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Full Length Research Paper

Oxidative stress pattern in hepatitis C patients co-infected with schistosomiasis

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This study was designed to investigate the role of hepatitis C virus (HCV)-induced oxidative stress in the pathogenesis of the disease with the measurement of tumor necrosis factor (TNF-α) and super oxide dismutase (SOD). Eighty patients from Hepatology Unit, Faculty of Medicine, Ain Shams University, were investigated. Thirty patients with bilharzial HCV and 30 patients with non-bilharzial HCV as compared to 20 healthy controls of the same age and sex ratio were investigated. The concentrations of liver enzymes [glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP)], bilirubin (Bil), albumin (Alb) and total protein (TP) as well as TNF-α and Mn-SOD were determined. The mean level of the different liver function tests in the three groups in the study showed that the levels of GOT, GPT and ALP were significantly higher in the HCV groups as compared to the control group ($p < 0.05$). While serum bilirubin, albumin and total protein were non-significantly decreased in the HCV groups as compared to the control group ($p > 0.05$). Furthermore, the mean level of TNF-α was significantly higher in the HCV groups as compared to the control group ($p < 0.001$) and SOD was significantly decreased in the HCV groups as compared to the control group ($p < 0.001$). There is a cause-effect relationship between increased levels of TNF-α and decreased levels of SOD, relative to progression of chronic HCV, especially with bilharzias co-infection. Supporting the view that oxidative damage plays a role in chronic HCV infection, also TNF-α establishes a positive auto regulatory loop that can amplify the inflammatory response and lead to chronic inflammation. More evidence indicates that HCV block apoptosis and prolong survival of the host cell in order to gain time for replication and increase viral progeny production.

Key words: Hepatitis C virus, tumor necrosis factor-alpha, superoxide dismutase, oxidative stress, schistosomiasis.

INTRODUCTION

Hepatitis C virus (HCV) is one of the main causative agents of chronic viral hepatitis. Chronic hepatitis C can progress to cirrhosis and eventually to hepatocellular carcinoma (HCC) over a period of 20 to 30 years. The mechanisms by which HCV causes cell damage are not well understood. Different mechanisms including immunological liver damage, direct cytotoxicity mediated by different viral product and inductions of oxidative stress have been suggested to play a pathogenic role in this infection (Boya et al., 1999). A healthy liver is able to regenerate most of its own cells when it becomes damaged. Tumor necrosis factor (TNF-α), is a pleiotropic cytokine that can produce oxidative stress by simulating the generation of reactive oxygen species (ROS) such as superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2). In the liver, TNF-α is involved in the pathophysiology of viral hepatitis and in the pathogenesis of abnormal hepatocellular function (Esteve et al., 2007).

HCV and schistosoma mansoni has negative impact on

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Abbreviations: HCV, Hepatitis C virus; **HCC,** hepatocellular carcinoma; **TNF-α,** tumor necrosis factor; **ROS,** reactive oxygen species; **SOD,** super oxide dismutase; **ELISA,** enzyme-linked immunosorbent assay; **PCR,** polymerase chain reaction; **HIV,** human immunodeficiency virus; **XOD,** xanthine oxidase; **CHC,** chronic active hepatitis C.

the course of liver disease. Co-infection leads to a more severe clinical courses, higher incidence of cirrhosis and poor response to interferon-α (INF-α) therapy. Individuals co-infected with HCV and schistomiasis displayed a type 2-predominance cytokine profile. Also, patients co infected with HCV and schistosomiasis exhibit a unique clinical, virological and histological pattern manifested by viral persistence with high HCV RNA titers, as well as higher necroinflammatory and fibrosis scores in their liver biopsy samples (Kamal et al., 2000).

HCV infection is associated with increased oxidative stress, which is marked by an increase in oxidants and a decrease in antioxidant capacity of the cells (Choi and Ou, 2006). Super oxide dismutase (SOD) is considered to be a first line of defense against oxidative stress. In addition to the contribution by chronic inflammation caused by infection, a direct induction of ROS/RNS and mitochondrial dysfunction by the virus is likely to occur. The role of ROS in liver fibrosis has been suggested by the ability of ROS to up-regulate the profibrogenic cytokine TGF-β (Packer, 2002). In the infected cells, proteins of the HCV core have been shown to augment the oxidative and nitrosative stress, lipid peroxidation, oxidized thioredoxin and antioxidant gene expression as well as to enhance sensitivity to toxins. Reactive oxygen species function as inflammatory mediators by activating expression of cytokins and therefore ROS may contribute to tissue injury by not only directly damaging the tissue but also by enhancing further leukocyte accumulation (Farinati et al., 2006). HCV is by itself and not only through inflammation able to induce ROS, an effect specific to this virus (Moriya et al., 2001). This free radical production, accompanied by oxidative genomic injury, constitutes the first step of a cascade of genomic and post genomic events that play an important role in HCC. Infection with HCV is associated with increased levels of ROS/RNS and decreased antioxidant levels in patients (Farinati et al., 2007).

