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ROLE OF INTERLEUKIN-8 AND OXIDATIVE STRESS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA INFECTED WITH HEPATITIS C VIRUS

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ABSTRACT

Background: The rate of hepatocellular carcinoma (HCC) is increasing in Egypt where the major risk factor is chronic infection with hepatitis C virus (HCV). The development of effective markers for the detection of HCC could have an impact on cancer mortality and significant public health implications worldwide. The objective of this study is to investigate the role of interleukin-8, some antioxidants and some trace elements in Egyptian patients with hepatocellular carcinoma infected with hepatitis C virus.

***Key words:** IL-8, HCC, HCV, Oxidative stress, Trace element.

Methods: This study comprised 40 patients with HCC (20 with cirrhosis and 20 without cirrhosis) and 20 patients with hepatitis C virus. They were 39 males and 21 females with age range from 22 to 71 years. Twenty apparently healthy volunteers with matched age and sex were taken as control group. Serum concentration level of IL-8 was measured using an enzyme-linked immunosorbent assay (ELISA). The serum antioxidants were measured using spectrophotometric analysis and trace elements by using atomic absorption spectrophotometry.

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Results: A highly significant elevation was found in interleukin-8, α-fetoprotein, iron and malondialdehyde in patients with HCC compared to control subjects. On the other hand, serum levels of reduced glutathione, catalase, superoxide dismutase, total antioxidant capacity and zinc were significantly decreased in patients with HCC compared to control subjects. A positive correlation was found between serum level of IL-8 and each of GSH (r=-0.534 and P= 0.000), SOD (r=-0.295 and P= 0.021), CAT (r=-0.545 and P= 0.000) and Zn (r=0.422 and P= 0.001) in all patients group.

Conclusion: The ability to measure IL-8 in the serum could be useful as a prognostic marker of HCC patients. The levels of antioxidants such as CAT, SOD and GSH in HCC patients when compared to control group played a vital and important role in the prevention of complications of liver cancer. Interleukin-8, some antioxidants (MDA, GSH, CAT and SOD) and some trace elements (Fe and Zn) are suggested to be simultaneously evaluated in order to enhance the detection of HCC.

INTRODUCTION

Hepatitis C virus (HCV) infection is a significant clinical problem throughout the world and it is a major cause of liver cirrhosis and liver cancer. Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world complicating liver cirrhosis in most cases. Its incidence is increasing worldwide ranging between 3% and 9% annual **[Filipowicz, (2010)]**. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients. The epidemiology of HCC is characterized by marked demographic and geographic variations **[Blum & Spangenberg, (2007) and Davis et al., (2008)].** The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt with a doubling in the incidence rate in the past 10 years **[Anwar et al., (2008)]**, where the major risk factors are chronic infections with hepatitis B and C viruses **[Ezzat et al., (2005)]**.

Other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides, and endemic infections in the community, such as chistosomiasis, may have additional roles in the etiology or progression of the disease **[Anwar et al., (2008)]**. On the other hand, these problems may be increases due to the high prevalence of hepatitis C viral infection in Egypt **[Hassan et al., (2001)]**.

Interleukin-8 is a small and soluble peptide (8-10 K Da) **[Brat et al., (2005)]**, it has sequence identity ranging from 24% to 46% with the other members of the CXC family. Expression of IL-8 can be induced, in some cases up to 100-fold, by IL-1, TNF-α, IL-6, interferon-γ, lipopolysaccharide, phytohemagglutinin, phorbol myristate acetate, reactive oxygen species, and other cellular stresses. Potent inhibitors of IL-8 production include dexamethasone, IL-4, and IL-10 **[Mukaida et al., (1994)]**. Serum level of IL-8 was found to be a prognostic marker in soft tissue sarcoma **[Rutkowski, et al. (2002)]**, B-cell chronic lymphocytic leukemia **[Molica, et al. (1999)]**, primary gastrointestinal non-Hodgkin's lymphoma **[Retzlaff et al., (2002)]** and malignant melanoma **[Ugurel et al., (2001)]**. IL-8 contributes to human cancer progression through potential mitogenic, and angiogenic functions **[Zekri et al.,(2010)]**. Its expression plays a critical role in the metastatic potential of human HCC more than in angiogenesis or tumor proliferation **[Kubo et al., (2005)]**.

The induction of cytokine synthesis was found to depend on the oxidative properties of H_2O_2 as it was inhibited by the addition of catalase, and to require de novo protein synthesis. It has been shown that HCV infection itself is also characterized by an increase in free radical formation manifested by increased hepatic and serum levels of products of lipid peroxidation **[De Maria et al., (1996)]**.

