

Paraoxonase1 (PON1) 55 and 192 Polymorphism in Egypt and its impact on the antioxidant status of patients with Bilharzial and Viral liver diseases

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ABSTRACT

Background/Aims: It is now known beyond doubt that viral hepatitis (caused by B or C virus) along with bilharzial infestation (mostly *S. mansoni*) are the most important factors responsible for the vast majority of morbidity and mortality in Egypt. Based upon the concept that oxidative stress plays a key role in the development and pathogenesis of chronic liver diseases, many of the factors known to have antioxidant properties are to be investigated for purpose of evaluating their role in the defense mechanisms against all oxidant brunts associated with liver diseases. Serum MDA was assayed as a famous marker of oxidative stress, while PON1 enzyme activities were investigated as a marker of antioxidant properties. The polymorphism at 55 and 192 position known to be associated with PON1 enzyme are also investigated in Egyptian normal and chronic liver patients, in order to elucidate whether such polymorphism has any effect on the enzyme activity, and consequently the state of hepatic affection. Whether routine assay of PON1 activity in chronic liver disease patients can be introduced as a non-invasive clue marker for diagnosis and rating the stage of hepatic affection is another aim of the present work. **Methods:** We studied 75 patients with chronic liver disease (25 patient with chronic Bilharziasis, 25 patients with chronic hepatitis C, 25 patients with mixed hepatitis C and bilharzial cirrhosis) and 25 apparently healthy controls. Serum paraoxonase activity and levels of the lipid peroxidation marker (serum malondialdehyde) were measured spectrophotometrically. PON1 genotyping at positions 55 and 192 were analyzed by PCR, restriction fragment length polymorphism and agarose gel electrophoresis. **Results:** the present work showed that chronic liver disease (viral and/or bilharzial) are associated with elevated oxidative stress (as indicated by increased MDA level) together with reduced PON1-activities, which is regarded as an antioxidant tool. The frequency of investigated polymorphism at 55 and 192 position were found to be of no statistical significance between patients and control groups. The MDA and PON1 values did correlate with standard liver function. **Conclusion:** The present work could introduce the assay of PON1 activity in the serum as a non-invasive, specific and reliable marker in a trial to assess the state of liver affection in chronic hepatic diseases.

INTRODUCTION

In Egypt, viral hepatitis along with *Schistosoma mansoni* infestation is the major cause of chronic liver disease¹. HCV infection is characterized by increased markers of oxidative stress². Lipid peroxidation products are increased in serum, peripheral blood mononuclear cells (PBMC), and liver specimen from hepatitis patients³. *Schistosoma mansoni* causes liver disease by inducing granulomatous inflammation. This favors formation of reactive oxygen species, including superoxide ions, hydrogen peroxide and hydroxyl radicals all of which may induce lipid peroxidation⁴, so the antioxidant status may play an important role in the development of chronic liver disease.

Paraoxonase is an ester hydrolase that is synthesized almost exclusively in the liver and which circulates in the plasma associated with HDL⁵. PON1 is the best studied member of a family of mammalian enzymes that includes PON2 and PON3, which share 60% sequence identity with PON1. PON1 and PON3 reside in the cholesterol-carrying particles HDL (good cholesterol), whereas PON2 is found in many tissues including the liver, Lungs, brain and heart⁶. PON₁ is a 355 amino acid glycoprotein with a molecular weight of approximately 43 kDa, and a serum concentration of about 50 mg/L⁷. PON1 degrades oxidized phospholipids in low-density lipoproteins (LDL) and HDL and, as such, plays a role in the organism's antioxidant system⁸⁻¹¹.

