenicity in chronic HCV (CHC) infection. Correlation between total microRNA-cDNA and HCV-RNA as well as their correlations with iron status and hepatopathogenesis will be evaluated. Methods, 118 patients with liver pathology were included in the study together with 22 of the healthy control. Their sera were tested for total microRNA-cDNA by nanoQuant, iron status with standard assay methods, HCV antibodies by enzyme linked immunosorbent assay (ELISA) and HCV-RNA by real time polymerase chain reaction (RT-PCR). Alpha-fetoprotein, routine liver function tests and platelets count were done. Results: HCV infection and/or its chronicity participate in porphyrogenicity, and thus, hepatopathogenesis, possibly via propagating an iron overload. This is because iron and ferritin levels in sera of all CHC patients were elevated but that of total iron binding capacity (TIBC) was down-regulated compared with those of the control. The highest elevations were reported in sera of HCC patients compared with those with liver cirrhosis. Simultaneously, the mean microRNA-cDNA and viral RNA levels were reduced in sera of the HCC group compared to the others but without any significant differences among the stages of liver pathology. Moreover, the individual results of microRNA-cDNA concentration were not significantly correlated with viral load and/or any of the parameters of iron status but serum iron was significantly correlated with ferritin in sera of all patients with chronic hepatitis. Therefore, one can conclude that only the presence of viremia but not viral load mediates porphyrogenicity as well as hepatopathogenicity in Egyptian patients, possibly via an iron overload mediated mechanism. Further, the role of miRNA in HCV pathogenesis must not be neglected.

Key words—ELISA, HCV, microRNA-cDNA, nanoQuant, RT-PCR.

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1 Introduction

Chronic hepatitis C virus (HCV) infection accounts for 170–200 million patients worldwide. Genotype-4 represents 12% -15% of the total global HCV infection. Shelbaya et al. [1] added that about 90% of this infection in Egyptian patients were of genotype-4 with a significant number of them are at high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC) [2].In Egypt, the prevalence of chronic hepatitis C (CHC) infection is 13.8% of whole population and about 80% of the patients with hepatocellular carcinoma have underling hepatitis C. HCV is an RNA virus and does not integrate with host genome and therefore, HCV induced hepato-carcinogenesis pursues a totally different mechanism causing imbalance between suppressors and proto-oncogenes and genomic integrity. However, the exact mechanism of HCC inducement still needs a full understanding of various steps involved in this process [3].

Moreover, chronic hepatitis C (CHC) does not affect the liver uniformly rendering liver biopsies to be questionable in some cases [2]. Thus, urgent need for new biomarkers for accurate diagnosis and treatment as well as prognosis of HCV is acceptable. Chronic hepatitis C infection is a risk factor associated with development of HCC [4]. The progression involves multiple steps to deregulate various signaling pathways and the host cells to acquire metastatic potential [5]. This demonstrates that HCV infection is a serious health problem recorded globally. Progression towards HCC involves multiple steps that ultimately lead to deregulation of various signaling pathways and help host cells to acquire metastatic potential in presence of surrounding micro-environment [5].

Undoubtedly, extracellular RNAs will constitute an excellent addition to classical noninvasive markers of liver pathology. However, standardization of sample preparation, quality assessment and quantification of circulating RNA molecules in urgently needed. It is also a prerequisite key for the adoption of cell-free RNA markers into clinical practice. [6]

MicroRNAs (miRNAs) are small non-coding RNAs that are consisting of 19–24 nucleotides long and are single-stranded. These control gene expression at both translational and/or posttranscriptional level via controlling mRNA translation [7]. The presence of them in tissue cells, and also in body fluids, as a result of leakage from cell necrosis, apoptosis or their active secretion through exosomes from cells together with their stabilities make them candidate targets [8].Moreover, several studies demonstrated that HCV needs some miRNAs for its replication [9].Generally, these molecules are important players in HCV pathogenesis, possibly via controlling signaling pathways in innate and adaptive immune response [9]. In this regard, miR-122 is the most abundant miRNA in normal liver parenchyma, accounting for more than 70% of the total miRNA in hepatocytes [10]. Also, plasma miR-122 and miR-21 had strong correlation with degree fibrosis in HCV genotype-4 patients; consequently they can be considered as potential biomarker for early detection of hepatic fibrosis [3].

