The association of Manganese superoxide dismutase gene polymorphism with the prognosis of Hepatitis C Virus related hepatocellular carcinoma patients

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ABSTRACT

Hepatitis C virus (HCV) is a hepatotropic RNA virus that causes acute and chronic hepatitis in humans with a high propensity for chronicity. If untreated, chronic hepatitis C can progress to cirrhosis and hepatocellular carcinoma (HCC) in a subset of patients. HCC is a prevalent disease in many populations worldwide. It causes many financial problems in treatment modalities with high mortality rates. In developing countries, the major concern in HCC frequently belongs to HCV long lasting infection. Chronic HCV infection mostly leads to hepatic cirrhosis before developing HCC. HCV infection induces overproduction of reactive oxygen species (ROS) in hepatocytes leading to their proliferation and triggers HCC. Manganese superoxide dismutase (MnSOD) is a nuclear-encoded antioxidant enzyme that neutralizes free radicals. Val16Ala single nucleotide polymorphism (SNP) is one of the most widely investigated SNPs in the MnSOD gene, where C to T transition resulting in the conversion of valine to alanine at amino acid 16 within the mitochondrial signaling sequence. This study was designed to investigate the association of MnSOD gene SNP (Val16Ala) with HCV and HCV-related HCC to elucidate its possible role in pathogenesis of HCC. The study included sixty individuals; they were classified into three groups; 20 patients with hepatitis C virus infection, 20 patients with hepatitis C virus-related HCC and 20 unrelated healthy volunteers who served as controls. Qualitative determination of Hepatitis C Virus antibody and quantitative determination of RNA-HCV by RT-PCR were done. MnSOD activity was evaluated using a superoxide dismutase assay kit. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method. The homozygous Ala/Ala (CC) was significantly more frequent in HCV-related HCC patients compared to HCV-infected patients and control group (67.5%, 12%, and 15% respectively, $p < 0.05$). Moreover, the C allele was more often associated with HCV-related HCC patients compared to HCV-infected patients and control group (50%, 10%, and 15% respectively, $p < 0.05$). MnSOD activity was significantly higher in HCV-related HCC patients and HCV-infected patients compared to control group (185.6± 49.3, 142.6± 27.3 and 66.25± 17.03 respectively, $p < 0.05$). In addition, MnSOD activity was significantly higher with CC and TC genotypes than TT genotypes in all HCV-infected patients with/without HCC ($p < 0.05$). There is increased susceptibility to HCC among HCV-infected patients with MnSOD gene SNP (Val16Ala). Serum MnSOD level was increased as HCV-related chronic liver disease progressed, especially among patients with HCC.

Keywords: Hepatitis C virus (HCV); Hepatocellular carcinoma (HCC); Reactive oxygen species (ROS); Manganese superoxide dismutase (MnSOD); Real time-polymerase chain reaction (RT-PCR); Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP); mitochondrial-targeting sequence (MTS); and single nucleotide polymorphism (SNP).

INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic RNA virus; it causes acute and chronic hepatitis (Alter & Seeff, 2000). HCV is an enveloped, positive sense, single stranded RNA virus from the family Flaviviridae and belongs to genus Hepacivirus (Robertson *et al.,* 1998). The majority of HCV isolates can be classified into six major groups designated genotypes 1-6, with subdivisions in each (subtypes a, b, c, etc.). In Egypt, a high homology of HCV subtypes, 90% of which is accounted for by genotype 4a, strongly suggests a local epidemic of HCV throughout the general population (Yasuhito *et al.,* 2004). HCV spreads primarily by blood-to-blood contact associated with intravenous drug abuse, poorly sterilized medical equipment, and transfusions. About 150–200 million people worldwide are infected with hepatitis C (Gravitz, 2011; WHO, 2011; Hanafiah *et al.,* 2013). Egypt has possibly the highest HCV prevalence worldwide (Mohamoud *et al.,* 2013), estimated among the general population to be around 14% (El-Zanaty & Way, 2009). If untreated, chronic hepatitis C can progress to cirrhosis and hepatocellular carcinoma (HCC) in a subset of patients (Alter & Seeff, 2000).

HCC is one of the most frequent visceral neoplasms world-wide (Parkin *et al.,* 2001), with an estimated 748,000 new cases and almost as many deaths (Jemal *et al.,* 2011). According to the number of new cases of cancer in humans each year, HCC is ranked as the sixth most common cancer over the world, the fifth in males and the seventh in females (Ferlay *et al.,* 2010). In Egypt, HCC is the second most common cancer in men and the sixth most common cancers in women (Globocan, 2008).