Alterations in circulating PON1 levels are associated with a variety of diseases involving oxidative stress¹²⁻¹⁴ which, as mentioned is associated with chronic hepatic derangement¹⁵⁻¹⁷

Serum paraoxonase gene is located at position q 21-q 22 on the long arm of chromosome 7, closely linked to the gene of cystic fibrosis. Human paraoxonase gene (PON1) shows two polymorphisms at its coding region (amino acid at positions 192 and 55) and 5 polymorphisms at the promoter regions of PON1 gene (positions 909, 824, 162, 126 and 107)¹⁸. The coding region of PON₁ gene contains two polymorphic sites: a leucine (L) methionine (M) transition at position 55 (55L/M), and glutamine (Q) to arginine (R) transition at position 192 (192Q/R)¹⁹. The polymorphisms affect the hydrolytic activity of the PON1 isoenzymes with respect to certain substrates, such as paraoxon and lipid peroxides²⁰. The dominant effect on activity is exerted by the PON1-192 polymorphism. However, the PON1-55 polymorphism also exerts a smaller, but significant, effect on activity²⁰.

Therefore, the idea of the current study was to measure PON1 activity and its relation to serum MDA, total proteins levels, AST and ALT activities in patients with chronic liver disease and determine whether or not PON1 activity can be a non-invasive marker of estimating the degree of liver damage in patients with chronic liver disease. Also, we investigated the PON1 gene polymorphism and its possible association with chronic liver disease.

METHODS

The present study was conducted in the Medical Biochemistry and Internal Medicine Departments, Faculty of Medicine, Zagazig University.

One hundred subjects were enrolled in this study. All were divided into 4 groups

Group I (Control group):

This group comprised 25 subjects (15 males and 10 females). Their age ranged from 25 to 50 years, with a mean value of 35.3 ± 6.5 years. All were volunteers from relatives, colleagues and patients attending the Out Patient Clinics for minor complaint e.g. minor superficial injuries, ear wax...etc. They suffered no illness that might interfere with the present study. No medications were received for the past few weeks.

Group II (Chronic Bilharzial patients):

Twenty five patients (13 males and 12 females) were included in that group. Their age ranged from 27 to 45 years, with a mean value of 34.2 ± 5 years. Bilharzial infestation was detected by assessing bilharzial antibodies (indirect haemagglutination test) and proved by rectal snip examination.

Group III (Chronic HCV group):

Twenty five patients (17 males and 8 females) were included in that group. Their age ranged from 30 to 50 years, with a mean value of 38.7 ± 6.4 years. Patients were diagnosed as having chronic HCV by ELISA antibody assessment and confirmed by the presence of HCV-RNA by RT-PCR estimation. All were negative for bilharzial infestation.

Group IV (Mixed bilharzial and HCV cirrhosis patients):

Twenty five patients (13 males and 12 females) were included in that group. Their age ranged from 35 to 60 years, with a mean value of 45.2 ± 6.9 years.

They were diagnosed as having both chronic pathological liver affection (HCV and bilharziasis) by history, clinical examination and serological investigation.

All these individuals were subjected to thorough history taking, routine general and abdominal medical examination, plain X-ray of chest and heart, ultasonographic assessment of abdominal organs and routine laboratory investigations (urine and stool analysis, complete blood count...etc)

Blood sampling and DNA extraction

Six ml fasting venous blood samples were withdrawn from all the subjects and divided into 2 portions:

1. Three ml were placed in glass tubes with K_2EDTA and genomic DNA was obtained from leukocytes using QIA amp DNA Blood Mini Kit supplied by Qiagen GmbH, (Hilden, Germany). The purified DNA was safely stored at $-20^\circ C$ for later use.
2. Three ml were collected into glass tubes with no anticoagulants, after being incubated at $37^\circ C$ for 20 minutes and centrifuged to obtain serum, then serum were separated and stored at $-20^\circ C$ till time of analysis.

Determination of PON Activity

PON1 activity assays were performed with 2 mmol L^{-1} paraoxon according to the method described by Mackness et al.^{21,22}. Initial hydrolysis rates of paraoxon (Sigma Chemical

Co, UK) were determined by measuring the released p-nitro-phenol at 405 nm at 37 °C on a Milton Roy Spectronic 3000 Array. The basal assay mixture included 2.0 mmol L⁻¹ paraoxon and 2.0 mmol L⁻¹ CaCl₂ in 0.1 mol L⁻¹ Tris-HCl buffer, pH = 8.0. To 350 µL of reagent mixture, 10 µL of serum was added. Enzyme activities were expressed in international units (U) per 1 litre of sera. An international unit is the amount of hydrolyzed substrate in µmol per minute.