The serum level of miR-122 strongly correlates with both serum liver enzymes ALT and AST in patients with HCV infection who did not develop de-compensated liver disease. In patients with persistently normal ALT levels, serum miR-122 did not differ from healthy control

[11]. Differential miRNA expression in tissue from HCV infected HCC specimens indicated 10 up regulated and 19 down regulated miRNAs [12]. Another study on HCV infected patients showed that 13 miRNAs were down regulated and were predicted to target genes related to immune response, antigen presentation, cell cycle, proteasome, and lipid metabolism signaling pathways [13]. Moreover, miR-21 can be used as a potential biomarker, for early detection of HCC if combined with vascular endothelial growth factor and α-fetoprotein (AFP) [3]. Once the role of miRNA signature is identified, early diagnosis of HCC can accept reality. In this regard and up till now, limited studies are available. However, validation of these miRNAs and their predicted targets are necessary for conclusive role of particular miRNA in HCV related HCC. MiRNA dys-regulation has been linked with initiation and progression of HCC but with poorly understood mechanisms [14]. Iron is the fundamental metal for our bodies and it is a cofactor for hemoglobin. It is a constituent of numerous enzymes including superoxide dismutase. It is also participate in a lot of enzymatic reactions and involved in many cellular processes including metabolic pathways [15]. Thus, both iron overload and iron deficiency can lead to cellular cytotoxicity, arrest of cell growth and/or cellular apoptosis [16]. Therefore, serum total miRNA-cDNA together with iron status in CHC virus patients were suggested to be assayed in sera of chronic hepatitis C patients with different stages of liver pathology; including HCC.

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2 MATERIALS AND METHODS

2.1 Patients and Blood sampling

2.1.1 Patients

This cross sectional observational study was performed on patients selected from the outpatient hepatology clinics and HCC early detection clinic, in Egyptian Liver Research Institute and Hospital (ELRIAH), Sherpin, Aldakahlia, Egypt, from April 2016 to April 2017. The study has been conducted on 118 participants. All patients tested positive for hepatitis C antibodies (HCV-Ab) and were negative for other chronic liver diseases. They had normal kidney function, normal glucose with no liver transplantation. None of the patients had received antiviral treatment before liver Fibroscan and blood sampling. Also, twenty two healthy volunteers with age and gender-matched to those of the patients were included (control group). They were free from any disease, especially liver disorders. Using Fibroscan, patients were categorized into those with minimal or no fibrosis, those with fibrosis and those with liver cirrhosis. HCV-related HCC were proved radiologically by abdominal US and Triphasic abdominal CT). All patients were enrolled in our study after signing an informed written consent. The study protocol was designed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution's human research committee, Faculty of Medicine, Mansoura University was obtained. Exclusion criteria included: Previous treatment of HCC (surgical, interventional or medical), Presence of other malignancies (e.g. cholangiocarcinoma, gastric, colorectal cancer).Patients with HCC on top of other causes rather than HCV, and patients on direct acting antiviral therapy for chronic HCV. All participants were subjected to full medical history, complete clinical examination, and full basal laboratory and radiological investigations.

2.1.2 Blood sampling

Six ml venous blood sample were withdrawn from each individual; of whom:

Serum sample collection

Four milliliters of venous blood were obtained, left to clot, centrifuged and the serum fraction was separated and either freshly used or stored at−80 °C until used.

Plasma sample collection

2 ml whole blood was poured onto EDTA.

2.2 Biochemical and immunological assays

2.2.1 Routine laboratory tests

Platelets count was done using D-cell 60 automated hematology analyzer (Sysmex X 1800 incorporation, Japan), Liver function tests including serum AST, ALT, albumin, bilirubin were done using automated Biochemistry analyzer (Cobas Integra 400, Roch, Switzerland).

2.2.2 Iron, ferritin and total iron binding capacity (TIBC)

Each of which were determined using standard colorimetric assay methods according to their enclosed pamphlets [Egyptian Company for Biotechnology (S.A.E), www.spectrum-diagnostics.com and e-mail:info@spectrum-diagnostics.com] using automated Biochemistry analyzer (Cobas Integra 400, Roch, Switzerland).

2.2.3 Serological markers

Serological markers for detecting HCV infection [hepatitis C antibodies (HCV Abs)] were estimated by ELISA (Merieux anti-HCV, version 4.0, Diasorin S.P.A. via Crescent no 13040 Saluggia (VC) – Italy).

