African J. Biol. Sci., 14 (2): 91-102 (2018)
ISSN 1687-4870 e- ISSN 2314-5501 (online)
www. ajbs.journals.ekb.eg E.mail: aasdjournal@yahoo.com

Can Serum Iron Parameters Represent Surrogate Markers for Chronic Hepatitis C-Based Hepatocellular Carcinoma?

Lamiaa Abdel-Latif[1], Gamal E Shiha[2,3], Waleed Samir[4] and Elsayed E A Toson[1]

1- Chemistry Department, Faculty of Science, Port Said University, Port Said, Egypt.
2- Internal Medicine Department, Faculty of Medicine, Mansoura Univ., Mansoura, Egypt.
3- Egyptian Liver Research Institute and Hospital (ELRIAH), Sherpin, Aldakahlia, Egypt.
4- Specialized Internal Medicine Hospital, Faculty of Medicine, Mansoura Univ., Egypt.

ABSTRACT

Iron homeostasis is of critical for human life. The pathogenesis of its accumulation in chronic hepatitis C (CHC) virus is not yet completely understood. Objectives: The aim is to investigate the possible correlations between serum metabolic markers of iron, HCV viral load, total micro-RNA-cDNA and the progression of liver disease into hepatocellular carcinoma (HCC). Patients and methods, A total of 126 patients with chronic hepatitis C virus were included in this study. Of them 22 patients were with HCC and 44 were CHC patients without HCC. In addition, a group of 30 healthy individuals were included and was served as control group. RNA was extracted and total microRNA was converted into-cDNA and was determined by nanoQuant, iron status with standard assay techniques. HCV antibodies were evaluated by enzyme linked immunosorbent assay (ELISA) and HCV-RNA by real time polymerase chain reaction (RT-PCR). Alpha-fetoprotein, routine liver function tests, international normalization ratio (INR) and platelets count were also done. Beside, HCV-related HCC were radiologically proved via abdominal US and Triphasic abdominal CT. Results: Markers of iron metabolism; including iron and ferritin were dramatically increased in sera of HCC patients compared with those of the non-HCC group. Conversely, the mean TIBC level was highly reduced in sera of HCC patients' group compared with that of the non-HCC individuals. Significant and positive correlations were found between the individual levels of iron markers with serum liver enzymes and bilirubin. On the other hand, negative correlations were found between the levels of these markers with both albumin and platelets, especially in the blood of HCC patients. Total miRNA-cDNA and viral RNA showed no significant difference among the two groups but the level of the former was significantly lowered in sera of HCC patients compared with those of the control group. In conclusion, these results suggest that serum markers of iron mediate liver disorders; especially HCC and can represent surrogate markers for the severity of such disorders. Also, the role viral molecules and/or micro-RNA in iron overload must not be neglected.

Key words: HCC, INR, TIBC and RT-PCR.

INTRODUCTION

In addition to causing liver diseases, chronic hepatitis C has a broad spectrum of extra-hepatic manifestations. Also, patients with long-lasting HCV infection are at major risk of developing hepatocellular carcinoma (HCC) (Dedania and Wu, 2015). Liver is the main iron reservoir in the body **(**Franchini *et al*., 2008; Vagu *et al*., 2013). Also, liver plays a fundamental role in iron metabolism. CHC infection frequently accumulates iron in the liver which may participate in liver injury and worsens its severity (Metwally *et al*., 2004) via the Fenton reaction producing

hydroxyl radicals (Fenton, 1894).These initiate a radical reaction that will ultimately damage the liver cells (Khare and Garg , 2015). Ferritin is an acute phase protein and its levels will be elevated in response to iron overload and systemic inflammation **(**Khare and Garg, 2015**).** Ferritin synthesis is induced by macrophages, and hepatocytes. Raised ferritin levels can be seen in iron overload conditions, inflammation and liver diseases (Walker et *al*., 2010).