Although chronic HCV infection is one major risk factor for HCC, the mechanisms by which HCV induces HCC remain uncertain (Levrero, 2006; Givern and Lemon, 2009). Some researchers concluded that in chronic hepatitis, immunity triggers the production of reactive oxygen species (ROS) (Muriel, 2009) and nitric oxide (NO) (García-Monzón *et al.,* 2000). Oxidative stress is initiated by inflammatory progressions that occur in hepatitis due to immunological mechanisms. In addition, in hepatitis C, viral proteins play a role in the induction of oxidative stress (Bureau *et al.,* 2001).

To guard the cells of the body against ROS, humans have a highly sophisticated antioxidant protection system. It involves a variety of endogenous and exogenous components, such as superoxide dismutase (SODs), glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions (Jacob, 1995).

SODs are enzymes that function to catalytically convert O^2 to oxygen (O_2) and hydrogen peroxide (H_2O_2) (Bertini & Magnani, 1998). Based on the metal cofactor they harbor, SODs are classified into four groups: iron SOD (Fe-SOD), manganese SOD (MnSOD), copper-zinc SOD (CuZnSOD), and nickel SOD (NiSOD). There are three forms of SOD in human tissue; constitutive cytoplasmic (CuZnSOD), inducible mitochondrial (MnSOD), and extracellular (NiSOD) (Liu *et al.,* 2000). The MnSOD is encoded by the same gene that is responsible for synthesis of a mitochondrial-targeting sequence (MTS) thus facilitating its import into the mitochondrial matrix (Wispe *et al.,* 1989). After cleavage of MTS, protein assembly takes place forming its active tetramer form that contains one manganese ion per subunit (Matsuda *et al.,* 1990).

The human MnSOD gene is located on chromosome 6q25 (Silva *et al.,* 2006). There are many SNPs this gene (Chung man Ho *et al.,* 2001) and have been related to carcinogenesis in the lung (Izutani *et al.,* 1998) oesophagus

(Toh *et al.,* 2000) colon (Kawaguchi *et al.,* 1990) and liver (Wan *et al.,* 1994).

Val16Ala SNP is one of the most extensively studied SNPs in the MnSOD gene, where C to T transition resulting in the conversion of valine to alanine at amino acid 16 within the mitochondrial signaling sequence (Rosenblum *et al.,* 1996).

This particular polymorphism occurs within the mitochondrial signaling sequence, thus affecting import of MnSOD into mitochondria. The 16Ala variant facilitates MnSOD targeting to the mitochondria. This finding was confirmed by Sutton and colleagues (2003) who noticed that the localization of 16Ala variant to the mitochondria was 30–40% more than the 16Val variant. This difference is related to the ά-helix structure of the 16Ala variant enabling its import into mitochondria and subsequently increasing its mitochondrial concentration and activity. On the other hand, the 16Val variant has a partial β-sheet structure so it is partially stuck inside the narrow inner membrane import pore and is consequently degraded by the proteasome. Moreover, the degradation of mRNA encoding the 16Val variant is faster than the Ala variant (Sutton *et al.,* 2005).

The current study was designed to investigate the association of MnSOD gene SNP (Val16Ala) with HCV and HCV-related HCC to elucidate its possible role in pathogenesis of HCC.

MATERIALS AND METHODS

Patients and controls

A total of 40 HCV-patients were enrolled in the study. They were recruited from the outpatient clinics of Zagazig University Hospital during the period of July 2013 to September 2014. According to their clinical diagnosis, patients were further divided into two subgroups (20 patients each) including patients with hepatitis C virus infection (group 1) and those with hepatitis C virus-related HCC (group 2). Twenty unrelated healthy blood bank donors served as a control group (group 3).

Group 1 and 2 fulfilled the inclusion criteria of chronic HCV infection with no other causes of liver disease (e.g., alcohol intake, drug abuse, HBV infection, Bilharizia infestation and fatty liver). Patients with hepatitis C virus-related HCC (group 2) were diagnosed by physical examination, ultrasonography (US) and alpha fetoprotein (AFP) assay. When these investigations suggested possible HCC, computed tomography (CT) and histopathology of a fine needle guided liver biopsy were done.

Blood sample was withdrawn from every participant under complete aseptic condition. Five ml of blood were collected from each studied individual; 1 ml of whole blood was collected in sterile K. Ethylene Diamine-Tetra Acetic Acid (K. EDTA) (solute form) containing tubes for DNA extraction, and the rest was left for 30-60 minutes for spontaneous clotting at room temperature then centrifuged at 3000 rpm for 10 minutes. Serum samples were separated into another set of tubes and kept frozen at -20 C for Anti-HCV, HCV RNA and MnSOD Activity enzyme.