HCV infection is characterized by increased markers of oxidative stress. Lipid peroxidation products are increased in serum, peripheral blood mononuclear cells (PBMC), and liver specimen from hepatitis C patients. In addition, there is a significant reduction of hepatic, plasmatic, and lymphocytic glutathione (GSH) levels in patients chronically infected by HCV, particularly with the 1b genotype **[Choi et al., (2004)]**.

On the other hand, trace elements are required for growth, maintenance of life and reproduction. A deficiency of these elements produces a functional impairment. Very small amounts of trace elements are necessary for optimal performance of a whole organism **[Saracoglu et al., (2009)]**.

The aim of this work is to evaluate the role of IL-8, some antioxidants and some trace elements in egyptian patients with hepatocellular carcinoma infected with HCV.

Subjects and methods

This study comprised 40 patients with hepatocellular carcinoma HCC (20 with cirrhosis and 20 without cirrhosis) and 20 patients with hepatitis C virus. They were 39 males and 21 females with age range from 22 to 71 years. They were selected from Gastroenterology-Surgical-Center, Mansoura University, Egypt. Twenty apparently healthy volunteers with matched age ranged from 18 to 73 years and matched sex (13 males and 7 females) were taken as control group. This study was approved by the local ethics committee, and a written consent was taken from patients.

Patients and controls were subjected to the following clinical and laboratory investigations: 1- Patient history taking with special stress on age, sex, presence of symptoms. 2- Thorough clinical examination, complete blood count and liver function tests (to exclude other diseases affecting the results) 3- Serum AFP was determined by a chemiluminescence method (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). 4- Serum IL-8 was determined using an Enzyme linked Immunosorbent Assay (ELISA) technique, and the IL-8 kit was obtained from Orgenium Laboratories Business unit, Finland, **[Matsuhuma K. Et al. (1989)]**. 5- Serum catalase was determined by the method of **[Aebi (1984)]**, serum reduced glutathione was determined by the method of **[Beutler et al. (1963)]** and serum superoxide dismutase by the method of **[Nishikimi et al. (1972)]**, while the lipid peroxidation levels was measured according to the method of **[Ohkawa et al. (1979)]** and serum total antioxidant capacity according to the method of **[Koracevic (2001)]** by using for each one a commercially available kit (Biodiagnostic, Egypt). 6-Trace elements were measured by using atomic absorption spectrophotometry by using Perkin Elemer 2380 instrument.

Statistical analysis was done using the statistical package of social sciences (SPSS) software version 11.5. Student's t-test was used to evaluate the difference between the means of two sets of data. P values < 0.05 were considered to indicate statistical significance.

RESULTS

The current study was undertaken to estimate the serum levels of interleukin-8 (IL-8) and of some micronutrients elements such as: zinc, copper and iron, as well as the serum activities of superoxide dismutase

(SOD) and catalase (CAT) enzymes also the concentration of total antioxidant capacity (TAC), glutathione (GSH) contents and lipid peroxidation in patients with HCC, with and without cirrhosis, HCV patients as well as in healthy control individuals.

Table 1 shows a significant increase in the level of interleukin-8 (IL-8) in HCV patients, in HCC without Cirrhosis patients and in HCC with Cirrhosis patients when compared to its level in the control group (p ▯ 0.001 for each group).

Table (1): Comparison between HCC with and without cirrhosis patients, HCV patients and healthy control group regarding IL-8 and AFP.

	Healthy Control N = 20	HCV patients N = 20	HCC Patients		P
			without Cirrhosis N = 20	with Cirrhosis N = 20	
IL-8 (pg/ml) Mean ± SD	2.86 ±0.80	7.92 ± 5.99	39.42 ±30.35	41.93 ±19.83	P1 < 0.001* P2 < 0.001* P3 = 0.757 P4 =0.001*
AFP (µg/L) Mean ± SD	2.52 ±0.59	2.43 ± 0.61	14.47 ±14.45	23.56 ±21.67	P1 = 0.001* P2 < 0.001* P3 = 0.120 P4 = 0.636

P1: significance between HCC without cirrhosis and healthy control.
P2: significance between HCC with cirrhosis and healthy control.
P3: significance between HCC with cirrhosis and HCC without cirrhosis patients.
P4: significance between HCV and healthy control.