The results of clinical studies which were designed to assess the impact of liver iron content on the risk of tumor development have remained controversial for some time. Further, it is known that common factors can affect both liver iron overload and the risk of cancer. Zhou *et al*. (1988) added that, the mechanisms underlying hepatic iron accumulation in chronic hepatitis C have not been fully elucidated. Reduction of the hepcidin transcription activity by hepatitis C virus (HCV)-induced reactive oxygen species (ROS) may in part account for iron loading, but the regulation of hepcidin is very complex and may depend on many variables, including the particular stage of the systemic and/or hepatic inflammatory conditions and the circulating transferrin-bound iron and intracellular iron stores. This might explain the variations in hepatic iron concentrations reported among patients with HCV-related chronic liver disease. However, even mild-to-moderate iron overload in the liver contributes to disease progression and hepatocarcinogenesis in chronic hepatitis C. Low hepcidin levels have been shown to trigger hepatic iron accumulation in these patients (Emerit et al., 2001). HCV suppresses hepcidin expression, increasing duodenal iron transport and macrophage iron release (Kohgo *et al.,* 2007).

At the molecular level, hepcidin binds to ferroportin, an iron exporter which are expressed by enterocytes and macrophages. Upon binding, induction of ferroportin internalization and degradation starts, thus preventing iron entry into plasma (Lavanchy, 2011). Such prevention enables the reduction of iron entry in the plasma compartment (Jaroszewicz *et al.*, 2010); De Domenico, 2009). Uchino *et al*. (2016) showed that both extremely high and low serum ferritin level was an independent risk factor for development of HCC in male patients with chronic hepatitis C. Uchino *et al.* (2018) reported that serum levels of ferritin were correlated with pathological iron deposition. However, such level was not a predictor of recurrence or survival among patients with HCC undergoing radiofrequency ablation (RFA).

Low level of hepcidin leads to iron overload which lead to inflammation and liver fibrosis (Hör and Schmidt, 2014). Studies in animal and in cellular models have suggested that HCV infection may directly modulate hepcidin expression by HCV-induced reactive oxygen species (ROS) (Castagna *et al.*, 2010).

MATERIALS AND METHODS

Patients and Blood sampling:

Patients:

This analytical retrospective 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 22 Patients with HCC, about 100 patients without HCC (Fibrotic and cirrhotic patients) and 30 individual as control group. 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. The exclusion criteria were patients with positive results for screening tests for other viral hepatitis infections (anti-HAV IgM, HBsAg, anti-HBc IgM) or HIV infection, hematological or non-viral chronic liver diseases, and patients previously treated for HCV infection. No formal sample size calculation was performed, all the study patients had to have active viral replication confirmed by detecting HCV-RNA, using real-time polymerase chain reaction assay (COBAS Taqman HCV Test, Roche.

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.

Biochemical and immunological assays:

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).

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

Each of which was determined using standard assay method according to their individual 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).

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).

Molecular analysis

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

RNA extraction:

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

HCV RNA quantization using RT-PCR:

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

Reverse transcription using miScript II RT Kit:

Only 5 x miScript HiSpec 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 RT for miRNA reverse transcription). Further, total miRNA was assayed using nanoQuant.

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).

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.

RESULTS

1. Total miRNA-cDNA, iron, ferritin and total iron binding capacity (TIBC) and levels in sera of HCC patients and control group:

1.1. Total MiRNA-cDNA levels:

The mean level of total miRNA-cDNA level of the control group was 31.7 ± 6.6 µg/dL and that of HCC patients was 26.6 ± 6.2 µg/dL. Thus, the mean difference was statistically and significantly lower than that of the control group (P<0.01, Table 1).

1.2. Serum iron levels:

The mean level of iron of the control group was 161.2 ± 27.3 µg/dL and that of HCC patients was 203 ± 30.6 µg/dL. Thus, the mean difference was statistically and significantly higher than that of the control group (P<0.0001, Table 1).

1.3. Serum ferritin levels:

The mean level of ferritin of the control group was 72.2 ± 41.9 µg/dL and that of HCC patients was 239.7 ± 128 µg/dL which was statistically significantly higher in sera of HCC patients compared with that of the control group (P<0.0001, Table 1).

1.4. Serum TIBC levels:

The mean serum level of TIBC of the control group was 493.0 ± 217.6 µg/dL and that of HCC patients was 298.4 ±108.1 µg/dL. In general, TIBC of the HCC patients was found to be highly significantly decreased than that of the control group (P=0.0008, Table1).