The aim of the current study was to investigate the role of HCV-induced oxidative stress in the pathogenesis of the disease with the measurement of TNF-alpha and superoxide dismutase. Furthermore, a cause affects relationship between TNF-alpha expression and SOD release. Finally, correlation of the measured parameters with the different clinic pathological factors was studied.

MATERIALS AND METHODS

This study was performed using 80 patients admitted at the Hepatology Diagnostic Unit, Faculty of Medicine, Ain Shams University, Egypt. The Ethical Committee of Faculty of Medicine approved this study. Demographic data and medical history were obtained at the beginning of the study. The study protocol was carried out in accordance with the Helsinki Declaration. All subjects were informed about the study and the written consent was obtained. Patients with chronic liver diseases were treated by supportive treatment as combined diuretics (furosemide [Lasix 40 mg daily]), (oral spironolactone [Aldactone 100 mg daily, Propranolol (Inderal 40 mg twice daily) and silymarin (legalon 140 mg three times daily). Groups of the study were categorized into the following groups:

Group I

Control group including 20 healthy normal individuals (mean age ± SD = 45.35 ±10.6; median age = 43.5 years; range 30 - 65).

Group II

Included 30 patients with bilharzial chronic HCV infection (mean age ± SD = 48.0 ± 9.47; median age = 45.5 years; range 27 - 65). The diagnosis was based on the presence of viable bilharzial ova in fecal material as well as serologic testing and liver histopathology.

Group III

Included 30 patients with non-bilharzial chronic HCV infection (mean age ± SD = 46.3 ± 8.86; median age = 46.5years; range 25 to 61). The diagnosis was based on a positive test for HCV antibodies by third generation Enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (PCR) for HCV RNA beside clinical, radiological, biochemical (elevated serum alanine aminotransferase levels for at least 6 months) and histopathological findings.

All patients were negative for hepatitis B surface antigen and Human immunodeficiency virus (HIV), and none had liver cirrhosis or renal disease. Other possible causes of hepatocellular injury, such as alcohol and drug-related injuries were also excluded.

Methods

Ten milliliters of venous blood were withdrawn from each individual under complete aseptic conditions and the blood samples were divided and put into two tubes. One for serum separation, and the other containing anticoagulant for the whole blood. Serum samples were separated and stored at -20°C until assayed for aspartate transferase, alanine transferase, alkaline phosphatase, total bilirubin, total protein, HCV-Ab, bilharzial-Ab and TNF-alpha. While whole blood samples were assayed for SOD levels. Liver function enzymes (AST, ALT and ALP) were assayed using QCA kits (Quimica Clinica Aplicada S.A. kits. Aspartado, Amposta, Spain) (Reitman and Frankel, 1957; Belfield and Goldberg, 1971). Total protein in serum was determined according to Gornall (1949). HCV diagnosis was carried out using ELISA (3rd generation) [Abbott laboratories, Chicago, IL USA] (Vrielink et al., 1997). Serum TNF-alpha levels were measured using ELISA Kit [ASSAYPRO, Assay Max Human Tumor Necrosis Factor alpha] (Corti et al., 1992) following the procedure recommended by the manufacturers (Bender MedSystem, Vienna, Austria). Detection limit was 2.3 pg/mL for TNF-α.

Estimation of SOD activity by ELISA

SOD catalyzes the dismutation of the superoxide radical (O_2-) into H_2O_2 and elemental O_2, and as such provides an important defense against the toxicity of superoxide radicals. Activity of SOD was measured according to the instructions from the ELISA reader's supplier. In this assay, O_2^- that were generated from the conversion of xanthine to uric acid and H_2O_2 by xanthine oxidase (XOD) convert WST-1 to WST-1 formazan. SODs in turn reduce superoxide ion concentrations and thereby lower the rate of WST-1 formazan formation. The extent of reduction in the appearance of WST-1 formazan is a measure of SOD activity in the experimental

Table 1. Different liver function in the examined groups.