There is a highly significantly increase ($p < 0.001$) in the level of α- fetoprotein (AFP) in HCC without Cirrhosis patients and in HCC with Cirrhosis patients when compared to control group. On the other hand, there is no significant difference between HCC with Cirrhosis patients and HCC without Cirrhosis patients regarding the levels of IL-8 and AFP.

Table (2): Comparison between HCC with and without cirrhosis patients, HCV patients and healthy control group regarding malondialdehyde, glutathione, catalase, total antioxidant capacity and superoxide dismutase.

	Healthy Control N = 20	HCV patients N = 20	HCC Patients		P
			without Cirrhosis N = 20	With Cirrhosis N = 20	
MDA (nmol/ml) Mean ± SD	7.77 ± 2.15	13.96 ± 4.72	14.10 ± 4.61	21.72±7.78	P1 < 0.001* P2 < 0.001* P3 < 0.001* P4 < 0.001*
GSH (mg/dl) Mean ± SD	921.20±20.21	782.30±18.94	651.10± 24.65	506.90±32.78	P1 < 0.001* P2 < 0.001* P3 < 0.001* P4 < 0.001*
CAT (U/L) Mean ± SD	9.23 ± 0.42	8.57 ± 0.34	6.01 ± 0.29	4.01 ± 0.27	P1 < 0.001* P2 < 0.001* P3 < 0.001* P4 < 0.001*
TAOC (mM/L) Mean ± SD	2.27 ± 0.80	2.11 ± 0.89	1.86 ± 0.43	1.94 ± 0.19	P1 = 0.05* P2 = 0.081 P3 = 0.463 P4 = 0.544
SOD (U/ml) Mean ± SD	284.70±94.37	277.63±78.79	199.78±93.68	203.35±85.84	P_1 = 0.006* P_2 = 0.007* P_3 = 0.900 P_4 = 0.798

P1: significance between HCC without cirrhosis and healthy control.
P2: significance between HCC with cirrhosis and healthy control.
P3: significance between HCC with cirrhosis and HCC without cirrhosis patients.
P4: significance between HCV and healthy control.

Table (3): Comparison between HCC with and without cirrhosis patients, HCV patients and healthy control group concerning the levels of some trace elements.

	Healthy Control N = 20	HCV patients N = 20	HCC Patients		P
			without Cirrhosis N = 20	with Cirrhosis N = 20	
Cu (mg/L) Mean ± SD	0.16 ± 0.04	0.17 ± 0.05	0.17 ± 0.07	0.17 ± 0.04	P1 = 0.644 P2 = 0.412 P3 = 0.913 P4 = 0.624
Fe (mg/L) Mean ± SD	0.85 ± 0.17	0.90 ± 0.34	1.11 ± 0.17	1.44 ± 0.95	P1 < 0.001* P2 = 0.010* P3 = 0.124 P4 = 0.562
Zn (mg/L) Mean ± SD	3.55 ± 0.30	3.21 ± 0.08	2.71 ± 0.30	1.98 ± 0.28	P1 < 0.001* P2 < 0.001* P3 < 0.001* P4 < 0.001*

P1: significance between HCC without cirrhosis and healthy control.
P2: significance between HCC with cirrhosis and healthy control.
P3: significance between HCC with cirrhosis and HCC without cirrhosis patients.
P4: significance between HCV and healthy control.

Table 2 shows a highly significant increase for malondialdehyde (MDA) in HCV patients, in HCC without Cirrhosis patients and in HCC with Cirrhosis patients when compared to control group ($p < 0.001$ for each group). Also, there is a highly significant increase (p 0.001) for MDA in HCC with Cirrhosis patients when compared to HCC without Cirrhosis patients.

A highly significant decrease for GSH and CAT in HCV patients, in HCC without cirrhosis patients and in HCC with Cirrhosis patients were also observed when compared to control group ($p < 0.001$ for each group). Also, there is a highly significant decrease for GSH and CAT in HCC with Cirrhosis patients when compared to HCC without Cirrhosis patients ($p < 0.001$ for each). There is a significant decrease for SOD in HCC with and without Cirrhosis patients when compared to control group ($p = 0.007$ and $p = 0.006$ respectively).

Table 3 shows a highly significant decrease for the serum level of zinc (Zn) in HCV patients and in HCC with and without Cirrhosis patients when compared to control group (p< 0.001 for each group). Also, a highly significant decrease was observed in the level of zinc (Zn) in HCC with Cirrhosis patients when compared to HCC without Cirrhosis patients ($p< 0.001$).

On the other hand, there is a significant increase in the level of the iron (Fe) in HCC with and without Cirrhosis patients when compared to control group ($p= 0.010$ and $p< 0.001$ respectively).