Table 1: Mean values of total miRNA-cDNA, iron, ferritin and total iron binding capacity (TIBC) in sera of HCC patients versus those of the control group:

Parameters	Control		HCC patients		
	N	**Mean ± SD**	**N**	**Mean ± SD**	
Total miRNA-cDNA	22	31.7 ± 6.6	21	26.6 ± 6.2	P <0.01
Iron	26	161.2 ± 27.3	22	203.0 ± 30.6	P1<0.0001
Ferritin	19	72.2 ± 41.9	22	239.7 ± 127.8	P1<0.0001
TIBC	16	493.0 ± 217.6	22	298.4 ±108.1	P=0.0008

Values were expressed as mean ± standard deviation (mean ±SD), n= number, p= probability (Significance Level) when HCC patients were compared to control group, HCC= hepatocellular carcinoma and miRNA- cDNA= microRNA-complementary DNA.

2. Liver function markers, platelets and alpha-fetoprotein (AFP) levels in sera of HCC patients and control group:

There were significant differences between control group and HCC patients with respect to their mean values of SGPT, SGOT, S. albumin, total bilirubin, count of platelets and alpha fetoprotein levels (Table 2).

Table 2: Mean values of the liver function markers, platelets count and alpha-fetoprotein (AFP) levels in sera of HCC patients versus those of the control group:

Parameters	Control group		HCC patients		
	N	**Mean ± SD**	**N**	**Mean ± SD**	
SGPT	27	21.4 ± 9.8	21	64.8 ± 38.6	p<0.0001
SGOT	30	23.8 ± 6.9	22	82.7 ± 31.7	P<0.0001
S. Albumin	30	4.5 ± 0.3	22	3.6 ± 0.6	p<0.0001
Total Bilirubin	28	0.45 ± 0.12	21	1.4 ± 0.9	P<0.0001
Platelets count	28	254.6 ± 55.9	22	98.5 ± 40.6	P<0.0001
Alpha fetoprotein	21	2.8 ± 0.5	22	168.1 ± 81.5	P<0.0001

Values were expressed as mean ± standard deviation (mean ±SD), n= number, p= probability (Significance Level) when the results of patients with hepatocellular carcinoma (HCC) were compared to those of the healthy control group.

3. Mean total microRNA-cDNA and viremia levels in sera of HCC patients versus those of non-HCC patients:

As shown in Table (3), total miRNA-cDNA levels were slightly decreased in sera of patients with HCC compared with that of non-HCC Patients. Viremia levels showed no statistically significant difference between these two groups of patients.

Table 3: Mean total microRNA-cDNA and viremia levels in sera of HCC patients versus those of non-HCC patients:

Parameters	Non-HCC patients		HCC patients		
	N	**Mean± SD**	**N**	**Mean ± SD**	
Viremia	63	5.5 ± 0.91	21	5.1 ± 0.9	P= NS
Total miRNA-cDNA	49	29.7 ± 10.8	21	26.6 ± 6.2	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.

4. Iron, ferritin and total iron binding capacity (TIBC) levels in sera of HCC patients and non-HCC patients:

4.1. Serum iron levels:

The mean level of iron of the non-HCC patients were 173.3 ± 25.3 µg/dL and that of HCC patients was 203 ± 30.6 µg/dL. Thus, the mean difference was statistically and significantly higher than that of the non-HCC patients (P<0.0001, Table 4).

4.2. Serum ferritin levels:

The mean level of ferritin of the non-HCC patients was 120.9 ±108 µg/dL and that of HCC patients was 239.7 ± 128 µg/dL which was statistically significantly higher in sera of HCC patients compared with that of non-HCC patients (P<0.0001 , Table 4).

4.3. Serum TIBC levels:

The mean serum level of TIBC of the non-HCC patients was 371 ± 204.9 µg/dL and that of HCC patients was 596.8 ± 216 µg/dL. In general, TIBC of the HCC patients was found to be highly significantly increased than that of the non-HCC patients (P=0.0001, Table 4).

Table 4: Mean values of iron, ferritin and total iron binding capacity (TIBC) levels in sera of HCC patients versus those of non-HCC patients.