Determination of PON genotype

PON1 genotypes were determined following PCR according to previously published protocols^{19,23}. For the polymorphism at position 192, sense primer 5'TATTGTTGCTGTGGGACCTGAG 3' and antisense primer 5' CACGCTAAACCCAAATACATCT C 3', which encompass the 192 polymorphic region of the human PON1 gene, were used. For the polymorphism at position 55, sense primer 5'GAAGAGTGATGTATA-GCCCCAG3' and antisense primer 5' TTTAATCC-AGAGCTAATGAAAGCC 3' were used. The PCR reaction mixture contained 100 ng of DNA template, 0.5 µM of each primer, 1.5 mM MgCl₂, 200 µM of each dNTP and 1 unit of TaqDNA polymerase (Qiagen GmbH, Hilden, Germany). After denaturing the DNA for 5 min at 95°C, the reaction mixture was subjected to 46 cycles of 1 min denaturation at 94°C, 30s of annealing at 61°C and 1 min of extension at 72°C. For the polymorphism at position 192, the 99 bp PCR product was digested with 8

units of AlwI restriction endonuclease (New England Biolabs, Cambridge, MA, U.S.A.) overnight at 37°C, and the digested products were separated by electrophoresis on a 3% (w/v) agarose gel and visualized using ethidium bromide. The R genotype (arginine) contains a unique AlwI restriction site which results in products of 66 and 33 bp, whereas the Q genotype (glutamine) would not be cut, allowing the PON1-192 genotype to be determined. For the PON1-55 polymorphism, the PCR reaction and cycling were the same as described above, except that 30 cycles were carried out. The PCR product (170 bp) was digested with NlaIII (New England Biolabs) in the presence of bovine serum albumin (BSA) (37 °C, overnight), and the digested products were separated and identified as above. The L genotype (leucine) does not contain an NlaIII site, whereas the M genotype (methionine) does contain an NlaIII site, giving rise to products of 126 and 44 bp. (Fig 2 and Fig.3).

Measurement of Serum MDA Concentration

Serum MDA levels were measured according to a method described elsewhere²⁴. The principle of the method was based on the spectrophotometric measurement of the color occurring during the reaction of thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of MDA-thiobarbituric acid complex and expressed in µmol/L. The traditional measures of liver function (serum alanine transaminase (ALT), and aspartate transaminase (AST) activities, as well

as total proteins) were analyzed with standard techniques using Milton Roy Spectronic 3000 Array.

RESULTS

The results of the biochemical measurement in different studied groups are shown in (table 1). Serum activities of liver enzymes were significantly increased in all patient groups.

Serum PON1 activities were found to be significantly lower in all the studied groups in comparison to control group ($P < 0.001$) (Fig.1).

Also, serum MDA levels were significantly increased in all groups in comparison to control.

PON1 activity was positively correlated with serum total proteins, in group II, III and IV ($r = 0.55, 0.54$ and 0.71 respectively) and ($P < 0.001$ in all groups) but not in group I (control group). There was significant negative correlation between serum PON1 activity and serum ALT in group II, III and IV ($r = -0.58, -0.49$ and -0.47 , respectively and $P < 0.001$ in group II and $P < 0.01$ in group III and IV). There was also significant negative correlation between serum PON1 activity and serum MDA in group I, II, III, IV ($r = -0.75, -0.64, -0.87$ and -0.69) and ($P < 0.001$) in all groups (Table 2).

Relationship between serum PON1 activity and genotypes

The distributions of the two polymorphisms in the control and in the patient groups with liver disease are shown in table (3). There were no significant differences in genotype or allele frequencies between cases and controls for either the PON1192 or PON155 polymorphisms. The mean decreases of serum PON1 activity in the patient groups relative to the control group were similar and independent of the genotype (Fig 4).