Parameter	Healthy subjects	Bilharzial HCV	Non Bilharzial HCV
AST (U/L)	27.85±7.47	64.5±46.77*	80.76±62.7*
ALT (U/L)	29.5±8.05	56.53±28.8*§	92.4±10.42*
ALP (U/L)	149.4±28.97	229.9±70.71*	241.96±123.18*
Bilirubin (mg/dl)	0.632±0.201	2.25±0.03	1.188±0.36§
Albumin (g/dl)	4.14±0.344	3.95±0.56	4.01±0.41
Total protein (g/dl)	7.08±0.211	6.94±0.5	6.94±0.43

Data are presented as mean ± SD, *, p <0.05 vs. control, §, p <0.05 vs. non bilharzial HCV.

Table 2. Mean level of SOD and TNF-α in the three examined groups.

Parameter	Healthy subjects	Bilharzial HCV	Non Bilharzial HCV
SOD(U/ml)	403.9±38.4	204.1±51.35*	229.6±42.4*
TNF-α (pg/ml)	1.19±0.72	12.05±12.32*	6.15±7.15*

Data are presented as mean ± SD, *, p <0.05 vs. control.

sample. The optical density was measured at 450 nm on an ELISA reader (Winterbourne et al., 1975). Serodiagnosis of schistosomiasis was done by indirect haemagglutination [Schistosomiasis Fumouze] (Doenhoff et al., 1981).

Quantitative estimation of viral load

Quantitative estimation of HCV RNA was performed according to the instructions from the supplier, and amplification and detection of the samples as well as the controls were done using the COBAS Amplicor Analyzer (Roche Diagnostics, Mannheim, Germany).

Liver biopsy

For patients who gave informed consent and who had pyruvate carboxylase activity above 60% (16 patients), ultrasound-guided liver biopsy was done by true-cut needle followed by histopathological examination of the specimen using haematoxylin and eosin, orcein stain, Pert's stain and periodic acid Schiff stain, and examined for staging and grading, according to the methods of Ishak et al. (1995).

Statistical analysis

All analysis were done using the Statistical Package for the Social Science (SPSS software version 16, Chicago, Illinois) on a personal computer. Data were presented as mean ± SD. Qualitative variables were assessed by Chi-square test. Non parametric measures were compared by Kruskal-Wallis one way analysis of variance with Post Hoc analysis using Mann-Whitney U test. Significant levels were P > 0.05 and P<0.00l highly significant. Correlation of variables was tested by the Pearson test. Receiver operating characteristic (ROC) curves were used to determine the threshold value for optimal sensitivity and specificity (Ishak et al., 1995). And cut-off values were calculated using the standard equations according to Sox et al. (1989).

RESULTS

The present work was carried out on 30 patients with bilharzial HCV, 30 patients non- bilharzial HCV and 20 healthy persons served as control group. The age of subjects ranged from 30 to 65 years in normal healthy control group, 27 to 65 years in bilharzial HCV group and 25 to 61 years in non-bilharzial HCV group. The mean age was 45.35 ± 10.6; 46.3 ± 8.86 and 48.0 ± 9.47 years in healthy subjects, non- bilharzial HCV group and bilharzial HCV group, respectively. The mean levels of the different liver enzymes AST, ALT and ALP were significantly higher in the HCV groups as compared to the control group (p <0.05). While serum bilirubin, albumin and total protein showed no statistical significant difference (p > 0.05) as shown in Table 1.

Among HCV groups (Table 2) TNF-α was significantly higher when compared with the control group (p <0.001), while SOD was significantly decreased (p < 0.001). However, no statistical significant difference was found on comparing both bilharzial and non-bilharzial HCV groups (Figure 1 and Tables 3 to 5).

The best cut off value of TNF-α was >2.3 pg/ml (area under the curve 0.881). Applying this cut off value, the overall sensitivity of TNF-α was 75%, and its specificity was 95%. The best cut off value of SOD was ≤296 U/ml (area under the curve 0.920). Applying this cut off value, the overall sensitivity of SOD was 80%, and its specificity was 100% as shown in Figure 2.

The overall sensitivity and specificity of TNF-α at cut off >2.3 pg/ml were 80 and 100%, respectively. The sensitivity and specificity of SOD at cut off at ≤296 U/ml were 75 and 95%, respectively. Furthermore, PPV, NPV and accuracy for TNF-α were 100, 62.5 and 77.9%, respectively; while for SOD it was 97.8, 55.9 and 90.8%, respectively (Table 6).