All studied groups were subjected to the following:

- Full history and clinical examination.
- Routine investigation including: CBC , liver function tests, PT, Bilharizia antibody titre, HBsAg, AFP, Kideny Function Tests, and Random blood suger, as well as CT and US.
- Determination of the qualitative of Hepatitis C Virus antibody and quantitative of HCV RNA for confirmation.

All patients with positive HCV infection confirmed by Anti-HCV 3.0 ELISA (The DIAKEY®, South Korea) for qualitative determination of Hepatitis C Virus antibody and by quantitative RT-

PCR (the Cobas Amplicor HCV monitor test, roche) for quantitative determination of RNA-HCV.

Manganese-SOD activity assay:

Manganese-SOD activity was measured using a superoxide dismutase assay kit **(Biodiagnostic, Egypt)**. According to the manufacturer's protocol, Phosphate Buffer (pH 8.5), Nitroblue tetrazolium (NBT) and NADH were mixed in ratio of (25+2.5+2.5 ml) to form working reagent. 50 µl of serum samples were added to 500 µl of working reagent and well mixed; then 50 µl of Phenazine methosulphate (PMS) were added to the reaction mixture. Absorbance change was read at 560 nm using spectrophotometer **(Jenway, 6505 UV|vis)** over 5 min, following the addition of PMS to the reaction mixture. The activity of MnSOD enzyme (units per millilitre) was estimated by the following formula:
SOD Activity: U|ml = % inhibition x 3.7

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the DNA Extraction mini kit according to the method described by Miller *et al.,* (1988) (iNtRON Biotechnology, Inc. Korea).The DNA concentrations were determined by spectrophotometric absorbance measurements of the extracts. Genotyping of the Val16Ala polymorphism was performed by PCR and restriction fragment length polymorphism analysis. The primers used were 5`-CAG CCC AGC CTG CGT AGA CGG-3` (forward) and 5`-CTT GGC CAA CGC CTC CTG GTA CTT-3`(reverse) (Gene ON, Deutschland, Germany) generating a PCR product of 267 bp (Figure 1,2,3). Polymerase chain reactions (PCRs) were carried out in a total volume of 20 µl, containing 5 µl of genomic DNA, 10µl of Master Mix PCR, 1 mmol/L $MgCl_2$, 2 µl H_2O, and 2 µl of each primer. (Gene ON, Deutschland, Germany). Twenty µl of mineral oils were added on the surface of this mixture to guard against prevent evaporation of the sample during heating. PCR amplifications were performed in a DNA thermal cycler according to the following cycling conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 45 sec, 54°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 5 min (Perkin Elmer-Cetus, USA). The amplified products were digested with restriction endonuclease BsaWI (New England Biolabs, France) at 56°C for a minimum of 2 h, according to the manufacturer's recommendation. The amplified products will be cleaved by the enzyme if a thymine base is present at position 47 nucleotide of the MTS of MnSOD gene producing two fragments of 183- and 84-base-pairs. The digestion products were visualized on 2.5% agarose gel. The pattern of the digestion products migration the gel allowed identification of the Val/Val (TT), Val/Ala (TC) and Ala/Ala (CC) genotypes.

Statistical analysis

Data were entered checked and analysed using Statistical Package for Sciences and Society (SPSS 12.0) (SPSS Inc., Chicago, IL, USA). Data are presented as tables and graphs, Quantities data were expressed as means ±SD and qualitative data were expressed as numbers and percentages. The statistical significance of difference was considered when $p < 0.05$.