A positive correlation was found between serum level of IL-8 and each of GSH ($r=-0.534$ and $p= 0.000$), SOD ($r=-0.295$ and $p= 0.021$), CAT ($r=-0.545$ and $p= 0.000$) and Zn ($r=0.422$ and $p= 0.001$) in all patients group.

DISCUSSION

Chronic hepatitis C virus infection is an important cause for the development of liver cirrhosis and hepatocellular carcinoma **[Seeff (2002)]**. There is overwhelming evidence suggesting the direct and indirect roles of HCV in the pathogenesis of HCC **[Tran (2008)]**. Most of acute and chronic liver diseases are characterized by inflammatory processes with enhanced expression of various pro- and anti-inflammatory cytokines in the liver **(Tilg & Kaser (2006)]**.

In our study HCC patients with and without cirrhosis show high levels of malondialdehyde (MDA) when compared with control subject. MDA, a product of polyunsaturated fatty acid peroxidation, was elevated in the liver and blood **[De Maria et al., (1996) and Paradis et al., (1997)]**. The peripheral blood mononuclear cells from patient of chronic hepatitis C had increased MDA concentrations this reflect lipid peroxidation **[Levent et al., (2006)]**.

Also, patients with HCV, HCC with and without cirrhosis show high significant decrease in the level of GSH, CAT and SOD when compared to control subjects. HCV can directly induce oxidative stress intracellularly in hepatocytes and associated with increased ROS, decreased intracellular and/or mitochondrial GSH content **[Abdalla et al., (2005) and Choi & Ou, (2006)]**. Moreover, **[Braticevici et al., (2009)]** reported that in chronic hepatitis C, ongoing hepatocytic

necrosis and inflammation are associated with an increased production of ROS. This oxidative stress induces hepatic damage leading to depletion of reduced glutathione (GSH). These results agree with the results obtained by **[Levent et al., (2006) and Osman et al., (2007)]**. They reported that GSH decrease in the blood serum of patients with chronic hepatitis C. This alternation of cellular redox state in patients with viral liver disease is potentiated by a correlated decrease in antioxidant enzymes and increase of free radical-mediated damage and apoptosis of liver cells **[Loguercio & Federico (2003)]**. **Lin and Yin, 2007** reported that the rapid energy metabolism and/or tumor growth occurred in HCC patients caused vitamins B depletion, which further down-regulated GSH synthesis. Definitely, the depletion of vitamins B and the decrease of GSH in HCC patients could impair many physiological functions and antioxidant defense, which might further facilitate cancer detrimental development.

Min et al., 2010 reveal a remarkable reduction of CAT in HCC patients and correlate with the degree of malignancy of the tumor suggesting the impairment of free radical scavenger system in hepatocarcinoma. This is due to exposure to ROS stress is significantly associated with catalase downregulation and methylation of the catalase promoter during the development of HCC and that changes in ROS stress markers and the decrease in CAT level may be due to over utilization of this enzymatic antioxidant to scavenge the products of reactive oxygen species.

In the other hand, **[Osman et al., (2007)]** reported that there is a decrease in SOD levels in liver tissue of patients with acute and chronic hepatitis accompanied by fatty degeneration. The decrease in SOD explains the depletion of antioxidant enzyme defense system. **Hadi et al., 1999** found lower activities of erythrocytes SOD in patients with cirrhosis compared with those obtained from control subjects; this is due to further peroxidative reactions in the erythrocytes, possibly due to peroxidation of some cellular structures sensitive to peroxidative attack. On the other hand, decreased activity of SOD could be due to over utilization of this enzymatic antioxidant to scavenge the products of lipid peroxidation or decrease in synthesize capacity of liver, and the antioxidant defense power of the patients with virus-originated HCC. Moreover, **[Yasuyama et al., (1988)]** found significant increases in plasma SOD activity in chronic hepatitis, autoimmune hepatitis, primary

biliary cirrhosis and hepatocellular carcinoma groups and this is due to increased release of injured hepatocytes. We speculate that oxidative damage plays an important role in the pathogenesis of liver diseases and changes in the oxidant-antioxidant balance may play a decisive role in the progression of liver damage and HCC.