The activities of SOD did not correlate with the HCV-RNA levels. The mean level of SOD was 0.144 ± 0.006 in the HCV group as compared to their respective control

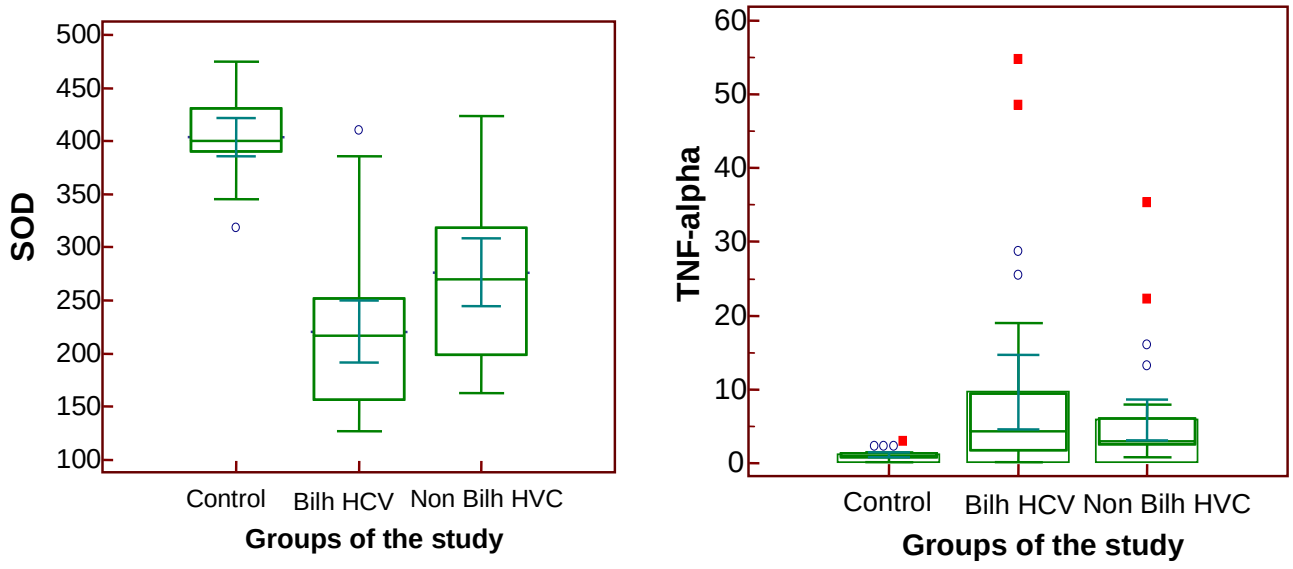

Figure 1. Multiple comparison graphs between the mean of SOD and TNF-α in different groups of the study.

Table 3. TNF-α concentration in the different studied groups.

Group	TNF-α (pg/ml)	
[a]Normal control (n=20)	Median	0.98
	Range	0.2 – 3
	Mean rank	17.62
[b]Non-bilharzial HCV (n=30)	Median	3
	Range	0.8 – 35.26
	Mean rank	48.65
[c]Bilharzial HCV (n=30)	Median	4.3
	Range	0.2 – 54.7
	Mean rank	47.60
Statistics	chi-square = 25.8709, P< 0.0001	

P value significant at < 0.05. Non-parametric Kruskal-Wallis test; [a]Statistically significant difference as compared to non-bilharzial HCV group by Mann-Whitney test, Z = 5.090, *P*< 0.0001; [b], Statistically significant difference as compared to bilharzial HCV group by Mann-Whitney test, Z = 0.651, *P* = 0.515; [c], Statistically significant difference as compared to the control group by Mann-Whitney test, Z = 3.971, *P* = 0.0001.

Table 4. Positivity rate of TNF-α in the different studied groups.

Group	No. of cases > 2.3 pg/ml (%)	No. of cases ≤2.3 pg/ml (%)
Normal control (n=20)	1 (5%)	19(95%)
Non-bilharzial HCV (n=30)	21 (70%)	9(30%)
Bilharzial HCV (n=30)	24 (80%)	6 (20%)
Statistics	X^2 = 30.691, P = 0.000	

Non-parametric chi-square test.

Table 5. SOD concentration in the different studied groups.