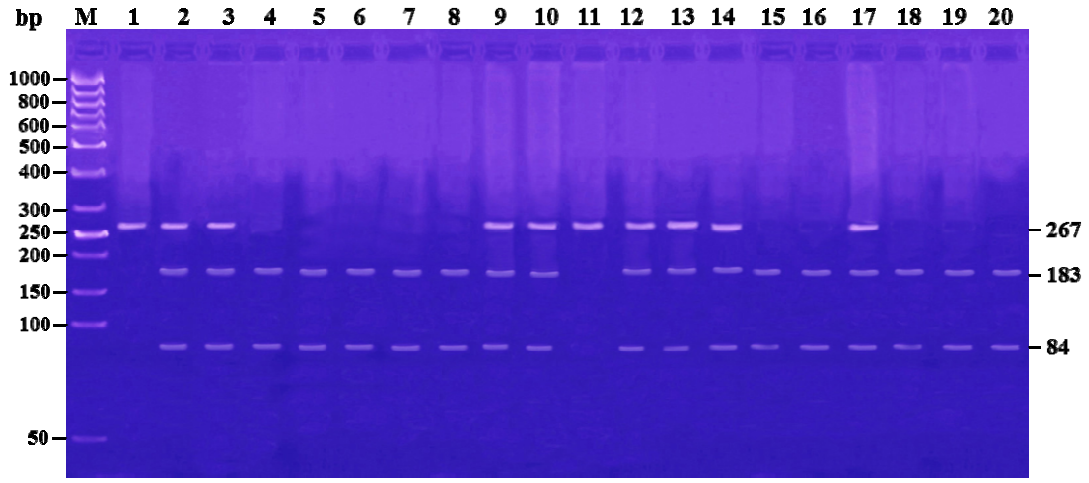

Figure (1): PCR- based RFLP assay. Representative agarose gel electrophoresis, findings of Polymorphism of MnSOD Ala 16Val gene in HCV group.

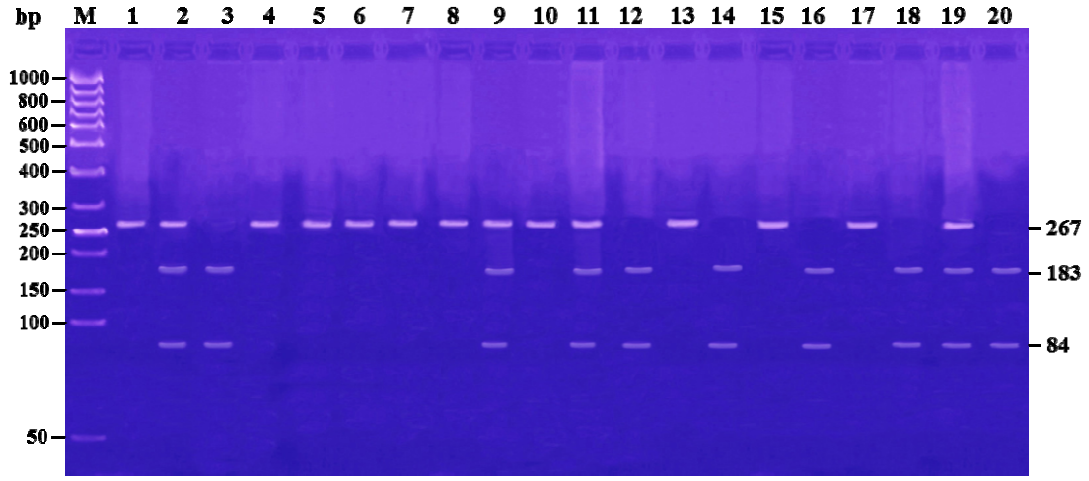

Figure (2): PCR- based RFLP assay. Representative agarose gel electrophoresis, findings of Polymorphism of MnSOD Ala 16Val gene in HCC group.

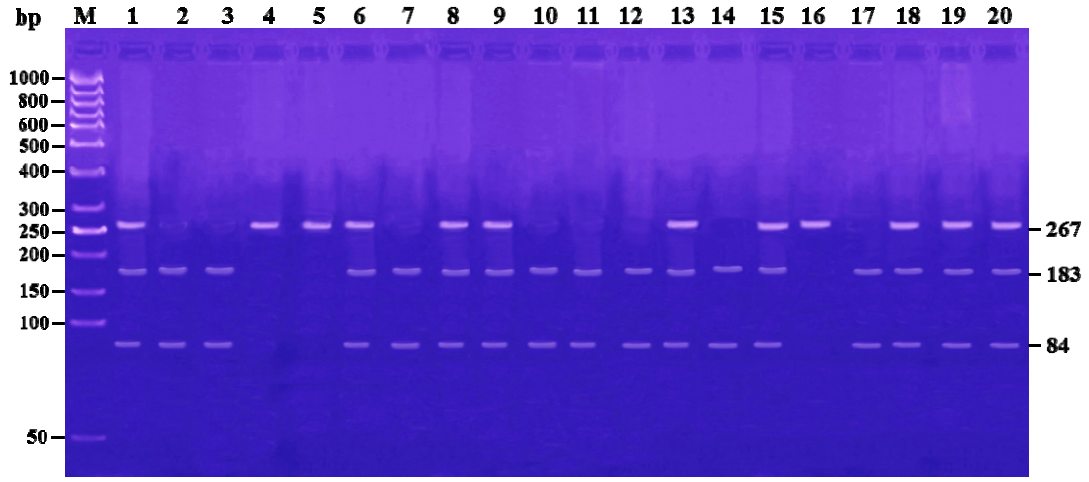

Figure (3): PCR- based RFLP assay. Representative agarose gel electrophoresis, findings of Polymorphism of MnSOD Val16Ala gene in control group.