The liver is the primary storage organ for iron and it is well documented that patients with chronic hepatitis C frequently show serum and hepatic iron overload. Hepcidin exclusively synthesized in the liver, is thought to be a key regulator for iron homeostasis and is induced by infection and inflammation **[Mastoi et al., (2009)]**. In the present study, there is a high significant increase in iron levels in HCC patients with and without cirrhosis compared to healthy control group. **Kato et al., 2007** reported that hepatic iron accumulation induces mitochondrial impairment and tumor development in the liver and iron excess is a risk factor for liver disease progression and HCC development in chronic HCV infection. Excess iron can be harmful and may be contribute to liver injury by increasing oxidative stress and leading to progressive liver inflammation and fibrosis in patients with HCV infection. So, the iron reduction is an effective therapeutic agent for decreasing the risk of HCC development in patients with HCV infection.

Sayed et al., 2005 found that the serum levels of zinc of the patients with chronic hepatitis, cirrhosis and HCC were lower than those of controls, which is matched with our findings. They also found that liver impairment in HCV related chronic liver disease is the main cause of blood decrease in zinc, independently of the nutritional status and that low liver zinc and high liver copper determine amount of fibrosis.

In the present study, HCC patients infected with HCV either with or without cirrhosis have higher levels of interleukin-8 (IL-8) when compared with control subject. This is agrees with **[Mihm et al., (2004)]**, they found that serum levels of the proinflammatory chemokine IL-8 in patients with chronic hepatitis C are significantly higher in comparison with healthy controls and are associated with cirrhosis and reported that the core protein of Hepatitis C Virus (HCV) activates the IL-8 promotor and HCV-E2 upregulates IL-8 production. Also, the increase level of serum IL-8 was correlating with the progression of liver disease in patients with advanced HCC with remote metastasis

[Tachibana et al., (2007)]. They also found that the expression of IL-8 may be augmented upon the malignant transformation of hepatocytes during the course of chronic HCV infection. The high levels of IL-8 encountered in serum may contribute to recruitment, trapping, and activation of neutrophils which can promote organ failure (liver) in both hepatocellular carcinoma and chronic active hepatitis. Also, the increase in IL-8 levels as the disease progressed from chronic hepatitis to liver cirrhosis and further to HCC, suggesting that the increase may be due not only to immune response against persistent HCV infection but also to the development of HCC **[Polyak et al., (2001)]**. IL-8 level was found to be a significant prognostic factor in terms of disease free survival and overall survival in patients with HCC, it was shown to be expressed in the cytoplasm of hepatoma cells and in vascular endothelial cells of tumors, suggesting that the angiogenetic activity of IL-8 may contribute to the growth of HCC **[Tachibana et al., (2007)]**. In brief, the core and NS5A proteins of HCV induce the expression of the IL-8 gene, and that serum IL-8 levels in chronic hepatitis C patients are associated with resistance to interferon treatment, suggesting that IL-8 plays an important role in the maintenance of persistent infection with HCV. The high-serum IL-8 levels in HCC patients may be caused by an excessive production in tumor cells and subsequent release into the circulation. These results suggest that IL-8 is central to the tumor progression of HCC. **Ren et al., 2003** show significant correlations of serum IL-8 levels with tumor size and tumor stage and suggest that IL-8 may be directly or indirectly involved in the progression of HCC and concluded that serum IL-8 may be a useful biological marker of tumor invasiveness and an independent prognostic factor for patients with HCC. **El-Tayeh et al., 2012** recommended that, Alpha fetoprotein (AFP) which is the golden marker for HCC is of low sensitivity, therefore, additional markers such as alpha-L-fucosidase (AFU), transforming growth factors alpha and beta (TGF-α and TGF-β) and interleukin-8 (IL-8) are suggested to be simultaneously evaluated in order to enhance the detection of HCC.

This study show a significant increase in the serum levels of AFP in HCC patients either with cirrhosis or without compared to healthy control subjects. This observation agrees with that shown by **[Arrieta et al., (2007), Rodriguez-Diaz et al., (2007) and Baig et al., (2009)]** they reported that the progressive elevation of alpha fetoprotein in patients

with liver cirrhosis is useful for the diagnosis of hepatocellular carcinoma and helpful in assessing problems in management of HCC and monitoring treatment regiments. In addition, AFP is also an indicator of HCC risks mostly in patients with cirrhosis and HCV/HBV infection.

In conclusion, the ability to measure IL-8 in the serum could be useful as a prognostic marker of HCC patients. The levels of antioxidants such as CAT, SOD and GSH in HCC patients when compared to control group played a vital and important role in the prevention of complications of liver cancer. Interleukin-8, some antioxidants (MDA, GSH, CAT and SOD) and some trace elements (Fe and Zn) are suggested to be simultaneously evaluated in order to enhance the detection of HCC.