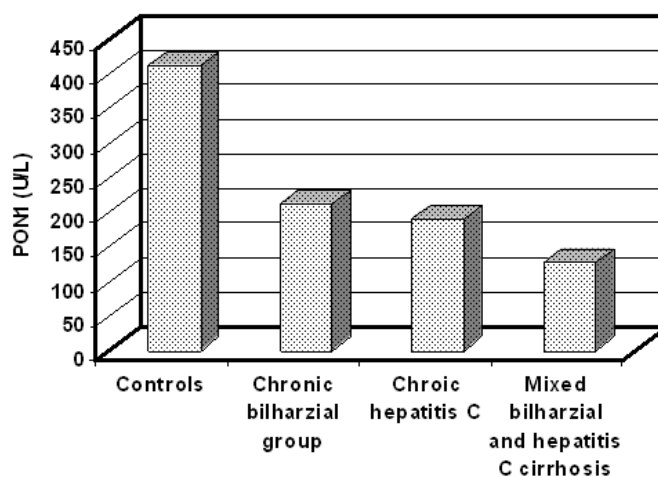
Multiple ANOVA showed that serum PON1 activities were significantly influenced ($P < 0.001$) by both patients group and the polymorphisms PON155 and PON1192. The plasma concentration of MDA was higher in patients carrying the RR isoform (QQ: $69.5 \pm 5.1 \mu\text{mol/L}$; QR: $52.3 \pm 9.4 \mu\text{mol/L}$; QQ: $43.3 \pm 9 \mu\text{mol/L}$; $P < 0.001$, RR vs. QR and QQ). In serum PON1 activity, it was the RR homozygote patients who were most able to hydrolyze paraoxon (QQ: $128.0 \pm 46.97 \text{ U/L}$; QR: $217 \pm 54.4 \text{ U/L}$; RR: $276.4 \pm 47.1 \text{ U/L}$; $P < 0.001$, RR and QR vs. QQ) (table 4).

The plasma concentration of MDA was higher in patients carrying the LL isoform ($P < 0.0001$, LL vs. ML and MM). In serum PON1 activity, it was the LL homozygote patients who were most able to hydrolyze paraoxon ($P < 0.001$, LL vs. ML and MM) (table 4).

Table 1. Results of the biochemical parameters in control and patient groups (Values are expressed as mean± SD)

Groups	Controls N=25	Chronic Bilharzial group N=25	Chronic hepatitis C N=25	Mixed bilharzial and hepatitisC cirrhosis N=25
PON1(U/L)	412.9±99.8	212.36±58.8*	191.7±75.3*	127.2±57.2*
MDA(μmol/L)	16.3±2.8	46.99±11.6*	51.5±11.96*	50.1±12.36*
ALT(U/L)	27.1±1.9	66.5±8.6*	88±10.8*	66.7±10.4*
AST(U/L)	23.4±6.8	32.8±10.3*	70.5±12.5*	63±21.1*
Total protein(g/dl)	7.1±0.3	6.9±0.5	7.1±0.3	6.9±0.7

*P<0.001 = Highly significant.

**Fig. (1):** Mean values ±SD of serum PON1 activity in all studied groups.**Table 2.** Correlation coefficient of serum PON1, serum MDA and standard liver function test.

	Controls N=25		Chronic Bilharzial group N=25		Chronic hepatitis C N=25		Mixed bilharzial and hepatitisC cirrhosis N=25	
	r	P	r	P	r	P	r	P
Total Protein	0.03	>0.05	0.55	<0.001*	0.54	<0.001*	0.71	<0.001*
ALT	-0.22	>0.05	-0.58	<0.001*	-0.49	<0.001*	-0.47	<0.001*
AST	-0.16	>0.05	-0.01	>0.05	0.17	>0.05	-0.17	>0.05
MDA	-0.75	<0.001*	-0.64	<0.001*	-0.87	<0.001*	-0.69	<0.001*

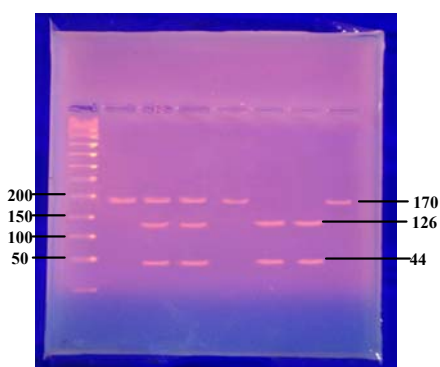


Fig. (2): Agarose gel electrophoresis pattern of PON1 55 polymorphism. Lane 1 Molecular weight marker, lane 2, 5 and 8 show the homozygous LL. Lane 3 and 4 heterozygotes ML. Lane 6 and 7 show the homozygote MM.