Figure 1, 2, 3. PCR/RFLP analysis of Val16Ala SNP of MnSOD gene revealed 50-bp low ladder DNA marker and three different patterns: the Val/Val homozygote showing a 183-bp fragment and a 84-bp fragment, the Ala/Ala homozygote presenting a 267-bp fragment, and the Val/Ala heterozygote characterized by fragments of 267 bp, 183 bp and 84 bp, respectively.

RESULT

Study population characteristics:

Characteristics of the study population are summarized in Table (1). The three studied groups were matched for age and gender. No significant differences between the studied groups concerning age and gender which indicate an adequate matching between the groups as regards age and gender ($P > 0.005$).The mean age was 50.65 ± 7.28, 54.8 ± 9.5 and 52.47±7.97 years (group1, 2 and 3 respectively).

Table (1): Characteristics of the study population

Characteristic	Group 1 HCV	Group2 HCC	Group 3 Control	P_v
Age				
Mean ± Sd years	50.65 ± 7.28	54.8 ± 9.5	52.47 ± 7.97	0.293
Range	38 – 63	43 - 81	38 - 70	
Gender				
Female	8 (40%)	10 (50%)	10 (50%)	0.765
Male	12 (60%)	10 (50%)	10 (50%)	

MnSOD genotypes and alleles frequencies in the study population:

The genotype distribution of the three groups is shown in Table (2). There was a significant difference between the studied groups concerning genotyping frequency for polymorphism of MnSOD Ala16Val gene ($P < 0.05$). There was a no significant difference between HCV group and control group ($P > 0.05$). There was a significant difference between HCC group and control group ($P < 0.05$) in one hand and between HCC group and HCV group in the other hand ($P < 0.05$).

The allele frequencies of the three studied groups are shown in Table (3). There were significant differences between the studied groups concerning allele frequency for polymorphism of MnSOD Ala16Val gene ($P < 0.05$). There was a no significant difference between HCV group and control group ($P > 0.05$), but There was a significant difference between HCC group and control group ($P < 0.05$) in one hand and between HCC group and HCV group in the other hand ($P < 0.05$).

Table (2): Genotypic frequencies of MnSODVal16Ala polymorphism in all studied groups.

	Group 1 HCV		Group 2 HCC		Group 3 Control		P_{v2}	P_{v3}	P_{v4}
Genotypes	**No**	**%**	**No**	**%**	**No**	**%**			
TT	**10**	**50**	**3**	**15**	**8**	**40**			
TC	**8**	**40**	**7**	**35**	**9**	**45**	**0.04**	**0.79**	**0.01**
CC	**2**	**10**	**10**	**50**	**3**	**15**			
	X^2=11.564				**P1= 0.021**				

P1: HCC, HCV and Control; P2: Control and HCC; P3: Control and HCV; P4: HCC and HCV.

Table (3): Allelic frequencies of MnSODVal16Ala polymorphism of in all studied groups:

	Group 1 HCV		Group 2 HCC		Group 3 Control		Pv1	Pv2	Pv3
	No	**%**	**No**	**%**	**No**	**%**			
T	**28**	**70**	**13**	**32.5**	**25**	**62.5**	**0.47**	**0.007**	**0.000**
C	**12**	**30**	**27**	**67.5**	**15**	**37.5**			
	X^2=12.73			**Pv = 0.02**					

P1: HCV versus Control, P2: HCC versus Control, and P3: HCC versus HCV.

MnSOD Enzyme Activity

As shown in Table (4), simple analysis of variance revealed a highly significant difference of the mean values of serum MnSOD level between all studied groups ($F = 62.9$, $P < 0.05$). There was a highly significant increase of MnSOD levels among HCC group and HCV group when each was compared with control group ($P < 0.05$), whereas there was a no significant difference between HCV group and HCC group ($P > 0.05$).

Also, there was a highly significant increase in the serum level MnSOD enzyme in homozygous CC and heterozygous TC than in homozygous TT in HCC group ($P < 0.05$), and there was a slightly significant increase in the serum level MnSOD enzyme in homozygous CC and heterozygous TC than in homozygous TT in HCV group ($P < 0.05$). There was no significant increase in the serum level MnSOD enzyme in homozygous CC and heterozygous TC than in homozygous TT in control group ($P < 0.05$), also there was a highly significant increase in its activity in HCC patients with CC and TC genotypes than in those with TT one ($P < 0.05$). In contrast, there was a non-significant increase in its activity among HCV group and healthy control group ($p > 0.05$). These findings are presented in Table (5).