Parameters	Non-HCC patients		HCC patients		
	N	Mean ± SD	N	Mean ± SD	
Iron	89	173.3 ± 25.3	22	203 ± 30.6	P<0.0001
Ferritin	104	120.9 ±108	22	239.7 ± 128	P<0.0001
TIBC	44	185.5 ±102.4	22	298.4 ±108.1	P<0.0001

Values were expressed as mean ± standard deviation (mean ±SD), n= number, p= probability (Significance Level) when the results of patients with hepatocellular carcinoma (HCC) were compared to those of the healthy control group.

5. Liver function markers, platelets and alpha-fetoprotein (AFP) levels in sera of HCC patients and non-HCC patients:

The mean activities of liver enzymes, total bilirubin and AFP levels were statistically and significantly elevated in the blood of HCC patients. On contrary, the mean level of serum albumin and count of platelets were significantly decreased (P<0.0001, Table 5).

Table 5: Mean values of the liver function markers, platelets count and alpha-fetoprotein (AFP) levels in sera of HCC patients versus those of non-HCC patients

Parameters	Non-HCC patients		HCC patients		
	N	Mean ± SD	N	Mean ± SD	
SGPT	88	52.0 ± 26.8	21	64.8 ± 38.6	P=0.07
SGOT	98	45.8 ± 22.1	22	82.7 ± 31.7	P<0.0001
S. Albumin	103	4.2 ± 0.5	22	3.6 ± 0.6	P<0.0001
Total Bilirubin	98	0.9 ± 0.53	21	1.4 ± 0.9	P<0.0009
Platelets count	104	175.4 ± 81.6	22	98.5 ± 40.6	P<0.0001
Alpha fetoprotein	43	9.4 ± 1.8	22	168.1 ± 81.5	P<0.0001

Values were expressed as mean ± standard deviation (mean ±SD), n= number, p= probability (Significance Level) when the results of patients with hepatocellular carcinoma (HCC) were compared to those of the healthy control group.

6. Correlation of total microRNA-cDNA and viremia with iron status and alpha fetoprotein in sera of all patients with chronic hepatitis C:

As shown in table 6, no significant correlations were found between total microRNA-cDNA and viremia with iron status and alpha fetoprotein in sera of all patients with chronic hepatitis C.

Table 6: Correlation of total microRNA-cDNA and viremia with iron status and alpha fetoprotein in sera of all patients with chronic hepatitis C

Parameters	Total RNA-cDNA	Log_PCR
Iron		
Correlation Coefficient	0.03	-0.12
Significance Level	0.8	0.33
Sample size	67	67
Ferritin		
Correlation Coefficient	0.03	-0.1
Significance Level	0.79	0.4
Sample size	70	80
TIBC		
Correlation Coefficient	0.19	0.1
Significance Level	0.13	0.58
Sample size	64	51
Alpha fetoprotein (AFP)		
Correlation Coefficient	-0.013	-0.19
Significance Level	0.91	0.22
Sample size	83	46

DISCUSSION

In the present study, iron excess was shown in sera of both CHC patients with HCC as well as in sera of non-HCC patients" group. The excess serum iron load in this study, confirms those which were reported by Fujita *et al*. (2007) who found excess hepatic iron in sera of patients with chronic hepatitis C. On contrary, Emerit *et al*. (2001) reported that, the causative factors of iron overload in the presence of the virus are not known.

The results of the present study confirm those of Siregar and Maail (2018) who found an increase in hepatic iron accumulation and iron related serum markers elevation. In the current study, the reduction in serum TIBC may be one of the causative factors of serum iron loading. This overload necessitates hepatic saturation with ionized iron. Therefore, one can render the progression of liver disorders to the relatively common oxidative stress mediated by the products of Fenton reaction (Fenton, 1894). This reaction produces hydroxyl radicals which initiate other radical reactions that will ultimately damage the liver cells (Khare and Garg, 2015). In this regard, Siregar and Maail, (Siregar and Maail, 2018) argue the increase in oxidative stress for the increment in iron loading in their patients. These increases strongly favor DNA damage, genetic instability, and tumorigenesis which are already the case in the present study. In this regard, iron loading was correlated with the parameters of liver function tests which reflects hepatic cellular inflammation, cellular membrane perforation with subsequent enzymatic elevations. The latter's enzymatic elevations reflects the severity of hepatic inflammation. The reduction in platelets formation, synthetic function (albumin) retardation and bilirubin elevations reflect liver cirrhosis and/or hepatic fibrogenesis and liver cells tumorigenesis in patients with chronic hepatitis C in the present study. Indeed, the results of Shibutani *et al.* (1991) Showed a

significant correlation between 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidatively generated DNA damage, and iron loading.