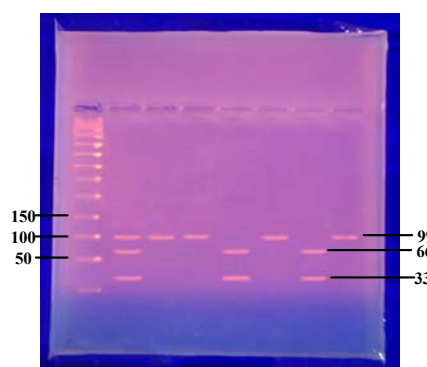


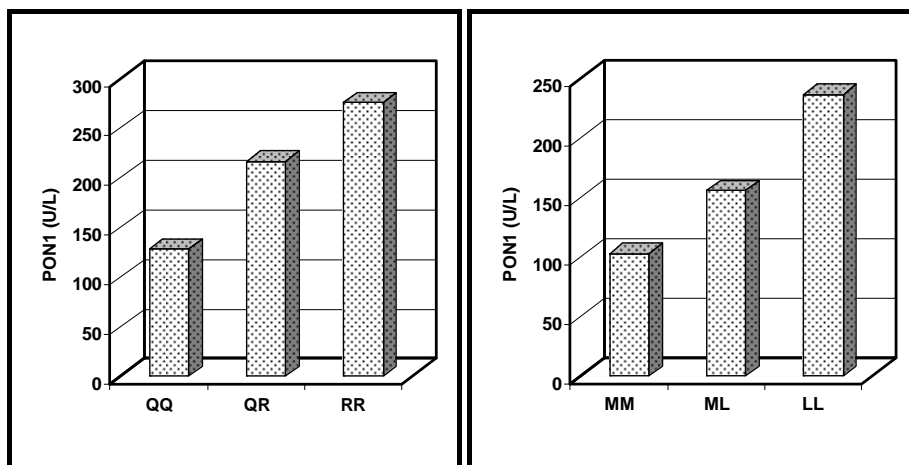
Fig.(3): Agarose gel electrophoresis pattern of PON1 192 polymorphism. Lane 1 Molecular weight marker, lane 2 show the heterozygotes QR. Lane 3, 4, 6 and 8 show the homozygous .QQ. Lane 5 and 7 show the homozygote RR.

Table 3. Distribution of PON1 genotypes in patients and control for polymorphism at positions groups 55 and 192.

	Controls N=25		Chronic Bilharzial group N=25		Chronic hepatitis C N=25		Mixed bilharzial and hepatitis C cirrhosis N=25	
PON1 192 genotype frequency								
QQ	12	48%	12	48%	13	52%	14	56%
QR	12	48%	11	44%	9	36%	8	32%
RR	1	4%	2	8%	3	12%	3	12%
PON1 192 allele frequency (Q/R)	72/28(%)		70/30(%)		70/30(%)		72/28(%)	
PON1 55 genotype frequency								
MM	3	12%	4	16%	4	16%	3	12%
ML	12	48%	12	48%	13	52%	11	44%
LL	10	40%	9	36%	8	32%	11	44%
PON1 55 allele frequency(M/L)	36/64(%)		40/60(%)		42/58(%)		38/62(%)	

Table 4: Serum levels of PON1 activity & MDA levels in relation to genotypes in all patient groups

	QQ N = 39	QR N = 28	RR N = 8	F	P
PON1	128.0±46.97	217±54.5	276.4±47.1	43.59	0.001
MDA	43.3±9	52.3±9.4	69.5±5.1	31.09	0.0001
	MM N = 11	ML N = 36	LL N = 28	F	P
PON1	103±42.1	155.7±52.4	236.5±64.4	27.64	0.001
MDA	36.9±4.8	48.1±8.3	56.2±13.3	14.6	0.0001

