

**Paraoxonase activity and gene polymorphism in colorectal cancer Egyptian patients**

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**Abstract:** Colorectal cancer (CRC) is a common cause of death worldwide, and represents the third most common form of cancer and the second leading cause of cancer-related death in the world. Human paraoxanase (PON1) is a Ca-dependent esterase synthesized in liver and related to high density lipoprotein (HDL) and protecting low density lipoprotein (LDL) by hydrolysis of lipid peroxides. This study aimed to evaluate the relation between CRC and PON1 enzyme activity and polymorphism. Fifty patients of both sexes diagnosed as CRC patients along with eighty healthy persons of matchable age and sex were enrolled in the study. The circulating levels of serum level of lipid profile, PON1 and Aryl esterase (ARE) enzymes were determined by spectrophotometer assays. PON1 gene polymorphism was done using polymerase chain reaction (PCR) technique. The present work showed significant reduction of the serum levels of HDL and triglycerides (TG) concomitant with significant elevation of the serum level of LDL in patients compared to controls. Plasma total cholesterol (TC) shows no significance difference between patients and controls. As regard PON1 gene polymorphism, the present study demonstrated that, the QQ genotype was the most frequent among the CRC patients and the controls (60%), followed by QR genotype (32%). The RR was the least frequent genotype in the two populations (8%). These finding indicated that the serum PON1 and ARE activities were significantly lower in CRC patients compared to healthy subjects concomitant with significant increase in the serum level of LDL and significant reduction of HDL and TG. Also there were significant difference of genotype distribution of PON1 between patients and control groups. These observations suggested the hypothesis that defects in the antioxidant system capacity and altered PON 1 activity may be involved in the pathogenesis of CRC.

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**Key words:** CRC, PON1 polymorphism, ARE enzymes and lipid profile.

**Abbreviations:** CRC, colorectal cancer; PON1, Paraoxonase; ARE, Aryl esterase; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides; TC, total cholesterol; PCR, polymerase chain reaction.

## 1. Introduction

Colorectal cancer (CRC) is a leading cause of death worldwide, with over 1 million of new cases and a half a million of deaths around the world every year. It is estimated that up to 10% of CRC cases can be attributed to hereditary factors of high penetrance leaving approximately 90% so-called sporadic CRC cases, which may be attributed to diet (Ferlay et al., 2010), lifestyle factors (Van Duijnhoven et al., 2009) and genetic factors of low penetrance (Gerber et al., 2009).

Genetic predisposition to CRC may involve polymorphic variations in genes encoding for detoxification enzymes. Genetic variations in these enzymes may alter the conversion rate of toxic/carcinogenic compounds ingested by food,

medication or life style habits such as smoking which subsequently might influence the levels of these compounds in the colonic lumen or mucosa, possibly altering the risk for CRC. In addition, it is suggested that reactive oxygen species (ROS) may also play a role in human cancer development. ROS may cause harm to surrounding tissue and this may influence the risk for CRC (Potteret al., 2008).

Human paraoxanase (PON1) is a Ca-dependent esterase, synthesized in liver, is related to high density lipoprotein (HDL) (Potter et al., 2008). PON1 has two main roles: detoxifying organophosphate compounds such as paraoxone and protecting LDL by hydrolysis of lipid peroxides (Poynter et al., 2005). Lipid peroxidation has really important roles in the control of the cell cycle in particular polyunsaturated fatty

acids (PUFA) are known to be vulnerable to free oxygen radical interaction creating lipid peroxidation (Park et al., 2009). The peroxidation products can interact with DNA bases to form exocyclic DNA base products which are exocyclic pyrimido-purines, cytotoxic and mutagenic (Poynter et al., 2009).

The aim of the present study was to determine the distribution of the PON1 192 genotypes and Q and R allelic frequencies in CRC patients compared with healthy controls. Also to identify the association between PON 1 genotypes and the clinical and biochemical variables in the studied populations.

## 2. Patients and methods

This study was carried out at Medical Biochemistry and General Surgery Departments, Sohag University, Alazhar University, Egypt during the summer months (May, June and July) of 2016. This study was approved from the ethics committee of Faculty of Medicine, Sohag University, Sohag, Egypt. Written informed consent was taken from each participant after explanation of study details.