REFERENCES

Abdalla MY, Ahmad IM, Spitz DR, Schmidt WN, Britigan BE (2005). Hepatitis C virus-core and non structural proteins lead to different effects on cellular antioxidant defenses. J Med Virol, 76: 489-97.

Aebi H (1984). Catalase in vitro. Meth. Enzymol. 105:121-6.

Anwar WA, Khaled HM, Amra HA, El-Nezami H, Loffredo CA (2008). Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities for prevention. Mutat Res., 659(1-2):176-84.

Arrieta O, Cacho B, Espinosa DM, Villavicencio AR, Estrada DF, Pedro NH (2007). The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. BMC Cancer. 7:28.

Baig JA, Alam JM, Mahmood SR, Baig M, Shaheen R, Sultana I and Waheed A (2009). Hepatocellular carcinoma (HCC) and diagnostic significance of α-fetoprotein (AFP). J Ayub Med Coll Abbottabad. 21(1):72-5.

Beutler E, Duron O, Kelly MB (1963). Improved method for the determination of blood glutathione. J Lab Clin Med. 61:882-8.

Blum H and Spangenberg H (2007). Hepatocellular Carcinoma: An Update. Arch Iranian Med, 10 (3): 361- 71.

Brat DJ, Bellail AC, Van Meir EG (2005). The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. J. Neurooncol. 7(2): 122-33.

Braticevici CF, Mohora M , Crețoiu D , Crețoiu S, Petrișor A, Usvat R, Ion DA(2009). Role of oxidative stress in the pathogenesis of chronic hepatitis C (CHC). Rom J Morphol Embryol, 50(3):407-12.

Choi J, James Ou J-H. (2006). Mechanisms of Liver Injury.III. Oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol Gastrointest Liver Physiol 290: G847-G851.

Choi J, Lee KJ, Zheng Y, Yamaga AK, Lai MMC, Ou JH (2004). Reactive oxygen species suppress hepatitis C virus RNA replication in human hepatoma cells. J Hepatol 39: 81-9.

Davis G, Dempster J, Meler J, Orr D, Walberg M, Brown B, Berger B, O'Connor J, Goldstein R (2008). Hepatocellular carcinoma: management of an increasingly common problem. Proc (Bayl Univ Med Cent). 21(3):266-80.

De Maria N, Colantoni A, Fagiuoli S, Liu GJ, Rogers BK, Farinati F, Van Thiel DH, Floyd RA (1996). Association between reactive oxygen species and disease activity in chronic hepatitis C. Free Radic Biol Med, 21(3):291–5.

El-Tayeh SF, Hussein TD, El-Houseini ME, Amer MA, El-Sherbini M, Elshemey WM (2012). Serological biomarkers of hepatocellular carcinoma in Egyptian patients. Dis Markers, 32(4):255-63.

Ezzat S, Abdel-Hamid M, Eissa SA, Mokhtar N, Labib NA, El-Ghorory L, Mikhail NN, Abdel-Hamid A, Hifnawy T, Strickland GT, Loffredo CA (2005). Associations of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt. Int J Hyg Environ Health. 208(5):329-39.

Filipowicz MS (2010). Interferon therapy of hepatitis C: Molecular insights into success and failure. Swiss Med Wkly. 140 (1–2):3-11.
Hadi YM, Kacmaz M, Öztürk HS, Durak I (1999). Antioxidant status of erythrocytes from patients with cirrhosis. Hepatogastroenterol. 46:2460-8.

Hassan MM, Zaghloul AS, El-Serag HB, Soliman O, Patt YZ, Chappell CL, Beasley RP, Hwang LY (2001). The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. J Clin Gastroenterol. 33(2):123-6.

Kato J, Miyanishi K, Kobune M, Nakamura T, Takada K, Takimoto R (2007). Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. J Gastroenterol. 42:830-6.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001). Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 54(5):356-61.

Kubo F, Ueno S, Hiwatashi K, Sakoda M, Kawaida K, Nuruki K, Aikou T (2005). Interleukin 8 in human hepatocellular carcinoma correlates with cancer cell invasion of vessels but not with tumor angiogenesis. Ann Surg Oncol. 12:800-7.

Levent G, Ali A, Ahmet A, Polat EC, Aytaç Ç, Ayse E, Ahmet S (2006). Oxidative stress and antioxidant defense in patients with chronic hepatitis C patients before and after pegylated interferon alfa-2b plus ribavirin therapy. J Transl Med. 4:25.