**Fig. (4):** Serum PON1 activity (U/L) in all patients group in relation to genotype

DISCUSSION

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease in most industrialized countries. There are ~170 million people in the world who are chronically infected by that virus. HCV viral infection frequently does not resolve, and ~80% of the infected individuals become chronic carriers who may then progress to severe liver diseases. Approximately 10–20% of chronically infected hepatitis C patients will develop severe liver

cirrhosis, and 1–5% will develop hepatocellular carcinoma (HCC) within two to three decades of infection. HCV infection is also, the single major cause of liver transplantation in the U.S.²⁵. Schistosomiasis is an important public health disease in many developing countries including Egypt. Hepatosplenic schistosomiasis is a serious manifestation of *S. mansoni* infection that may lead to irreversible sequelae²⁶. The prevalence of HCV antibodies was greater in Egyptian patients with bilharziasis due to HCV

transmission during antibilharzial parenteral therapy or due to depressed cell mediated immunity in bilharzial patients²⁷. It was proved that the state of oxidative stress at the sites of inflammation is responsible to a large extent for cellular damage and progression of fibrosis in chronic liver diseases²⁸⁻³⁰. Evidence of oxidative stress has been detected in almost all the clinical and experimental conditions of chronic liver disease, often in association with decreased antioxidant defenses³¹⁻³³. Therefore, antioxidant therapy is a promising strategy for treating or preventing oxidative stress related liver diseases.

Aviram et al.³⁴ demonstrated that paraoxonase possesses an esterase and peroxidase like actions. PON1 peroxidase-like activity reduces L-OOH to L-OH and prevents initiation of LDL oxidation. PON1 can also interact with and remove specific lipid peroxides by acting stoichiometrically as a 'suicidal enzyme' with oxidized lipids as a 'biological buffer'. Consequently, the lowering of lipid peroxidation by PON1 can lead to alleviation of hepatic damage in the initial phase³⁵.

It is accepted that arylesterase and PON1 activities are functions of a single enzyme³⁶. In addition, *in vitro* biochemical studies indicate that hepatic and serum PON1 share many pharmacodynamic properties, as K_m , V_{max} and effects of activators and inhibitors, etc. and which support the concept of a common identity for both paraoxonases³⁷.

Early studies had observed a significant decrease of serum arylesterase activity in patients with liver cirrhosis³⁸⁻⁴⁰. Recently, there has

been an increasing interest in paraoxonase and arylesterase as potential biomarkers for chronic liver impairment⁴¹⁻⁴³. Decreased activities of both enzymes may contribute to liver dysfunction by effect on lipid metabolism and peroxidation⁴³. Kilic et al.⁴² found significant decrease in paraoxonase and arylesterase activities in patients with chronic hepatitis B virus infection. Moreover, paraoxonase plays a role in the regulation of oxidative stress and hepatic cell apoptosis events that are closely related to liver fibrosis⁴⁴.

In the present study we investigated the importance of PON1 activity measurement in assessment of the degree of liver damage as well as the effect of PON1 55 and 192 polymorphism in patients with chronic liver disease. Serum PON1 activity is reduced in patients with chronic liver disease and that decrease is related to the degree of liver damage as stated by that its level decrease in patients with combined bilharzial and hepatitis C cirrhosis more than in the other two patients groups. The decrease in serum PON1 activity in patients with chronic liver disease could be accepted as an evidence of increased lipid peroxidation since it was shown that a decrease in liver microsomal PON1 activity is an early biochemical change related to lipid peroxidation and liver injury observed in rats with carbon tetrachloride (CCL4)-induced cirrhosis⁴⁵. Additionally, same authors monitored the modulation of these processes by the dietary supplementation with zinc, a metal that possesses antioxidant and anti-fibrogenic properties. These findings are consistent with those of Ali

(2004)⁴⁶ who found that the activities of paraoxonase and arylesterase were partially restored in liver and serum by chronic administration of zinc as compared to control mice in experimental *S. mansoni* model. Also, elevated level of PON1 significantly reduced the extent of liver injury caused by CCL4 administration³⁵. It has been reported that PON1 is inactivated after hydrolyzing lipid peroxides³⁴ and this is confirmed in our study by the increased levels of serum MDA in all patient groups.