### 2.1. Patients

Fifty newly diagnosed CRC Egyptian patients admitted to the outpatient clinic of surgery were prospectively included in the study. Final diagnosis of each patient was confirmed by the microscopic evaluation of colonoscopic biopsy samples, followed by total excision of tumors and histopathological examination. Any patient under antioxidant drugs was excluded. The following pathologic findings were assessed: according to modified Dukes classification (stage A = 8 cases, stage B = 25 cases, stage, C = 10 cases and stage D = 7 cases).

### 2.2. Control group

Eighty healthy control subjects of corresponding gender and age were also enrolled for comparison.

### 2.3. Sample preparation

From each participant, 5 mL of fasting blood was drawn and distributed into serum and EDTA tubes. Serum was obtained from serum tubes after centrifugation at 4000 rpm for 10 min for determination of Lipogram, PON1 and ARE activity. The plasma from the EDTA tubes were stored at -80°C for DNA extraction.

## 2.4. Methods

### 2.4.1. Determination of lipid profile

Total plasma cholesterol was determined quantitatively using enzymatic colorimetric method. To 10 µL of the serum, 1000 µL of cholesterol reagent was added. The amount of triglycerides in 10 µL of the EDTA plasma was quantified colorimetrically using 1000 µL of the triglyceride reagent. For HDL determination 500 µL of the plasma was added to 1000 µL of HDL precipitating reagent and wait for 10 minutes. After centrifugation, 100 µL HDL

cholesterol esters in the supernatant was added to 1000 µL of cholesterol reagent and determined spectrophotometrically. The LDL level in the plasma was calculated using the Friedewald Formula, LDL-C = total cholesterol - (HDL cholesterol + triglycerides/5) mg/dl.

### 2.4.2. Determination of PON1 activity

PON1 activity was measured by adding 20 µl of serum to tris buffer (100 mmol/l, pH 8.0) containing 2 mmol/l CaCl<sub>2</sub> and 1 mmol/l paraoxon (O, O-diethyl-O-nitrophenyl phosphate (Sigma) The rate of generation of P-nitrophenol was determined at 405 nm, 37°C over 50 seconds after 1 minute lag time with the use of spectrophotometer (Abbott et al., 1995).

### 2.4.3. Determination of aryl esterase activity

Aryl esterase activity was measured using phenyl acetate as a substrate. The reaction mixture contained 750 µl of 0.1 mol/l Tris-HCl (pH 8.5), 1 mmol/l CaCl<sub>2</sub>, 125 µl of 12 mmol/l phenyl acetate and 125 µl of diluted serum (1:10 diluted with water). Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37°C. Enzyme activities were expressed in international units per 1 liter of serum (U/l). (Hardy et al., 2001).

### 2.4.4. Polymorphism analysis

DNA extraction: Genomic DNA was extracted from whole heparinized blood samples, using (CinnaPure DNA) Cat No. PR881612. Tehran). The DNA samples were stored at -80°C until needed for further analysis.

Protocol: Before starting samples was mixed and transferred to plastic tubes by pipetting and centrifugation for 10 min. 100 uL of the sample was added to a sterile 1.5 ml polypropylene tube (Ebindorve). Then add 400 uL lysis buffer and vortex at max speed for 20 sec. after that add 300 uL precipitation solution and vortex at max speed for 5 sec. The solution was transferred to a spin column with collection tube by pipetting and centrifuge the tube at 13.000rpm for 1min. Discard collection tube and place spin column in new collection tube and add 400 uL wash buffer 11 to the spin column and centrifuge at again for 1 min. Discard flow through. Wash the spin column with 400 uL of wash buffer 11 by centrifugation at (13.000rpm) for 1 min. Discard flow-through two times with repeat the centrifugation step. Carefully transfer the column to a new 1.5 ml tube and add 50 uL elution buffer in the center of the column and then centrifuge for 1 min to elute the DNA. Primer base, Oligo Name: PON1 192 F, Oligo number: 31216B3-963D10 84/136, 5' TAT TGT TGC TGT GGG ACC TGA G 3', 84/136. DNA, OligoName: PON1 192 R Oligo number: 31216B3-963E10 85/136, 5' CAC GCT AAA CCC AAA TAC ATC TC 3'.Restriction enzyme: Cat.NO: PR8252C.

Composition of PCR Master Mix (2X) 0.08 units/ $\mu$ l taq DNA polymerase in reaction buffer, 3 mM  $MgCl_2$ , 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP.