Lin CC, Yin MC (2007). B vitamins deficiency and decreased anti-oxidative state in patients with liver cancer. Eur J Nutr 46:293-9.

Loguercio C, Federico A (2003). Oxidative stress in viral and alcoholic hepatitis. Free Radic Biol Med. 34:1-10.

Mastoi AA, Devrajani BR, Rahopoto Q, Memon SA, Qureshi GA (2009). Anti-Oxidant Activity and Metabolic Disturbances in Hepatitis C Patients with or without Diabet Melllitus. World Applied Sciences Journal. 7(8): 971-7.

Matsuhuma K. Et al. (1989). Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. J. Exp. Med. 169:1485-1490.

Mihm U, Herrmann E , Sarrazin U, Wagner MV, Kronenberger B, Zeuzem S, Sarrazin Ch (2004). Association of serum interleukin-8 with virologic response to antiviral therapy in patients with chronic hepatitis C. J Hepatol 40:845-52.

Min JY, Lim SO, Jung G (2010). Downregulation of catalase by reactive oxygen species via hypermethylation of CpG island II on the catalase promoter. FEBS Letters. 584:2427-32.

Molica S, Vitelli G, Levato D, Levato L, Dattilo A, Gandolfo GM (1999). Clinicobiological implications of increased serum levels of interleukin-8 in B-cell chronic lymphocytic leukemia. Haematol. 84: 208-11.

Mukaida N, Morita M, Ishikawa Y, Rice N, Okamoto S, Kasahara T, Matsushima K (1994). Novel mechanism of glucocorticoidmediated gene repression. Nuclear factor-kappa B is target for glucocorticoid-mediated interleukin 8 gene repression. J. Biol. Chem. 269:13289-95.

Nishikimi M, Appaji N, Yagi K (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 46(2):849-54.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95(2): 351-8.

Osman HG, gabr OM, Lotfy S, Gabr S (2007). Serum levels of bcl-2 and cellular oxidative stress in patients with viral hepatitis. Indian J. Med. Microbiol. 25 (4): 323-9.

Paradis V, Mathurin P, Kollinger M, Imbert-Bismut F, Charlotte F, Piton A (1997). In situ detection of lipid peroxidation in chronic hepatitis C: correlation with pathological features. J Clin Pathol. 50:401-6.

Polyak SJ, Khabar KSA, Rezeiq M, Gretch DR (2001). Elevated levels of interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. J Virol. 75:6209-11.

Ren Y, Poon RT, Tsui HT, Chen WH, Li Z, Lau C, Yu WC, Fan ST (2003). Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. Clin Cancer Res. 1:5996-6001.

Retzlaff S, Padro T, Koch P, Oelmann E, Lugering N, Mesters RM, Berdel WE (2002). Interleukin 8 and Flt3 ligand as markers of advanced disease in primary gastrointestinal non-Hodgkin's lymphoma. Oncol Rep. 9: 525-7.

Rodriguez-Diaz JL, Rosas-Camargo V, Vega-Vega O, Morales-Espinosa D, Martinez-Tlahuel JL, Gamboa-Dominguez A, Arrieta O (2007). Clinical and Pathological Factors associated with the development of hepatocellular carcinoma in patients with hepatitis virus-related cirrhosis: a long-term follow-up study. Clin Oncol.19(3):197-203.

Rutkowski P, Kaminska J, Kowalska M, Ruka W, Steffen J (2002). Cytokine serum levels in soft tissue sarcoma patients: correlations with clinicopathological features and prognosis. Int. J. Cancer. 100: 463-71.
Saracoglu S, Tuzen M, Soylak M (2009). Evaluation of trace element contents of dried apricot samples from Turkey. J Hazard Mater. 167:647-52.

Sayed HA, El Ayyat A, El Dusoki H, Zoheiry M, Mohamed S, Hassan M, El Assaly N, Awad A, El Ansary M, Saad A, El Karim AA (2005). A cross sectional study of hepatitis B, C, some trace elements, heavy metals, aflatoxin B1 and schistosomiasis in a rural population, Egypt. J Egypt Public Health Assoc. 80(3-4):355-88.

Seeff LB (2002). Natural history of chronic hepatitis C. J Hepatol. 36: S35-46.

Tachibana Y, Nakamoto Y, Mukaida N, Kaneko S (2007). Intrahepatic interleukin-8 production during disease progression of chronic hepatitis C. Cancer Lett. 251(1):36-42.

Tilg H, Kaser A (2006). How to modulate inflammatory cytokines in liver diseases. Liver Int. 26(9):1029-39.