In the present study, dramatic increases in both iron and ferritin were reported in sera of HCC patients than those in sera of non-HCC group. On contrary, transferring activity; as was reflected by TIBC was decreased with the increase in serum load of iron and ferritin. This may be due to the decrease in TIBC. Such decrease can leave ionized iron to be available for excessive production of free radicals with subsequent formation of oxidative stress, DNA modification and initiation of hepatocarcinogenesis. (Siregar and Maail, 2018; Shibutani et al., 1991). Therefore, one can suggest that iron loading and oxidative stress can also participate in reduction of hepcidin as was reported by Castagna *et al.* (2010) who also suggested that HCV infection in animal and in cellular models may directly modulate hepcidin expression via HCV-induced reactive oxygen species (ROS). This mechanism can triggers further iron accumulation in HCV chronically infected patients. The HCV-mediated TIBC is a key protein in iron metabolism, therefore its reduction in sera of the patients of the present study, and may also be hepcidin, can lead to iron deposition in the liver and/or higher levels of non-transferrin-bound iron in the blood stream (Hör and Schmidt, 2014). Since TIBC is a key protein in iron metabolism, one can expect that lowering of hepcidin level may leads to iron deposition in the liver and/or higher levels of non-transferrin-bound iron in the bloodstream (Hör and Schmidt,2014).

Milic *et al.* (2005) added that, liver disease decreases its synthetic functions; including, albumin and prothrombin-related proteins as well as hepcidin. Again, the non-bound iron mediates hydroxyl radicals flux and other reactive oxygen species (ROS). As a result, the phospholipids containing cell membrane will be undergo peroxidation, amino acid side chains oxidation, DNA breaks, and protein fragmentation. Taken together, iron-induced cellular damage may participate in hepatopathogenesis in patients with HCC on top of CHC, as was reported in the present study. These results confirm that of Furutani *et al.* (2006) who reported importance of oxidative stress and subsequent mitochondrial injury synergistically induced by iron loading and HCV proteins in the development of HCC.

The beneficial effects of iron chelators and/or hepcidin antagonist administration on the pathogenesis of iron overloading in mouse models, lead one to confirm the participation of iron in liver pathogenesis. Also, Siregar and Maail (2018) showed that ferritin levels were increased with the progression of Child-Pugh class ($p<0.001$), i.e. with the increase in the severity of liver disease. The results of the present study confirm their findings in that the mean ferritin levels were increased in sera of patients with HCC than those of non-HCC. In 2015, Sekine et al. (2014) reported that HCV core protein up-regulates iron uptake into the mitochondria, and thus, exacerbates oxidative stress (lipid peroxidation and reduction in GSH and NADPH concentrations) as well as hepatic toxicity (Siregar and Maail, 2018). Since our patients are chronically infected with HCV, one can expect the incorporation of viral proteins as well as viral RNA in iron loading, oxidative stress and hepatic toxicity; including HCC. This is the case in our patients, as viral RNA and miRNA-cDNA did not differ among the two groups but those of iron markers did. These outhers added that, serum ferritin and transferrin levels seem to play an important role to determine the severity of liver fibrosis and necro-inflammatory activity. Nakano *et al*. (2018) added that core protein of HCV may

account for the susceptibility of HCV-infected individuals to develop porphyria cutanea tarda (PCT). In their study, high prevalence of HCV infection was found among patients with PCT. These mediate a simultaneous increase in iron overload, oxidative stress and enhancement of hepatic disorders including fibrosis, cirrhosis and/or HCC, a mechanism which may be expected in the present study.