Another possible explanation may be related to the fact that alteration in HDL structure and composition can affect PON1 activity⁴⁷. It is known that patients with liver disease often present with lecithin: cholesterol acyl transferase deficiency⁴⁸. As a consequence, cholesterol esters synthesis is impaired and HDL shows great changes in shape and structure⁴⁹. Such abnormalities may affect serum PON1 activity.

In our study, serum MDA level was significantly increased in patients with chronic liver disease in relation to control group. Increased serum MDA level is one of the well known indicators of lipid peroxidation. Increased MDA levels and decreased serum PON1 activity reflect increased oxidative damage in patients with chronic liver disease.

There is a wide inter-individual variation in PON1 concentration and activity. That variation is for apart determined by common genetic variants (polymorphism) in the PON1 gene⁵⁰. Regarding the effect of PON1 polymorphisms on serum PON1 activity, the effects of the PON1 192 and PON155 polymorphisms were the

same for both patients and control groups. Thus, QQ homozygotes have the lowest paraoxonase activity, while QR heterozygotes have intermediate activity and RR homozygotes have the highest activity. Also, MM homozygotes had significantly lower PON1 activity than LM heterozygotes, who in turn had lower PON1 activity than LL homozygotes. These results are in agreement with those reported by Sorenson et al.³⁶; Mackness et al.⁵¹; Ferre et al.⁵².

In the present study the serum level of MDA varies with different PON1 genotypes and it was higher in patients carrying the RR isoform in PON1 192 genotypes and patients carrying the LL isoform in PON155 genotypes. We thus think that PON1 192 RR and PON1 55 LL alleles are more susceptible to develop disease associated with increased oxidative stress. However, this could not be ascertained because, our results indicated that the genotype and allele frequencies were not statistically different between patients with chronic bilharziasis, patients with chronic hepatitis C and patients with mixed bilharzial and hepatitis C cirrhosis or between all patients groups and control.

PON1 hydrolyses lipid peroxides and there is evidence to suggest that PON1Q is more efficient than PON1R^{51,53}.

One study identified a significant association between PON1192 polymorphism and the presence of hepatitis C virus infection⁵⁴. They demonstrated that patients with chronic hepatitis had a higher frequency of the RR isoform of the 192 polymorphism than healthy

subjects and thus subjects carrying the R allele might be more susceptible to developing disease involving an increased oxidative stress⁵⁴. What may appear to be a paradox in this study is that the genotype associated with a higher frequency in patients with hepatitis is also, associated with a higher PON1 activity in serum. One possible explanation for this could be that the enzyme has multiple active sites serving different purposes, one of which relates to the lipid peroxide decoupling. In support of this hypothesis, it has been demonstrated that the PON1 active site for protection against lipid peroxidation requires a free sulfhydryl group at cysteine-284, whereas the enzyme activity against paraoxon does not⁵³.

In an experimental study of Zhang et al.³⁵ intramuscular electrotransfer of hPON1Q gene led to efficient expression of the PON1 in mice. Elevated level of PON1 significantly reduced the extent of liver injury caused by CCL₄ administration. In addition, the hPON1Q gene transferred mice also, exhibited lower degrees of oxidative stress, reflected by reduced serum MDA concentration, in contrast to their control counterparts. These data provide compelling and mechanistic evidence for the importance of PON1 in regulation of liver injury.

However, in our study the genotype and the allele frequency were statistically nonsignificant between patients with chronic liver disease and controls and this could be probably due to lower number of participants, ethnic difference and to heterogeneous etiology of their diseases.

In conclusion, the present study indicated that decrease in the activities of paraoxonase may play a role in the pathogenesis of chronic liver disease. In addition, serum paraoxonase activity measurement may be a beneficial tool for assessing the degree of liver damage in patients suffering chronic hepatic diseases. Further studies are required to gain further insight on the role of PON1 polymorphism in relation to chronic liver diseases.