**Polymerase Chain Reaction (PCR):** The extracted DNA samples were amplified by PCR with the PON 1 192 primers. The primer sequences were 5' TAT TGT TGC TGT GGG ACC TGA G 3' for the forward primer and 5' CAC GCT AAA CCC AAA TAC ATC TC 3' for the reverse primer respectively. The PCR was performed for 46 cycles in a Gene Pro thermal cycler (Hangzhou Bioer Technology Co. Ltd., China). The conditions for the PCR were as follows: initial denaturation was done at 95 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 61 °C for 30 sec, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The thermal cycler was set to hold the products at 4 °C until they were retrieved at the end of the 46 cycles. The products were then resolved on 3% agarose gel.

**Restriction fragment length polymorphism (RFLP) Analysis:** RFLP analysis was done on DNA samples of 50 CRC patients and 80 controls. To 5  $\mu$ L of the PCR product in an eppendorf tube, nine  $\mu$ L of nuclease-free water, one  $\mu$ L of 10x buffer Tango (Thermo Fisher Scientific Inc.) and 0.5  $\mu$ L of 2u/ $\mu$ L BspPI (AlwI) restriction enzyme (Thermo Fisher Scientific Inc.) were added. The mixture was tapped gently and spun down for 5 seconds. It was then incubated for 2hrs at 55°C in a water bath. The RFLP products obtained after the restriction enzyme digestion were resolved on 3% agarose gel.

**Agarose gel electrophoresis:** The PCR and RFLP products were separated on 3% agarose gel (Thermo Fisher Scientific Inc.) which was prepared with 1 X tris acetate diamino ethanetetra- acetic acid (TAE) buffer. The gel was poured and allowed to set. It was placed in an electrophoretic chamber which filled with 750 ml of 1 X TAE buffer containing 20  $\mu$ L of 10 mg/mL ethidium bromide solution (Sigma Chemical, USA; Appendix C 1.3). An O'Gene ruler 50 bp DNA ladder (Thermo Fisher Scientific Inc.) was ran alongside the PCR products in the first well. The electrophoresis was performed using 56 mAmp electric current at a potential difference of 80 volts for 90 min. The gel was visualized with an ultraviolet transilluminator and photographed with a photoman (Uvitec, Cambridge, UK). The images were printed with a video copy processor.

### 2.5. Statistical analyses:

Using SPSS software version 16, the data were statistically analyzed. In normally distributed groups the results were presented with mean and SD. The significance of the differences between groups was determined by Student's unpaired t-test for normal distributions, and by the Mann-Whitney U-test in

abnormal distribution. The association between PON1 192 genotype and the development of malignancy was examined by calculating odds ratio. The distribution of genotypes were tested for Hardy Weinberg equilibrium (HWE) (with  $df = N - 2$ ) P- value of 0.05 were considered statistically significant. The persons correlation analysis were used to assess the relationship between different parameters

### 3. Results

As regard age as shown in table (1) there was no significant value in CRC patients compared to controls ( $58.44 \pm 3.70$  VS  $58.54 \pm 5.97$ ; P0.909). In the present study as shown in table (4) and figures (1, 2 and 3), the serum level of HDL and TG were significantly decrease in patients compared with controls ( $33.24 \pm 2.67$  vs  $52.16 \pm 5.73$ ; P 0.0001\*) & ( $143.80 \pm 3.79$  vs  $150.57 \pm 6.77$ ; P 0.001\* ) respectively. The serum levels of LDL & TC show significant increase in CRC patients compared with controls ( $152.28 \pm 2.82$  vs  $87.67 \pm 7.34$ ; P 0.0001\*) & ( $194.92 \pm 198.69$  vs  $165.52 \pm 3.47$ ). Our study as shown in (table 4 and Fig. 4 and 5) shows significant reduction of serum levels of PON1 in CRC patients compared with control group ( $272.60 \pm 118.2$  vs  $394.09 \pm 81.84$ ; P <0.001\*\*\*) and also significant reduction of serum level of ARE in CRC patient compared to controls ( $202.04 \pm 33.97$  vs  $228.40 \pm 13.17$ ; P <0.001\*\*\*)).