Group		SOD (U/ml)
[a]Normal control (n=20)	Median	400
	Range	318 – 475
	Mean rank	65.70
[b]Non-bilharzial HCV (n=30)	Median	270
	Range	163 – 424
	Mean rank	25.23
[c]Bilharzial HCV (n=30)	Median	216
	Range	127 – 410
	Mean rank	38.97
Statistics	chi-square = 36.5990, P < 0.0001	

P value significant at <0.05. Non-parametric Kruskal-Wallis test; a, Statistically significant difference as compared to non-bilharzial HCV group by Mann-Whitney test, Z = 4.487, *P* < 0.0001; [b], Statistically significant difference as compared to bilharzial HCV group by Mann-Whitney test, Z = 2.669, *P* = 0.0076; [c], Statistically significant difference as compared to the control group by Mann-Whitney test, Z = 5.496, *P*< 0.0001.

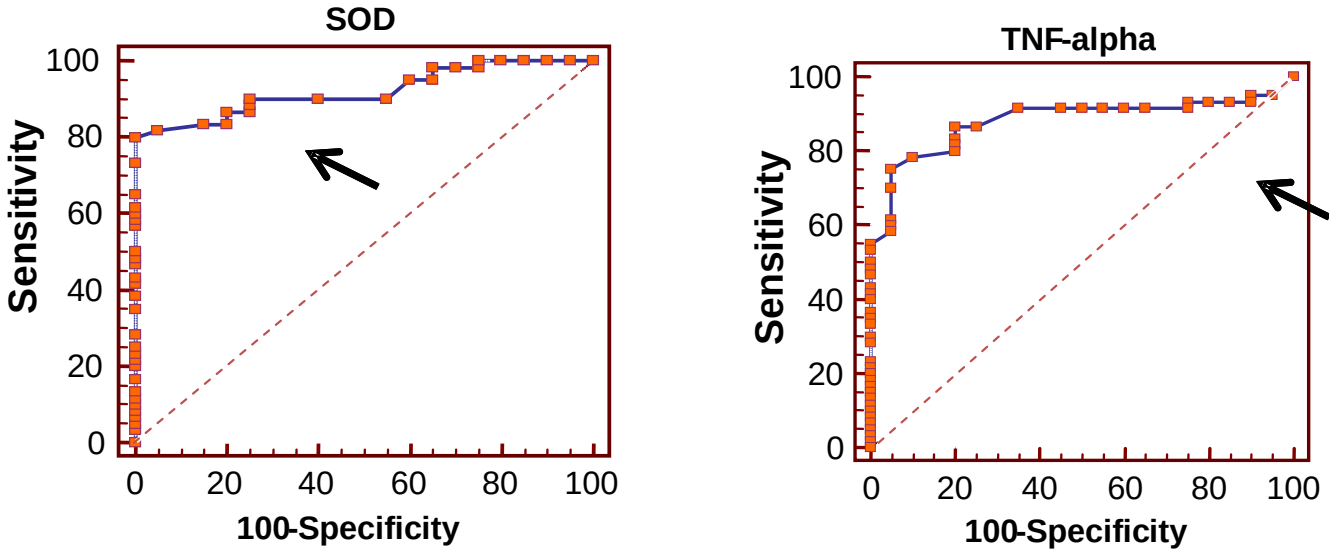

Figure 2. ROC curve analysis for SOD and TNF-α.

Table 6. The overall sensitivity, specificity, PPV, NPV and accuracy of TNF-α and SOD when tested independently (bilharzial and non bilharzial HCV versus healthy control).

Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
TNF-α	80	100	100	62.5	77.9
SOD	75	95	97.8	55.9	90.8

(P>0.05, data not shown).

DISCUSSION

The present work was designed to determine the diagnostic accuracy of biomarkers like: TNF-α and Mn-SOD and correlate levels of biological serum markers like: AST, ALT, AP, T. Bil, TP and Alb), in the detection of the prognosis and severity of inflammation of liver due to HCV mono-infection or when co-infected with *Schistosoma mansoni*. In addition, we attempted to establish associations between the investigated parameters. In this present study, the mean age ± SD of