These observations lead one to suggest that the induction of intracellular porphyrin metabolism via the core protein of HCV may account for the susceptibility of HCV-infected individuals to develop porphyria cutanea tarda (PCT) as was reported by Nakano *et al*. (2018). In their study, high prevalence of hepatitis C virus (HCV) infection was found among patients with PCT. These mediate a simultaneous increase in iron overload, oxidative stress and enhancement of hepatic disorders including fibrosis, cirrhosis and/or HCC, a mechanism which may be expected in the present study. In this regard, severe necro-inflammatory activity; together with hepatic parenchymal disease was directly correlated with markers of iron status; namely serum iron, ferritin ($P < 0.001$) and TIBC levels ($P < 0.05$) (Vagu *et al*., 2008).

A possible explanation for these elevations is that a necroinflammatory hepatic status can participate in the release of iron and ferritin from damaged hepatocytes, a process sustained also by the concomitant high serum activities of liver enzymes and lowering in albumin levels in sera of patients with HCC compared with those of non-HCC, in the present study (Price and Kowdley , 2009).Taken together, one can conclude that, the presence of viral proteins as well as viral RNA and/or micro-RNA, irrespective of their levels can participate in iron over load. The latter mediate HCC formation. Also, the role of viral molecules and/or micro-RNA in iron overload must not be neglected.

REFERENCES

Castagna, A.; Campostrini, N.; Zaninotto, F. and Girelli, D. (2010). Hepcidin assay in serum by SELDI-TOF-MS and other approaches. J. Proteomics, 73(3):527-536..

De Domenico**,** I.; Lo**,** E.; Ward**,** D.M. and Kaplan**,** J. (2009). Hepcidin-induced internalization of ferroportin requires binding and cooperative interaction with Jak2.Proc Natl Acad Sci (USA), 106(10): 3800–3805.

Dedania, B. and Wu, G.Y. (2015). Dermatologic Extrahepatic Manifestations of Hepatitis C. J. Clin. and Translational Hepatol., 3(2):127–33.

Emerit, J.; Beaumont, C. and Trivin, F. (2001). Iron metabolism, free radicals, and oxidative injury. Biomed. Pharmacother., 55:333–339.

Fenton, H.J.H. (1894). Oxidation of tartaric acid in presence of iron. J. Chem. Soc., 65: 899–910.

Franchini, M.; Targher, G.; Capra, F.; Montagnana, M. and Lippi, G. (2008).The effect of iron depletion on chronic hepatitis C virus infection. Hepatol. Int.,2(3):335–40.

Fujita, N.; Horiike, S.; Sugimoto, R., et al. (2007). Hepatic oxidative DNA damage correlates with iron overload in chronic hepatitis C patients. Free Radic. Biol. Med., 42: 353–362.

Furutani, T.; Hino, K.; Okuda, M. et al. (2006).Hepatic iron overload induces hepatocellular carcinoma in transgenic mice

expressing the hepatitis C virus polyprotein. Gastroenterology, 130: 2087–2098.

Hör, W.H. and Schmidt, A. (2014). Low hepcidin triggers hepatic iron accumulation in patients with hepatitis C. Nephrol. Dial Transplant., 29(6):1141-1144.

Jaroszewicz, J.; Rogalska, M.; Flisiak, I. and Flisiak, R. (2010). Successful antiviral therapy is associated with a decrease of serum prohepcidicin in chronic hepatitis C. World. J. Gastroenterol., 16(14): 1747-1752.].

Khare, S. and Garg, V.K. (2015). Serum iron and TIBC parameters in chronic liver disease, Scholars Journal of Applied Medical Sciences (SJAMS) , 3(5E):2128-2131.

Kohgo, Y.; Ikuta, K.; Ohtake, T.; Torimoto, Y. and Kato, J. (2007). Iron overload and co-factors with special reference to alcohol, hepatitis C virus infection and steatosis/insulin resistance. World J. Gastroenterol. ,13:4699–4706

Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect, 17(2): 107–115.].

Metwally, M.A.; Zein, C.O. and Zein, N.N. (2004).Clinical significance of hepatic iron deposition and serum ron values in patients with chronic hepatitis C infection. Am J. Gastroenterol., 99(2):286–91.