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مدى الاختلاف الجيني لإنزيم الباراكسونيز-1، ٥٥، ١٩٢ في الأشخاص المصريين وتأثير ذلك على مضادات الأكسدة في أمراض الكبد الفيروسية أو الناشئة عن الإصابة بالبلهارسيا أمل فتحي غريب^١، رحاب احمد كرم^١، إسماعيل زكى النجار^١، محمد إمام فرغلي^٢ قسمي الكيمياء الحيوية الطبية^١ والأمراض المتوطنة^٢ كلية الطب- جامعة الزقازيق

تعتبر الإصابة بالالتهاب الكبدي الفيروسي (بي وسي) وكذلك الإصابة بالبلهارسيا المعوية من العوامل الأساسية المسؤولة عن كثير من الحالات المرضية ومعدلات الوفيات في مصر، وقد وجد أن الشوارد الحرة الأكسجينية والنيتروجينية تلعب دوراً هاماً إما في إحداث أو تطور أمراض الكبد ولذلك فإنه يتم دراسة كثير من مضادات الأكسدة لتحديد دورها في الوقاية أو علاج أمراض الكبد المزمنة.

ويعتبر إنزيم الباراكسونيز أحد مضادات الأكسدة الذي يصنع في الكبد ويفرز عن طريقه إلي الدم مصاحباً للبروتينات الدهنية عالية الكثافة و لذلك فإن الهدف من هذا البحث هو دراسة نشاط إنزيم الباراكسونيز في مصل دم المصابين ببعض أمراض الكبد وكذلك دراسة الطفرة الجينية لجين الباراكسونيز عند (+٥٥ و+١٩٢) وعلاقة تأثير هذا التغيير علي وظيفة الإنزيم كمضاد للأكسدة في بعض أمراض الكبد المزمنة.

تم إجراء هذه الدراسة في قسمي الكيمياء الحيوية الطبية والأمراض المتوطنة وقد اشتملت علي مائة شخص تم تقسيمهم كالآتي :

المجموعة الأولى ٢٥ شخصاً من الأصحاء كمجموعة ضابطة، المجموعة الثانية ٢٥ شخصاً مصابين بمرض البلهارسيا المعوية المزمنة، المجموعة الثالثة ٢٥ شخصاً مصابين بمرض الالتهاب الكبدي الفيروسي (سي) والمجموعة الرابعة ٢٥ شخصاً مصابين بتليف الكبد الناتج عن كلاً من البلهارسيا المعوية المزمنة و الالتهاب الكبدي الفيروسي (سي) وقد تم قياس نشاط إنزيم الباراكسونيز وكذلك مستوي المألون ثنائي الأدهيد في الدم باستخدام الطرق الضوئية وكذلك اختبار وجود تحورات لجين الباراكسونيز عند (+٥٥ و+١٩٢) والكشف عنها باستخدام التفاعل التسلسلي عديد البلمرة.

وأظهرت نتائج البحث التالي :-

يوجد انخفاض ذو دلالة إحصائية في نشاط إنزيم الباراكسونيز في جميع المجموعات مقارنة بالأصحاء وكذلك يوجد ارتفاع ذو دلالة إحصائية في مستوي المألون ثنائي الأدهيد في جميع المجموعات مقارنة بالأصحاء وتبين عدم وجود أي علاقة ارتباط ذات دلالة إحصائية بين التحور الجيني لجين الباراكسونيز (+٥٥ و+١٩٢) بين المجموعات المرضية والمجموعة الضابطة وقد تبين وجود تناسب طردي ذو دلالة إحصائية بين إنزيم الباراكسونيز بالدم ونسبة البروتين الكلية كما تبين وجود تناسب عكسي ذو دلالة إحصائية بين إنزيم الباراكسونيز بالدم ومستوي المألون داي الأدهيد في الدم .

ومن هذا نستخلص أن دراسة نشاط إنزيم الباراكسونيز بالدم يمكن أن يعتبر من الإختبارات التشخيصية لقياس نسبة إصابة الكبد في بعض أمراض الكبد المزمنة وكذلك نوصي بعمل دراسات أخرى لتوضيح دور إنزيم الباراكسونيز وكذلك التحور الجيني لهذا الإنزيم في أمراض الكبد المختلفة .