2.3 Molecular analysis

2.3.1 RT-PCR for detecting HCV infection and cDNA-miRNA assays

2.3.1.1 RNA extraction

RNA was extracted from serum using QIAzolLysis Reagent according to the manufacturer's instructions. The RNA purity was assessed by the RNA concentration and quantified by NanoDrop ND-1000 (Nanodrop, United States).

2.3.1.2 HCV RNA quantization using RT-PCR

HCV RNA was quantized by quantitative RT-PCR using fully automated analyzer (Cobas amplified, Taqman48 analyzer, Roch Switzerland).

2.3.1.3 Reverse transcription using miScript II RT Kit

Only 5 x miScriptHiSpec Buffer had been used for preparation of cDNA for real-time PCR with miScript miRNA PCR Arrays. Single-stranded cDNAs were generated using the RT kit according to the manufacturer's directions (miScript miRNA PCR system, miRneasy mini kit for miRNA extraction, miScript RTII for miRNA

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reverse transcription. Further, total miRNA was assayed using nanoQuant.

2.4 Fibroscan and ultrasonography

Liver stiffness [expressed in kilopascals (kPa)] was measured by transient elastography (Fibroscan; Echosens SA, Paris, France). The results obtained were ten valid readings with a success rate of at least 60% and an interquartile range under 30% of the median value. Fibroscan results ranges from 2.5 to 75 kPa. Healthy people without liver disease have a liver scarring reading less than 7.0 kPa (median is 5.3 kPa). A person with chronic hepatitis C and a liver stiffness more than 14 kPa has nearly a 90% probability of having cirrhosis, while patients with liver stiffness more than 7 kPa have around an 85% probability of at least significant fibrosis. Patients were classified according to Fibroscan median into: F0 (no fibrosis, 0-5 kPa), F1 (minimal fibrosis without septa, 5.1- 7 kPa), F2 (moderate fibrosis with few septa, 7.1-10 kPa), F3 (severe fibrosis with numerous septa but without cirrhosis, 10.1-17.5 kPa) and F4 (cirrhosis, 17.5-75 kPa).

2.5 Statistical analysis

All statistical analyses were performed by Medcalc software (version 14.8.1.; Medcalc Software Bvba, Ostend, Belgium). Continuous variables were expressed as mean± standard deviation (SD). Comparisons of markers as well as routine laboratory tests and stages of fibrosis were analyzed using a two-sided P value. A value of $P < 0.05$ was considered statistically significant. Person's correlation coefficient was used in establishing correlation among parameters.

3 Results

3.1 Total microRNA-cDNA and viremia in chronic hepatitis C patients

As shown in table 1, total miRNA- cDNA levels were slightly decreased in sera of patients infected with HCV compared with that of healthy individuals. The highest reduction was in sera of hepatocellular carcinoma (HCC) patients. Simultaneously, viremia was decreased in sera of HCC patients, group with no significant differences among groups.

TABLE 1: TOTAL MICRORNA-CDNA VERSUS VIREMIA AND LIVER PATHOLOGY ASSESSED BY FIBROSCAN IN SERA OF CHRONIC HEPATITIS C PATIENTS.

Groups	Total microRNA-cDNA	Viremia
Control		
Mean ± SD	31.7 ± 6.6	-
N	22	
All patients		
Mean ± SD	28.6 ± 9.7	5.4 ± 0.93
n	70	84
p	P= NS	
Fibrotics		
Mean ± SD	29.0 ± 9.7	5.7 ± 0.5
n	24	6
p	P= NS	P= NS
Cirrohtics		
Mean ± SD	30.0 ± 12.0	5.7 ± 0.8
n	25	33
p	P= NS	P= NS
HCC Patients		
Mean ± SD	26.6 ± 6.2	5.1 ± 0.9
n	21	21
p	P = 0.01	P= NS

Values were expressed as mean ± standard deviation (mean ±SD), n= number, p= probability (Significance Level), NS= non-significant *and miRNA- cDNA= microRNA-complementary DNA.*

3.2 Iron status in chronic hepatitis C patients

3.2.1 Serum iron levels

The mean level of iron of the healthy control was 161± 27.3 μg/dL and that of all patients was 179.2±28.8 μg/dL. Thus, the mean difference was statistically and significantly higher than that of the control value ($P<0.004$, table 2).