Table (4): MnSOD Enzyme Activity

MnSOD	Group 1 HCV	Group 2 HCC	Group 3 Control	P1	P2	P3
Mean ± SD	142.6± 27.3	185.6± 49.3	66.25± 17.03	0.000	0.000	0.1
Range	88.3 – 191.7	84.9 - 271.7	42.26 – 103			
P = < 0.001						

P1: Control and HCV; P2: Control and HCC; P3: HCC and HCV.

Table (5): Serum MnSOD (U/ml) and different genotypes in all studied groups:

	TT	TC	CC	TC + CC	F	P1	T	P2
Control	**8 cases**	**9 cases**	**3 cases**	**12 cases**				
Mean ± SD	**74.5 ± 22.7**	**57.8 ± 8.3**	**69.4 ± 8.3**	**60.7 ± 9.5**	**2.4**	**0.12**	**1.9**	**0.07**
Range	**42.3 – 103**	**48.3 – 70.8**	**62.3 – 78.5**	**84.9 - 121.3**				
HCV	**10 cases**	**8 cases**	**2 cases**	**10 cases**				
Mean ± SD	**130.3 ± 25.9**	**148.7 ± 21.6**	**179.2 ± 17.7**	**154.8 ± 23.7**	**3.9**	**0.03**	**2.2**	**0.04**
Range	**88.3 – 168.3**	**128.3 – 183.3**	**166.7 – 191.7**	**128.3 – 191.7**				
HCC	**3 cases**	**7 cases**	**10 cases**	**17 cases**				
Mean ± SD	**102.3 ± 18.2**	**168.7 ± 8.7**	**222.5 ± 30.7**	**200.3 ± 36.2**	**32.2**	**.000**	**4.5**	**.000**
Range	**84.9 - 121.3**	**158.2 – 180.67**	**185.3 – 271.7**	**158.2 – 271.7**				

F, P1 (TT, TC, and CC), and T, P2 (TT and TC+CC).

Correlation between RNA-HCV in HCV group and HCC group

Table 6 shows The HCV RNA titers in HCC and HCV group. There was no significant difference between HCV group and HCC group in HCV RNA levels. Serum HCV RNA does not correlate with HCC ($P > 0.05$) as shown in figure 4.

In addition, no correlation was found between HCV-RNA titer and MnSOD activity in both HCV group and HCC group ($r_1 = 0.169$, $P_1 > 0.05$; $r_2 = 0.14$, $p_2 > 0.05$) as shown in figure 5.

Table (6): HCV RNA titers**:**

	HCV	HCC	R	Pv
HCV RNA Median Range	$4.246X10^5$ $3555 – 15.2x10^6$	$4.59x10^5$ $19154 – 5.1x10^6$	-0.204939	P > 0.05

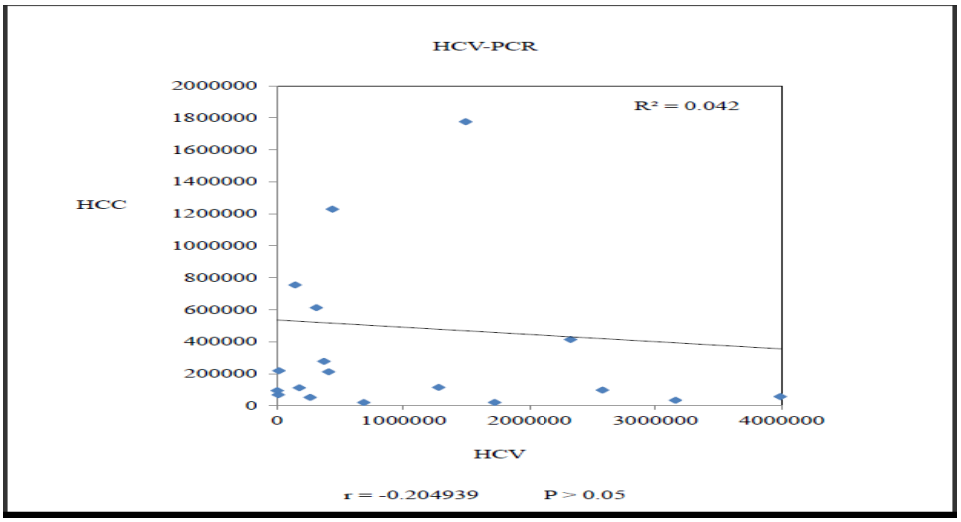

Figure 4: Correlation between RNA-HCV in HCV group (group 2) and HCC group (group 3)

Table (7): Correlation between serum manganese superoxide dismutase and serum RNA-HCV in HCV group and HCC group:

	HCV	HCC	r_1	P_1	r_2	P_2
MnSOD	142.6± 27.3 88.3 – 191.7	185.6± 49.3 84.9 - 271.7	0.169	>0.05	0.14	>0.05
HCV-RNA	$4.246X10^5$ 3555 – $15.2x10^6$	$4.59x10^5$ 19154 – $5.1x10^6$				

r_1, P_1: Correlation between serum manganese superoxide dismutase and RNA-HCV in HCV and r_2, P_2: Correlation between serum manganese superoxide dismutase and RNA-HCV in HCC.