the different studied groups was 45.35 ± 10.6 in healthy normal, 46.3 ± 8.86 in non-bilharzial HCV group and 48.0 ± 9.47 in bilharzial HCV group (ranged from 30 to 65, 25 to 61 and 27 to 65 years, respectively). Age at infection has been shown to influence the outcome of hepatitis C and patients infected after the fourth decade has a higher risk of disease progression (El-Zanaty and Way, 2008). Conventional liver function tests reflect hepatocytes damage as ALT and AST, whereas biliary obstructions can be studied by bilirubin and ALP and biosynthetic function by albumin and total protein. Although, these tests provide important information on some aspects of liver function, they do not assess the activity of the fibrogenic process, but can indicate the stage of fibrosis (Gressner et al., 2007; Rossi et al., 2007). All these factors either reflect liver function and/or severity of inflammation of liver in the presence of HCV infection only or co-infected with schistosomal parasite. In comparing the HCV groups with the control group, the present work revealed a statistically significant difference in the levels of AST, ALT and ALP in HCV groups ($p < 0.01$), while no such significant difference was shown with serum bilirubin, albumin and total protein. Elevated levels of fibrosis serum markers; AST and ALP, in HCV patients, both bilharzial and non bilharzial as compared to normal control, suggests that these markers could differentiate co-infected from the mono-infected patients (Shahzad, 2009). In comparing the mean levels of different biochemical tests in both HCV groups, it was shown that the levels of ALT and bilirubin were significantly higher in the bilharzial-HCV group ($p < 0.05$). While serum AST, alkaline phosphatase, albumin and total protein showed no statistically significant difference. Higher levels of ALT and total bilirubin in HCV patients with elevated alpha-fetoprotein (AFP) associated with severity of inflammation and advanced fibrosis was also shown by Tai et al. (2009). Tumor necrosis factor-alpha (TNF-α) is a cytokine that may act as an endogenous tumor promoter and is raised 5 to 10 folds during chronic inflammatory liver disease. Liver injury is reportedly associated with a chronic inflammatory response involving TNF-α and interleukin-1b (IL-1b). The former plays a central role in liver injury, triggering the production of other cytokines that in turn recruit inflammatory cells, promote fibrogenesis and further activate oxidative burst (Ramadori and Armbrust, 2001). The data obtained from our study revealed that TNF-α was elevated in the HCV groups when compared with the control group with mean levels (1.19 ± 0.72, 12.05 ± 12.32 and 6.15 ± 7.15) and $P < 0.001$, in normal, bilharzial and non-bilharzial groups, respectively. Most of the reported studies (El-Shahat and Kadry, 2009) revealed that elevated levels of TNF-α in serum have been found in patients with hepatitis C, and correlate with the degree of inflammation. A statistically significant difference was observed by comparing the levels of TNF-α within the three studied groups (chi-square = 25.8709, $P < 0.001$). Hepatitis C infection is associated with increased transcriptional expression of the TNF-α gene in the liver with high serum levels of TNF-α (Larrea et al., 1996). Activation of TNF-α has a pivotal role in the inflammatory process of chronic hepatitis C, and TNF-α levels correlate with the degree of inflammation (Stephen et al., 2001). In the current study, there was a statistically significant difference in the positivity rates of TNF-α. In the three studied groups, the positivity rates of TNF-α in the voided samples of the control, non-bilharzial HCV and bilharzial HCV groups were 1 (5%), 21 (70%) and 24 (80%), respectively (chi-square = 30.691, $P < 0.001$). These results were in concordance with an Egyptian study by Farag et al. (2011). Using ROC curve, the best cut off value of TNF-α was 2.3 pg/ml with the overall sensitivity of 75%, and specificity of 95%. Patients with mild liver inflammation have elevated serum TNF-α levels, suggesting that this cytokine could be used as a sensitive predictor of liver inflammation (Neuman et al., 2002). Patients with chronic hepatitis C often exhibit increased production of TNF-α, a cytokine that can produce oxidative stress by stimulating the release of reactive oxygen species (ROS), such as superoxide radical and hydrogen peroxide (Keigo et al., 2006). Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals, are produced during aerobic metabolism. Thus, oxidative stress has emerged as a key player in the development and the progression of many pathological conditions, including HCV-induced pathogenesis of liver. Our work revealed a statistically significant decrease in the mean levels of SOD in the HCV groups as compared to the control group ($P < 0.001$). Levels of SOD were significantly decreased in chronic hepatitis C patients (Gorenek et al., 2006). In this study, when comparing both bilharzial and non-bilharzial HCV groups, no statistically significant differences were observed in the level of SOD. HCV core expression does not show a major change in SOD levels, suggesting a non-significant impact of core on oxidative stress in liver diseases (Wang-Sheng et al., 2000). The activities of SOD did not correlate with the HCV-RNA levels (Simula and De Re, 2011). Using ROC curve, the best cut off value of SOD was ≤296 U/ml with the overall sensitivity of SOD 80%, and its specificity 100%. Oxidant stress, as reflected in blood by a wide range of pro- and anti-oxidant markers, is a significant feature of hepatitis C infection (Jain et al., 2002). In the present study, the positivity rates of SOD in the voided samples of the control, non-bilharzial HCV and bilharzial HCV groups were 0 (0%), 22 (73.3%) and 26 (86.7%), respectively (chi-square = 36.5990, $P < 0.0001$). These findings are in agreement with that of Farinati et al. (2007). Our study demonstrated that SOD decreased, correlating with severity of inflammatory process in adults with chronic active hepatitis. Also, it was found that SOD activity was significantly low in chronic active hepatitis C (CHC) patients. On the contrary, some author has detected high