3.2.2 Serum ferritin levels

The mean level of ferritin of the healthy control was 72.2±41.9 µg/dL and that of all patients was 141.7±120 µg/dL which was statistically significantly higher in sera of all patients compared with that of the control subjects ($P<0.013$, table 2).

3.2.3 Serum TIBC levels

The mean serum level of TIBC of the healthy control was 493±217.6 µg/dL and that of all patients was 223.2±116.6 µg/dL. In general, TIBC of the patients was found to be highly significantly decreased than that of the control value ($P<0.0001$, table2).

TABLE 2: MEAN LEVELS OF IRON, FERRITIN AND TOTAL IRON BINDING CAPACITY (TIBC) IN THE SERA OF ALL HCV PATIENTS VERSUS THOSE OF THE CONTROL SUBJECTS.

Parameter	Control		All patients	
	N	Mean ± SD	N	Mean ± SD
Iron	26	161.2± 27.3	111	179.2 ± 28.8 P = 0.004
Ferritin	19	72.2 ± 41.9	126	141.7 ± 120 p< 0.013
TIBC	16	493± 217.6	66	223.2± 116.6 p<0.0001

Values were expressed as mean ± standard deviation (mean ±SD). n= number and p= probability. TIBC= total iron binding capacity.

3.3 Correlation of total microRNA-cDNA and viremia with iron status in sera of all patients with chronic hepatitis C.

The results of table 3 showed non-significant correlations among parameters.

TABLE 3: CORRELATION OF TOTAL MICRORNA-CDNA AND VIREMIA WITH IRON STATUS AND SERA OF ALL PATIENTS WITH CHRONIC HEPATITIS C.

Parameters	Iron	Ferritin	TIBC
miRNA-cDNA			
Correlation Coefficient	-0.09	0.03	0.13
Significance Level	0.4	0.8	0.27
Sample size	84	83	80
Log-PCR			
Correlation Coefficient	-0.12	-0.1	0.08
Significance Level	0.33	0.4	0.58
Sample size	67	80	51

Log PCR = Log *HCV-RNA estimated by polymerase chain reaction= PCR, miRNA*- cDNA = microRNA-complementary DNA and TIBC= total iron binding capacity.

3.4 Correlation of total microRNA-cDNA, viremia and iron status with routine liver function tests and platelets counts in blood of all patients with chronic hepatitis C.

The results of table 4 showed that iron and ferritin were highly significant and positively correlated with SGPT, SGOT and bilirubin levels, and negatively correlated with albumin and platelets in sera of all patients with chronic hepatitis C. And TIBC was significantly correlated with bilirubin and platelets. Also, miRNA-cDNA was significantly correlated with albumin and bilirubin. Log PCR showed non-significant correlation.

TABLE 4: CORRELATION OF TOTAL MICRORNA-CDNA, VIREMIA AND IRON STATUS WITH ROUTINE LIVER FUNCTION TESTS AND PLATELETS COUNTS IN BLOOD OF ALL PATIENTS WITH CHRONIC HEPATITIS C.

Parameters	Parameters of liver function tests				Platelets count
	SGPT	SGOT	Albumin	Bilirubin	
Iron					
Correlation Coefficient	0.331	0.360	-0.21	0.21	-0.25
Significance Level	0.0003	<0.0001	0.02	0.02	0.004
Sample size	114	128	130	124	128
Ferritin					
Correlation Coefficient	0.22	0.2	-0.25	0.3	-0.26
Significance Level	0.01	0.02	0.0035	0.0002	0.002
Sample size	120	132	138	129	136
TIBC					
Correlation Coefficient	-0.18	-0.16	0.19	-0.25	0.27
Significance Level	0.14	0.17	0.098	0.035	0.02
Sample size	67	74	76	71	76
miRNA-cDNA					
Correlation Coefficient	0.03	-0.16	0.22	-0.34	0.03
Significance Level	0.78	0.14	0.04	0.002	0.82
Sample size	76	84	86	79	85
Log-PCR					
Correlation Coefficient	0.003	-0.02	0.06	-0.04	0.06
Significance Level	0.98	0.86	0.61	0.73	0.59
Sample size	69	78	83	79	84

Log PCR = Log HCV-RNA estimated by polymerase chain reaction= PCR, miRNA- cDNA = microRNA-complementary DNA and TIBC= total iron binding capacity.