Milic, F.; Mikolasevic, I.; Orlic, L.; Devcic, E.; Starcevic-Cizmarevic, N.; Stimac, D.; Kapovic, M. and Ristic, S. (2016). The Role of Iron and Iron Overload in Chronic Liver Disease. Med. Sci. Monit., 22: 2144-2151

Nakano, T.; Moriya, K.; Koike. K. and Horie, T. (2018). Hepatitis C virus core protein triggers abnormal porphyrin metabolism in human hepatocellular carcinoma cells. PLoS ONE, 13(6): e0198345.)

Price, L. and Kowdley, K.V. (2009).The role of iron in the pathophysiology and treatment of chronic hepatitis C. Can. J. Gastroenterol., 23(12):822– 828.

Saito, H.; Fujimoto, Y.; Ohtake, T.; Suzuki, Y.; Sakurai, S.; Hosoki, Y.; Ikuta, K.; Torimoto, Y. and Kohgo, Y. (2005). Up-regulation of transferrin receptor 1 in chronic hepatitis C: Implication in excess hepatic iron accumulation. Hepatol Res., 31:203–210.

Sekine, S.; Ito, K.; Watanabe, H.; Nakano, T.; Moriya, K.; Shintani, Y.; et al. (2015). Mitochondrial iron accumulation exacerbates hepatic toxicity caused by hepatitis C virus core protein. Toxicology and applied pharmacology, 282(3):237–43. Epub 2014/12/30. pmid:25545986.

Shibutani, S.; Takeshita, M. and Grollman, A.P. (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxo-7-hydrodeoxyguanosine (8-oxodG). Nature, 349: 431–434.

Siregar, G.A. and Maail, W. (2018). Serum iron parameters in liver cirrhosis. Earth and Environmental Science, 125(1):012217.

Uchino, K.; Tateishi, R.; Fujiwara, N.; Minami, T.; Sato, M.; Enooku, K.; et al. (2016). Impact of serum ferritin level on hepatocarcinogenesis in chronic hepatitis C patients. Hepatology research: the official journal of the Japan Society of Hepatology, 46(4):259–268.

Can Serum Iron Parameters Represent Surrogate Markers for Chronic Hepatitis C-Based Hepatocellular Carcinoma?

Uchino, K.; Tateishi, R.; Nakagomi, R.; Fujiwara, N.; Minami, T.; Sato, M.; et al. (2018). Serum levels of ferritin do not affect the prognosis of patients with hepatocellular carcinoma undergoing radiofrequency ablation. PLoS ONE, 13(7): e0200943.

Vagu, C.; Sultana, C. and Ruta, S. (2013).Serum iron markers in patients with chronic hepatitis C infection. Hepatology Monthly, 13(10): e13136.

Walker, N.M.; Stuart, K.A.; Ryan, R.J.; Desai, S.; Saab, S.; Nicol, J.A. and Crawford, D.H.G. (2010). Serum ferritin concentration predicts mortality in patients awaiting liver transplantation. Hepatol., 51(5) 1683–91.

Zhou, X.D.; Stahlhut, M.W.; Hann, H.L. and London, W.T. (1988). Serum ferritin in hepatocellular carcinoma. Hepatogastroenterology, 35(1):1-4.

هل يمكن ان تمثل دلالات الحديد فى مصل الدم دلالات بديلة لسرطان الكبد القائم على وجود التهاب كبدى مزمن س؟

لمياء عبد أللطيف[1] ، جمال ألسيد شيحة[2, 3] ، وليد سمير[4] ، ألشحات أبومسلم طوسون[1]