SOD levels in CHC patients. The reduction in the amount of SOD reflects both a decrease in the synthesize capacity of liver, and the antioxidant defense power of the patients with chronic HCV (Irshad et al., 2002).

It can be argued that decrease in the antioxidant levels may be an early marker of the oxidative stress. Lipid peroxides formed can be chemotactic for the neutrophils causing increased inflammation, which further drives oxidant-mediated injury in the liver (Dikici et al., 2005).

In the viral hepatitis, virus also infects the peripheral lymphocytes. The infected lymphocytes produce interferon to stimulate healthy cells against viruses. The pathogenetic mechanisms through which HCV causes cell damage remain obscure, although it has been suggested that the oxidative stress may play a pathogenetic role in this infection. The patients with chronic hepatitis C exhibit an increased production of TNF-α, a cytokine that can produce oxidative stress by stimulating the generation of oxygen ROS (Levent et al., 2006).

PPV, NPV and accuracy for SOD were 100, 62.5 and 85%, respectively. In comparing TNF-α and SOD levels in chronic HCV patients, we found that overall sensitivity and specificity of SOD (80 and 100%, respectively) were better than those of TNF-α (75 and 95%). Also, PPV, NPV and accuracy for SOD (100, 62.5 and 85%) were better than those of TNF-α which were 97.8, 55.9 and 81.3%, respectively. In conclusion, our study revealed that the production of TNF-α in patients with HCV infection was accompanied by a reduction in the activity of antioxidant enzyme, superoxide dismutase (SOD) when compared with healthy control group. Therefore, oxidative stress could play a role in the pathogenesis of HCV infection.

REFERENCES

Belfield A, Goldberg DM, (1971). Colorimetric determination of alkaline phosphatase activity. Enzyme 12:561-568.

Boya P, Pena A, Beloqui O, Larrea E, Conchillo M, Castelruiz Y, Civeira M P, Prieto J (1999). Antioxidant status and glutathione metabolism in peripheral blood mononuclear cells from patients with chronic hepatitis Clin. J. Hepatol. 31:808-814.

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

Corti A, Fassino J, Marcucci F, Barbanti E, Cassani G (1992). Oligometric tumor necrosis factor-α slowly converts into the reactive forms at bioactive levels. Biochem J. 284:905-910.

Dikici I, Mehmetoglu I, Dikici N, Bitirgen M, Kurban S (2005). Investigation of oxidative stress and some antioxidants in patients with acute and chronic viral hepatitis B and the effect of interferon-α treatment. Clin Biochem. 33(12):1141-1144.

Doenhoff MJ, Pearson S, Dunne DW, Bickle Q, Lucas S, Bain J (1981). Immunological control of hepatotoxicity and parasite egg excretion in Schistosoma mansoni infections. Trans R Soc. Trop. Med. Hyg. 75:41-53.

El-Shahat ME, Kadry A (2009). Cellular immune response to infection by different genotypes of hepatitis C virus. Indian J. Clin. Biochem. 24(3):234-240

El-Zanaty F, Way A (2008). Egypt Demographic and Health Survey, Cairo, Egypt: Ministry of Health and Population.

Esteve E, Botas P, Delgado E (2007). Soluble TNF-α receptor 2 produced by alternative splicing is paradoxically associated with markers of liver injury. Clin. Immunol. 123:89-94.

Farag NH, Rashed H, Hassan M, Darweesh A, Shehata M, Hassanein T, Mills PJ (2011). Hepatitis C infection, Cognition, and inflammation in an Egyptian sample. J. Med. Virol. 83(2):261-266.

Farinati F, Cardin R, Bortolami M (2007). Hepatitis C virus: from oxygen free radicals to hepatocellular carcinoma. J. Viral Hepat. 14:821-829.

Farinati F, Cardin R, Bortolami M, Guido M, Rugge M (2006). Oxidative damage, pro-inflammatory cytokines, TGF-α and c-myc in chronic HCV-related hepatitis and cirrhosis . World J Gastroenterol. 12(13):2065-2069.