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3.5 Discriminant power of iron status in differentiating the stages of liver disorder

3.5.1 Serum iron levels

The mean iron level in sera of patients with no/minimal fibrosis was 160± 18.8µg/dL. Also, the mean level of iron was 187.3±24.9µg/dL in sera of fibrotic patients and it was 168.2±23µg/dL in sera of cirrhotic patients. In addition, the mean iron level in sera of patients with HCC was 203±30.6 µg/dL. Thus, significant iron overload was developed in sera of the fibrotic and HCC groups compared with that of the healthy control group ($p< 0.0003$ and $P<0.0001$, respectively and table 5).

3.5.2 Serum ferritin levels

The mean value of ferritin in sera of the healthy control was 72.2±41.9µg/dL and that of patients with no/minimal fibrosis was 86.3± 69.5µg/dL. Also, the mean value of ferritin in sera of fibrotic patients was 134.5±87 µg/dL, and was 111±84 µg/dL in sera of cirrhotic patients. Finally, ferritin mean level was 239.7±127.8 µg/dL in sera of HCC patients, group. Statistically, the mean ferritin concentration was significantly increased in sera of the fibrotic and HCC groups ($P=0.0013$ and $p<0.0001$ respectively) when compared with that of the control one (Table 5).

3.5.3 Serum TIBC levels

The mean value of TIBC in sera of the healthy control was 493±217.6 µg/dL and those of fibrotic and cirrhotic patients were 187±75.8 µg/dL, and 184±122µg/dL, respectively. Finally, TIBC of HCC patients was298±108µg/dL. Statistically, the mean serum TIBC concentration was significantly decreased in sera of the three groups ($P<0.0001$) when compared with that of the control one, especially in cirrhotic individuals (Table 5).

TABLE 5: MEAN VALUES OF IRON, FERRITIN AND TOTAL IRON BINDING CAPACITY (TIBC) LEVELS IN SERA OF HCV PATIENTS WITH VARIOUS STAGES OF LIVER DISORDERS WHEN COMPARED WITH THOSE OF THE CONTROL GROUP.

Parameters	Control group		No/minimal fibrosis(F0,F1)		Fibrotic patients(F2,F3)		Cirrhotic patients(F4)		HCC patients	
	N	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean± SD	n	Mean ± SD
Iron	26	161.2 ± 27.3	24	160.1±18.8 P1=NS *P=NS	34	187.3±24.9 P1<0.0003 P2<0.002	31	168.2 ± 23 P1= NS	22	203 ± 30.6 P1<0.0001 P2<0.0001
Ferritin	19	72.2 ± 41.9	30	86.3 ± 69.5 P1=NS P2=NS	36	134.5 ± 87 P1<0.0013 P2=NS	38	111 ± 84 P1= 0.06	22	239.7 ± 127.8 P1<0.0001 P2<0.0001
TIBC	16	493± 217.6	-	-	20	187± 75.8 P<0.0001 P2=0.9	24	184± 122 P<0.0001	22	298±108 P<0.0008 P2<0.0017

Values were expressed as mean± standard deviation (mean ±SD), .n= number, P= probability and NS= non-significant. If P1<0.05=significant when the mean levels of the individual groups were compared with those of the control). P2<0.05 = significant when the mean levels of the individual groups were compared with those of patients with liver cirrhosis.

4 DISCUSSION

In this study, the mean serum levels of both iron and ferritin were increased but that of TIBC was decreased in sera of all HCV patients compared with those of the healthy controls. The highest elevations in the formers and the lowest one of the latter were reported in sera of HCC patients, indicating bad outcomes of HCV pathogenesis with the progression of the disease. Therefore, one can suggest that HCV infection can disturb iron status. Such disturbances may exeggravates HCV pathogenesis and mediate viral persistence with 3 possible mechanisms. The first is that iron may worsen HCV infection outcomes by participating in oxidative stress in cells other than hepatocytes which appears to cause irreversible mitochondrial damage which was associated with the course of hepatic fibrosis [17]. Second, the essentiality of iron, as a nutrient for nearly all cells, including HCV, renders it to affect such viral replication. Thus, the increases in viral load in HCV-infected human hepatocytes, cell line support the second mechanism [18]. A third mechanism which was reported by may also be suggested to be included in this study. Such mechanisms include direct interaction of iron with the Th1-mediated effector mechanisms via formation of nitric oxide and the production of TNF-α [19]. The increase of iron overload in this study with its involvement in Fenton reaction [20] can produce free radicals. These can simultaneously be involved in the production of TNF-α. The latter induce Th2-cells to produce anti-inflammatory cytokines which suppress the fighting capacity of infectious diseases, including HCV [21] and [22].