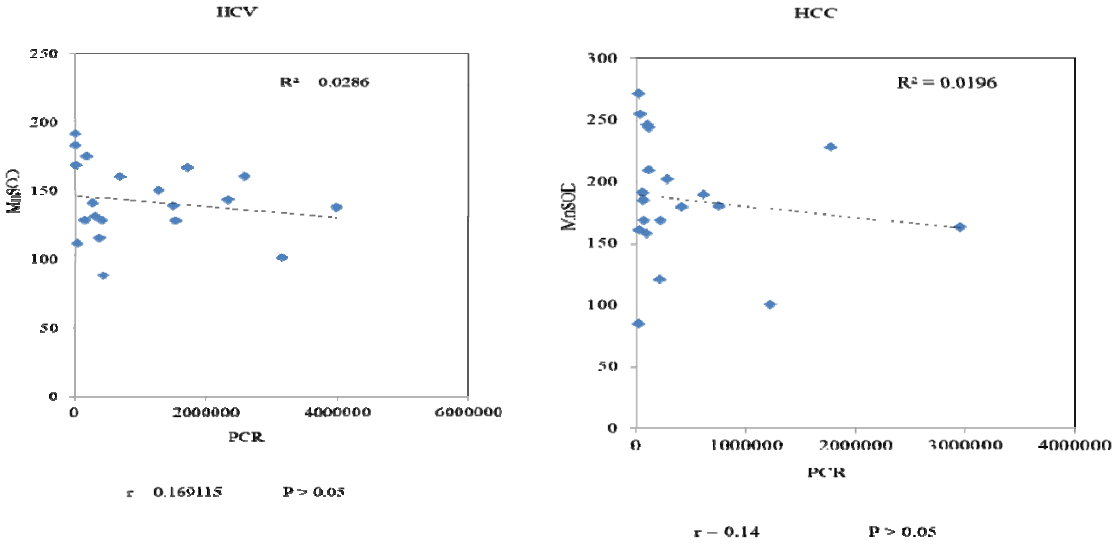

Figure 5: Correlation between MnSOD and serum RNA-HCV in HCV group (group 2) and HCC group (group 3)

DISCUSSION

HCV infection is the main contributor to the etiology of HCC. A rising incidence of HCC has been largely related to increased HCV infections in the general population during the preceding 50 to 60 years (Kiyosawa *et al.*, 2004).

In Egypt, hepatocellular carcinoma (HCC) is the second most common cancer in men and the 6th most common

cancers in women (Globocan 2008). Hospital-based studies from Egypt revealed that HCC represents more than 95% of liver cancers, HCV infection reportedly being the cause of more than 75% of these cases (El Zayadi *et al.,* 2005; Lehman & Wilson, 2009).

Oxidative stress is one of the mechanisms, by which HCV stimulates proliferation of hepatocytes and leads to HCC (Tamai *et al.,* 2011). Antioxidants are substances that neutralize free radicals or their actions (Sies, 1996). Manganese superoxide dismutase (MnSOD) is a vital antioxidant enzyme that catalyzes the conversion of superoxide radicals (O2 •-) to hydrogen peroxide and molecular oxygen in the mitochondria (Weisiger and Fridovich, 1973).

In this study, The HCV RNA titers were analysed, revealed no significant difference of HCV RNA between HCV group and HCC group (P>0.005).

Different studies in diverse populations and cancer types have resulted in identifying conflicting roles for the Val16Ala MnSOD polymorphism and cancer risk (Bag and Bag, 2008).

Several reports have shown that serum MnSOD levels are associated with various clinical findings, such as fibrosis and hepatic oxidative stress (Clemente *et al.,* 2007; Nahon *et al.,* 2007; Qadri *et al.,* 2004).

So, level of serum MnSOD activity and polymorphism in MnSOD gene were the parameter assayed and evaluated in the present study.

Concerning the association between MnSOD gene polymorphism with the prognosis of hepatitis C Virus related hepatocellular carcinoma patients using RFLP method.