1 - قسم الكيمياء- كلية العلوم- جامعة بورسعيد- مصر

2- قسم الباطنة - كلية الطب جامعة المنصورة

3- مستشفي ومعهد بحوث الكبد المصري- شربين- محافظة الدقهلية - مصر

4- مستشفي الباطنة التخصصى- كلية الطب - جامعة المنصورة

المستخلص

يلعب تنظيم مستوي الحديد فى الجسم دورا رئيسيا في حياة الانسان. وحتى الان فان الاثار الضاره لتراكم الحديد بسبب الاصابة بفيروس الالتهاب الكبدى الوبائي س لم يتم فهمها بالقدر الكافي. **ولذلك فان الهدف** من هذه الدراسه هو دراسة الارتباطات المحتملة بين دلالات حالة الحديد والحمل الفيروسي س والمجموع الكلى لمستوى الاحماض الريبوزية الصغيرة (على مستوي الحامض النووى الدى اوكسي ريبوزى المكمل Total micro-RNA-cDNA)، وحدوث سرطانا في الكبد بسبب تقدم حالة المرض. **وقد** تم اجراء هذه الدراسة على عدد 126 مريضا بفيروس الالتهاب الكبدى الوبائي س من بينهم عدد 22 مريضا بسرطان الكبد الناشئ عن الاصابة المزمنة بفيروس الالتهاب الكبدى الوبائي س. اضف الي ذلك فقد تضمنت الدراسة عدد ثلاثون فردا من الاصحاء الذين لا يعانون من اى امراض كبدية كمجموعة ضابطة. ولذلك تم استخلاص الحامض النووي الريبوزى الكلي بما في ذلك الاحماض الريبوزية الصغيرة الكلية والتى تم تحويلها جميعا الى ألاحامض النووى الدى اوكسي ريبوزية المكملة باستخدام تقنية ال RT-PCR وكذلك تم تقدير الحامض النووى الريبوزي ألخاص بالفيروس بتقنية تفاعل البلمرة المتسلسل (PCR). وكذلك تم تقدير مستويات دلالات التمثيل الغذائى للحديد (الحديد، الفيريتين و القدرة الكلية للارتباط بالحديد (TIBC). كما تم تقدير مستوي الالفا-فيتوبروتين - كدلالة اورام - وتقييم وظائف ألكبد وزمن البروثرومبين - ومنه تم حساب قيمة international normalization ratio، (INR) المقابلة. وايضا تم عمل عد للصفائح الدموية فى دماء المرضي. وكذلك تم توقيع ألكشف ألاكلينيكى على المرضى وعمل أشعة تليفزيونية ، أشعة مقطعية وأشعة فيبروسكان لتقييم حدة المرض. وقد أتضح من النتائج وجود زيادة عالية في مستويات دلالات التمثيل الغذائى للحديد (الحديد، الفيريتين و القدرة الكلية للارتباط بالحديد) فى مرضى سرطان الكبد مقارنة بمجموعة مرضى الالتهاب الكبدي الوبائى المزمن (س) والذين لا يعانون من وجود أورام. كما وجدت ارتباطات ايجابية وذات دلالة احصائية بين دلالات التمثيل الغذائى للحديد مع مستوى نشاط انزيمات الكبد ومع نسبة الصفراء فى الجسم. وعلى الجانب الاخر وجدت ارتباطات سالبة بين دلالات التمثيل الغذائى للحديد مع مستوى الزلال فى مصل الدم وعدد الصفائح الدموية فى الدم ككل (Whole blood). وعلى العكس ، فقد وجد ان المجموع الكلى لمستوى الاحماض الريبوزية الصغيرة (Total micro-RNA-cDNA) وكذلك الحامض النووى الريبوزي الخاص بالفيروس (HCV-RNA) لا يرتبطا ارتباطات ذات دلالات احصائية مع دلالات التمثيل الغذائى للحديد ولكن نقص Total micro-RNA-cDNA فى مصل مرضى سرطان الكبد مقارنة بمجموعة الاصحاء. وخلصت نتائج هذه الدراسة الى ان دلالات الحديد فى الجسم تلعب دورا هاما فى ميكانيكية تقدم حالة الكبد وحدوث سرطان فيه. وعليه فان دلالات التمثيل الغذائى للحديد تعتبر دلالات بديلة (Surrogate Markers) لتقييم حدة المرض الكبدي وخاصة سرطان الكبد القائم على وجود الفيروس (س). وايضا فان لمستوى الحامض النووى الريبوزى للفيروس (س) وكذلك الاحماض الريبوزية الصغيرة (micro-RNA) - على مستوي ال Total micro-RNA-cDNA- دورا لا ينكر فى تقدم حالة الكبد وحدوث السرطان الكبدي القائم على وجود عدوى مسبقة بالفيروس (س).