Garrick [23] revealed that HCV alters expression of the iron-uptake receptor; namely, transferrin receptor 1 (TfR1) and the latter mediates HCV entry, and thus, HCV infection. Human iron transporters manage iron carefully because tissues need iron for critical functions, but too much iron increases the risk of reactive oxygen species. Iron acquisition and trafficking depends largely on the transferrin cycle [24].

The capacity to store iron in ferritin is essential for mammal's life. Poly(C)-Binding Protein 1 (PCBP1) can function as a cytosolic iron chaperone in the delivery of iron to ferritin [25]. Thus, PCBP1 with its paralog, PCBP2, can function as a cytosolic iron chaperone in the delivery of iron to ferritin and could contribute to ferritin iron loading. Depletion of PCBP1 in human cells inhibits ferritin iron loading and increases cytosolic iron pools [25]. This may be the case in the present study. This is because iron homeostasis play a role in the regulation of miRNA biogenesis and the alteration in the expression of miRNA might contribute directly to the molecular pathogenesis of human diseases associated with disrupted iron homeostasis [26]; including liver. The correlation between , iron, ferritin and TIBC with the parameters of liver function tests and platelets from one end and total miRNA-cDNA levels was correlated with albumin and bilirubin from the other end. In this regard, a use of iron chelators was suggested to modulate the activity of miRNA pathway in molecular pathogenesis. [24]In this study, total miRNA-cDNA was reduced in sera of patients with HCC but elevated in sera of patients with liver fibrosis and cirrhosis but without significant differences among these groups and the control one. These results added that miRNAs are important players in HCV infection and pathogenesis. Thus, targeting them with miRNA antagonist is of possible treatment as was reported by Gebert [27].

In this study, total miRNA-cDNA was reduced in sera of patients with HCC but elevated in sera of patients with liver fibrosis and cirrhosis but without any significant differences among these groups and the control group. Also, total miRNA-cDNA was positively correlated with albumin and negatively correlated with bilirubin. These results indicate that miRNAs are important players in HCV infection and its pathogenesis. Thus, targeting them with miRNA antagonist may be a possible treatment mechanism

as was reported by Gebert [27].Also, potential involvement of microRNA in the molecular pathogenesis of cancers has been recognized by Inui et al.[28].On the same line of results, differential expression of miRNAs in cirrhotic and non-cirrhotic HCCs was reported by Salvi et al[29].Moreover, Gragnani et al.,[30] suggested that miRNA profiling in HCV-related malignancies is a high impact issue but with little of research papers that dedicate to this specific viral etiology completely.[30]Generally, miRNAs can both mediate oncogenicity as well as tumor suppressors.[31].

Circulating miRNAs have been demonstrated to be very specific and stable in human serum and plasma. In addition, circulating miRNAs display consistent profiles between healthy individuals and significantly altered levels in disease conditions [32]. The elevation in total miRNA-cDNA in sera of CHC virus patients than that of healthy control can confirm the latter findings. These characteristics of circulating miRNAs established their potential value as biomarkers for detection and prediction of the progression of liver disease in HCV infection [32].

A majority of CHC patients progress to end stage liver diseases including liver cirrhosis and HCC. Once chronic HCV infection is established, chronic inflammation, insulin resistance, steatosis, oxidative stress, and continuing liver fibrosis are produced. [33] Herein, these changes are possibly mediated via iron overload and imbalance in its homeostasis. Also, Irshad et al. [33] added that viral particles interact with several cellular proteins to modulate cell signaling. Such modulation disturb the interplay of various reaction cascades with a possibility of genomic imbalance, normal reactions disturbance, and cell cycle and apoptosis abnormalities with subsequent facilitation of hepatocarcinogenesis, which is the case in this study.

5 Conclusion

The presence of viremia but not viral load mediates porphyrogenicity with subsequent iron overloading and hepatopathogenesis in Egyptian patients; with CHC virus. MiRNA mediated mechanism may also be included.

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