In this study, the analysis of the three groups showed that MnSOD Ala/Ala genotype is increased in HCC group in comparison to HCV group and healthy controls, respectively. This results matched with Ezzikouri *et al.,* (2008), who found that Ala/Ala genotype has been associated with a 5.09-fold increase in HCC risk among Moroccan patients, and Amany Ibrahim *et al.,* (2010), who found that there is an evidence of association between Ala16Val MnSOD polymorphism and HCC occurrence in HCV-infected Egyptian patients.

However, other studies revealed that the Ala/ Ala genotype was similarly distributed in patients with HCV-related cirrhosis and normal individuals. Thus, there was no association between this genotype and risk of HCC or even death (Nahon *et al.,* 2007; Martin *et al.,* 2009). This finding is explained by the presence of other polymorphisms in the transport coding sequence and in other detoxifying genes (Van Landeghem *et al.,* 1999). In addition, genetic diversity and differences in gene-environment interactions may be reflected on the impact of the MnSOD polymorphism (Nahon *et al.,* 2007).

MnSOD is primarily localized to the mitochondrial matrix (Matés *et al.,* 1999). Therefore, MnSOD may be an indicator of mitochondrial disorders that are induced by oxidative stress. In the present study, the MnSOD enzyme that was examined had different origins in the mitochondria and cytoplasm.

Regarding the serum MnSOD levels the results were analysed using simply analysis of variance revealed a highly significant difference of the mean values of serum MnSOD level among all studied groups ($F = 62.9$, $P < 0.001$). There was a highly statistically significant difference increase of MnSOD levels among HCC group and HCV group when each was compared with control ($P < 0.001$), where as there was a no significant difference between HCV group and HCC group ($P = 0.11$).

In this study, serum MnSOD levels were significantly higher in the HCC group than in both control group and HCV group. It is known that in humans, MnSOD activity is higher in the liver compared to other tissues (Westman & Marklund, 1981). Furthermore, ROS are

overproduced by Kupffer cells and inflammatory cells in liver disease (Jaeschke *et al.,* 2002; Loguercio & Federico, 2003). The increased MnSOD expression reflects hepatocyte oxidative stress and correlates with decreased hepatic function, increased hepatic fibrosis and ROS production by inflammatory cells in liver cirrhosis. These features represents the main background characteristics causing HCC and may be linked with the indirect effects of liver cancer progression. These associations may also explain why serum MnSOD levels predicted the overall survival of patients with HCC. These results are in agreement Clemente *et al.,* (2007), who reported a significant increase of serum MnSOD activity in patients with cirrhosis with or without HCC compared with controls. Tamai *et al.,* (2011) who found that The serum MnSOD levels was significantly higher in patients with HCV-related HCC than in patients without HCC, Amany Ibrahim *et al.,* (2010) reported that serum MnSOD activity was significantly higher in those patients compared to control subjects.

In this study, there was a highly significant increase in the serum level MnSOD enzyme in homozygous CC and heterozygous TC than in homozygous TT in HCC group also there was a highly significant increase in its activity in HCC patients with CC and TC genotypes than in those with TT one. Similarly, Sutton *et al.,* (2005), reported increased MnSOD activity with the Ala/Ala genotype in a human hepatoma cell line.

On the contrary, other researchers estimated the activity of MnSOD red blood cells and cryopreserved liver cells in normal individuals. MnSOD activity was lower Ala/Ala and Val/Ala genotypes compared to Val/Val genotype. This difference may be due to the variation in the transport efficiency and activity of MnSOD between normal tissue and lesional and perilesional tissue of cancer specimen (Bastaki *et al.,* 2006; Martin *et al.,* 2009),.

Serum MnSOD is reported to be biomarker of oxidative stress in several diseases, including liver disease (Deng *et al.,* 2011; Fujimoto *et al.,* 2010; Takami *et al.,* 2010). Serum MnSOD is potential clinical biomarker that predict patient prognosis in HCV-related HCC (Tamai *et al.,* 2011). Therefore, clinicians should consider using MnSOD as diagnostic biomarker for early detection of HCC or as additional marker in HCC surveillance program using ultrasonography or AFP. Also, it is very important to know if these markers decrease in response to HCC treatment and reductions in tumor burden.. This marker also may have utility in patients on a transplant waiting list who are treated with neo-adjuvant therapy for tumor downstaging.

In conclusion, HCC risk is elevated among HCV-infected patients with MnSOD Ala/Ala genotype. Serum MnSOD level increased as HCV-related chronic liver disease progressed, especially among patients with HCC. These findings recommend that serum MnSOD level is not only a potential biomarker for HCV-related liver disease, but may also be used as a prognostic marker in HCC.

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