Tran G (2008). The role of hepatitis C virus in the pathogenesis of hepatocellular carcinoma. Biosci. Horiz. 1(2):167-75.

Ugurel S, Rappl G, Tilgen W, Reinhold U (2001). Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. J Clin Oncol. 19:577-83.

Yasuyama T, Inoue K, Kojima T, Sasaki H (1988). Activities, electrophoretic profiles and immunolocalization of superoxide dismutase in human liver specimens. Jpn J Med. 27:34-41.

Zekri AR, Alam El-Din HM, Bahnassy AA, Zayed NA, Mohamed WS, El-Masry SH, Gouda SK, Esmat G (2010). Serum levels of soluble Fas, soluble tumor necrosis factor-receptor II, interleukin-2 receptor and interleukin-8 as early predictors of hepatocellular carcinoma in Egyptian patients with hepatitis C virus genotype-4. Comp Hepatol. 9(1):1.

دور إنترليوكين- ٨ وبعض مضادات الأكسدة فى مرضي سرطان الكبد المصريين المصابين بالإلتهاب الكبدي الفيروسي

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إن الاصابة بالالتهاب الكبدى المزمن(سي أو بي) وأيضا التعرض للسموم الفطرية المعروفة بالأفلاتوكسين تعتبر من أهم وأكثر العوامل التى تساعد على حدوث الاصابة بمرض سرطان الخلايا الكبدية. كما أن الشقوق الحرة تلعب دورا هاما فى الإصابة بالكثير من الأمراض حيث أن ازديادها يحدث نقصان فى معدل مضادات الأكسدة مما ينتج عنه تلف فى الأنسجة. و يعتقد أن ظهور مرض سرطان الخلايا الكبدية يرتبط بحدوث خلل ما بين إنتاج هذه الشقوق الحرة و التخلص منها بصورة آمنة.

لقد أجريت هذه الدراسة على ٦٠ مريضا حيث تم تقسيمهم إلى ٤٠ مريضًا يعانون من سرطان الخلايا الكبدية ، و٢٠ مريضا مصابين بالالتهاب الكبدى الوبائى سي . هذا بالاضافة الى ٢٠ شخصا متطوعا من الأصحاء كمجموعة ضابطة.

وتم إخضاع جميع الحالات لفحص التاريخ المرضي، وإجراء فحص إكلينيكي شامل، وعمل الاختبارات المعملية التالية لكل من هذه الحالات والمجموعة الضابطة وهى : صورة دم، وظائف كبد، تعيين المستوى المصلى للـ IL-8 وللألفافيتوبروتين بواسطة الـ ELISA وتعيين مستوى مضادات الأكسدة بواسطة التحليل الضوئي ، أما تعيين مستوى العناصر الضئيلة بواسطة الامتصاص الذري الطيفي. ومن خلال هذه الدراسة تم التوصل للنتائج الآتية:

-إرتفاع معنوي في مستوى الإنترليوكين-٨ والحديد والالفافيتوبروتين و المالونديالدهيد وإنخفاض معنوي فى مستوى الجلوتاثيون المختزل و إنزيم الكتاليز و إنزيم سوبرأكسيد ديسميوتيز والسعة الكلية لمضادات الأكسده والزنك لدى المرضى المصابين بالالتهاب الكبدى

الوبائي سي ومرضي سرطان الخلايا الكبدية المصابين والغير مصابين بالتليف الكبدى مقارنة بالمجموعة الضابطة.

- وجود علاقة طردية بين مستوى مصل الإنترليوكين-٨ وكل من الجلوتاثيون المختزل وإنزيم الكتاليز و إنزيم سوبرأكسيد ديسميوتيز والزنك لدى كل المرضى.

نستنتج من هذه الدراسة أن الإنترليوكين-٨ يعتبر مؤشرا هاما فى تشخيص مرضي سرطان الخلايا الكبدية. كما أن إنخفاض معدل مضادات الأكسدة مثل السوبرأكسيددبسميوتيز و الكتاليز و الجلوتاثيون المختزل عن معدلاتها الطبيعية عند مقارنتها بالأصحاء يؤكد أنها تلعب دوراً حيوياً و هاماً فى الوقاية من خطورة و مضاعفات مرض سرطان الكبد. وأخيرا توصى هذه الدراسة بضرورة قياس مستوى الإنترليوكين-٨ الى جانب بعض مضادات الاكسدة (MDA, GSH, CAT and SOD) وبعض العناصر النادرة (Fe and Zn) من أجل تحسين الكشف عن سرطان الكبد.