Gorenek L, Ali A, Ahmet A, Polat EC, Aytac C, 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. Trans. Med. 4:25.

Gornall AC, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 17:751-766.

Gressner OA, Weiskirchen R, Gressner AM (2007). Biomarkers of hepatic fibrosis, fibrogenesis and genetic pre-disposition pending between fiction and reality. J Cell Mol. Med. (11):1031-1051.

Irshad M, Chaudhuri, Joshi Y K (2002). Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. Hepatol. Res. 23:178-184.

Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN (1995). Histological grading and staging of chronic hepatitis. J. Hepatol. 22:696-699.

Jain SK, Pemberton PW, Smith A, McMahon RF, Burrows PC, Aboutwerat, A, Warnes T W (2002). Oxidative stress in chronic hepatitis C: not just a feature of late stage disease. J Hepatol. 36:805-811.

Kamal SM, Madwar MA, Bianchi L, Tawil AE, Fawzi R, Peters T, Rasenack JW (2000) Clinical, virological and histopathological features: Long term follow up in patients with chronic hepatitis C co-infected with *Schistosoma mansonii*. Liver 20:281-289.

Keigo M, Kevin TH, Cheng CK, King-Song J, Vicky MH, Michael MC (2006). Hepatitis C Virus triggers mitochondrial permeability transition with production of reactive oxygen species, leading to DNA damage and STAT3 activation. J. Virol. 80(14):7199-7207.

Larrea E, Garcia N, Qian C, Civeira MP, Prieto J (1996). Tumor necrosis factor alpha gene expression and the response to interferon in chronic hepatitis C. Hepatology 23:210-217.

Levent G, Ali A, Ahmet A, Polat EC, Aytaç C, Ays 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. Trans. Med. 4(1):25-31.

Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T Kimura S, and Koike K (2001). Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res. 61:4365-4370.

Neuman MG, Benhamou JP, Bourliere M (2002). Serum TNF-α & TGF-B1 levels in chronic hepatitis C patients are immunomodulated by therapy. Cytokine 17:108-117.

Packer L, (2002). Methods in Enzymology, 2nd Ed. Volume 349. San Diego, California Academic Press, p. 121.

Ramadori G, Armbrust T (2001). Cytokines in the liver. Eur. J. Gastroenterol. Hepatol. 13:777-784.

Reitman S, Frankel S, (1957). Colorimetric determination of serum GOT (glutamic oxaloacetic transaminase) GPT (glutamic pyruvic transaminase) activity. Am. J. Clin. Pathol. 28:56.

Rossi E, Adams LA, Bulsara M, Jeffrey GP (2007). Assessing liver fibrosis with serum marker models. Clin. Biochem. Rev. 28:3-10.

Shahzad A (2009). Non- invasive evaluation of liver fibrosis in patients with chronic hepatitis B and C. J. Egyptian Soc. Parasitol. 39(1):183-190.

Simula M, De Re V (2011). Hepatitis C virus-induced oxidative stress and mitochondrial dysfunction . A focus on recent advances in proteomic. 83(2):261-266.

Sox HC, Blatt MA, Higgins MC and Marton KI (1989). Medical decision making London, Butterworth. pp. 67-146.

Stephen J, Polyak1, Khalid SA, Khabar MR, David RG (2001). Elevated

levels of Interleukin-8 in serum are associated with hepatitis C virus infection and resistance to interferon therapy. J. Virol. 75(13):6209-6211.
Tai WC, Hu TH, Wang JH, Hung, Chao-Hung, Lu SN, Changchien Chi-Shin, Lee CM (2009). Clinical implications of alpha fetoprotein in chronic hepatitis C. J. Formos. Med. Assoc. 108(3):210.
Vrielink H, Reesink HW, Zaaijer HL, Scholten E Kremer LC (1997). Look back of anti-HCV ELISA-positive, HCV-RNA PCR-negative donors and recipients of their blood products. Biol. Sci. 72(2):67-70.
Wang-Sheng K, Chih-Hung G, Maw-Sheng Y, Li-Yun L, Guoo-Shyng W, Hsu Pei-Chung C, Mei-Ching L, Chia-Yeh L (2000). Blood micronutrient, oxidative stress, and viral load in patients with chronic hepatitis C . World J. Gastroenterol. 11(30):4697-4702.
Winterbourne CC, Hawkines RE, Brain M, Carell RW, (1975). The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med. 85:337-341.