The distribution pattern of the PON 1 Q192R genotypes (QQ, QR and RR) in CRC patients group of the present study was similar to what was observed in the control group (as shown in table 3 and figure 6). The number of QQ genotype observed in CRC patients and control populations was higher than the QR genotype. QR genotype was the least frequent. The distribution of the Q and R alleles was significantly different in the two populations. In CRC patients, the frequency of the Q allele was higher than what was observed in the control population; whereas the R allele was more frequent in the control population.

Table (5) shows the relation between PON192 with different parameters in patients group. It was observed that there was no statistically significant difference in age between 3 genotype, the QR genotype recorded higher values in TC level than QQ and RR genotypes and these differences were statistically significant, RR genotype recorded higher values in HDL-c level than QQ and QR genotypes and these differences were statistically significant, RR genotype recorded higher values in LDL-c level than QQ and QR genotypes and these differences were not statistically significant. The QQ genotype recorded higher values in TG and ARE levels than QR and RR genotypes and these differences were statistically

significant, The RR genotype recorded higher values in PON1 levels than QQ and QR genotypes and these

differences were statistically significant.

Table (1): Comparison between the two studied groups according to age

	Patients (n=50)	Control (n = 80)	t	p
Age (years)				
Min. – Max.	52.0 – 65.0	51.0 – 68.0		
Mean ± SD	58.44 ± 3.70	58.54 ± 5.97	0.115	0.909

t: Student t-test

Table (2): The observed and expected values of the genotype frequencies among the studied cases groups.

Genotype	Observed	Expected		p
PON1 192				
QQ	30	28.9	0.754	0.385
QR	16	18.2		
RR	4	2.9		

Table (3): Distribution of PON1 (Q192R) genotype frequency and alleles frequency in the CRC patients and controls.

Enzyme	Genotype	CRC patients (n = 50)	Control (n = 80)	Odds ratio	Confidence interval 95%	P value
PON-1 192	QQ	30 (60.0)	20 (25.0)	4.500	2.106 – 9.613	<0.001*
	QR	16 (32.0)	36 (45.0)	0.575	0.275 – 1.205	0.141
	RR	4 (8.0)	24 (30.0)	0.203	0.066 – 0.627	0.003*
	Q Allele	76 (76.0)	76 (47.5)	3.500	2.011 – 6.091	<0.001*
	R Allele	24 (24.0)	84 (52.5)	0.286	0.164 – 0.497	<0.001*

$\chi^2$ : Chi square test

\*: Statistically significant at  $p \leq 0.05$

Table (4): Comparison of mean values of lipid profile, PON-1 and ARE activity among diseased group compared with control group.

	Patients (n=50)	Control (n = 80)	Test of sig.	p
TC (mg/dl)				
Min. – Max.	143.0 – 1158.0	161.0 – 186.0	Z = 8.462*	<0.001*
Mean ± SD	194.92 ± 198.69	165.52 ± 3.47		
HDL-c (mg/dl)				
Min. – Max.	30.0 – 40.0	44.0 – 60.60	t = 25.437*	<0.001*
Mean ± SD	33.24 ± 2.67	52.16 ± 5.73		
LDL-C (mg/dl)				
Min. – Max.	150.0 – 160.0	73.60 – 105.0	t = 70.799*	<0.001*
Mean ± SD	152.28 ± 2.82	87.67 ± 7.34		
TG (mg/dl)				
Min. – Max.	140.0 – 160.0	140.90 – 160.0	Z = 6.232	<0.001*
Mean ± SD	143.80 ± 3.79	150.57 ± 6.77		
PON-1 (U/L)				
Min. – Max.	128.0 – 487.0	280.70 – 567.90	Z = 4.614*	<0.001*
Mean ± SD	272.60 ± 118.82	394.09 ± 81.84		
ARE activity (U/L)				
Min. – Max.	190.0 – 437.0	200.0 – 254.0	Z = 9.226*	<0.001*
Mean ± SD	202.04 ± 33.97	228.40 ± 13.17		

t: Student t-test

MW: Mann Whitney test

\*: Statistically significant at  $p \leq 0.05$

Table (5): Relation between PON192 with different parameters in patients group:

Item	PON192			Test of sig.	p
	QQ (n = 30)	QR (n = 16)	RR (n = 4)		
Age					
Min. – Max.	52.0 – 65.0	54.0 – 64.0	59.0 – 61.0	F = 0.404	0.670
Mean ± SD	58.40 ± 4.06	58.13 ± 3.44	60.0 ± 1.15		
TC (mg/dl)					
Min. – Max.	145.0 – 162.0	143.0 – 1158.0	145.0 – 155.0	KW $\chi^2 = 6.875^*$	0.032*
Mean ± SD	156.13 <sup>a</sup> ± 6.44	278.88 <sup>ab</sup> ± 343.23	150.0 <sup>b</sup> ± 5.77		
Sig. bet. Grbs	p <sub>1</sub> = 0.064, p <sub>2</sub> = 0.042*, p <sub>3</sub> = 0.148				
HDL-c (mg/dl)					
Min. – Max.	30.0 – 33.0	34.0 – 37.0	39.0 – 40.0	F = 145.778*	<0.001*
Mean ± SD	31.47 <sup>a</sup> ± 1.04	35.0 <sup>b</sup> ± 1.03	39.50 <sup>c</sup> ± 0.58		
Sig. bet. Grbs	p <sub>1</sub> < 0.001*, p <sub>2</sub> < 0.001*, p <sub>3</sub> < 0.001*				
LDL-C (mg/dl)					
Min. – Max.	150.0 – 160.0	150.0 – 155.0	151.0 – 155.0	F = 3.178	0.051
Mean ± SD	152.93 <sup>a</sup> ± 3.14	150.88 <sup>b</sup> ± 1.67	153.0 <sup>ab</sup> ± 2.31		
TG (mg/dl)					
Min. – Max.	141.0 – 160.0	140.0 – 143.0	144.0 – 144.0	KW $\chi^2 = 17.118^*$	<0.001*
Mean ± SD	144.93 <sup>a</sup> ± 4.46	141.63 <sup>b</sup> ± 0.89	144.0 <sup>a</sup> ± 0.0		
Sig. bet. Grbs	p <sub>1</sub> < 0.001*, p <sub>2</sub> = 0.699, p <sub>3</sub> < 0.001*				
ARE activity (U/L)					
Min. – Max.	196.0 – 437.0	190.0 – 198.0	195.0 – 199.0	KW $\chi^2 = 37.729^*$	<0.001*
Mean ± SD	206.27 <sup>a</sup> ± 43.59	195.38 <sup>b</sup> ± 2.42	197.0 <sup>c</sup> ± 2.31		
Sig. bet. Grbs	p <sub>1</sub> < 0.001*, p <sub>2</sub> = 0.366, p <sub>3</sub> = 0.385				
PON-1 (U/L)					
Min. – Max.	128.0 – 210.0	390.0 – 398.0	480.0 – 487.0	KW $\chi^2 = 22.435^*$	<0.001*
Mean ± SD	179.87 <sup>a</sup> ± 26.79	393.75 <sup>b</sup> ± 3.21	483.50 <sup>ab</sup> ± 4.04		
Sig. bet. Grbs	p <sub>1</sub> < 0.001*, p <sub>2</sub> < 0.001, p <sub>3</sub> < 0.001*				

Different superscripts are significant

F: F test (ANOVA), Sig. bet. grps was done using Post Hoc Test (LSD)

<sup>KW</sup> $\chi^2$ : Chi square for Kruskal Wallis test, Sig. bet. grps was done using Mann Whitney test

p<sub>1</sub>: p value for comparing between QQ and QR

p<sub>2</sub>: p value for comparing between QQ and RR

p<sub>3</sub>: p value for comparing between QR and RR

\*: Statistically significant at p ≤ 0.05

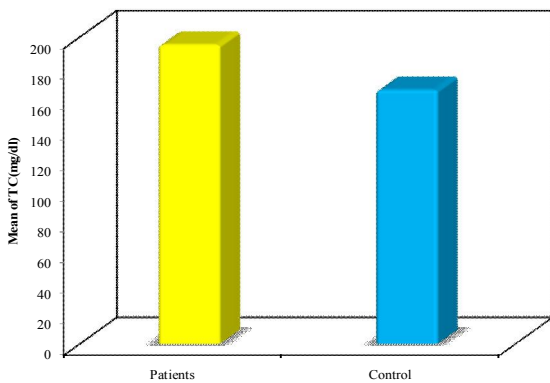


Figure (1): Comparison between the two studied groups according to TC

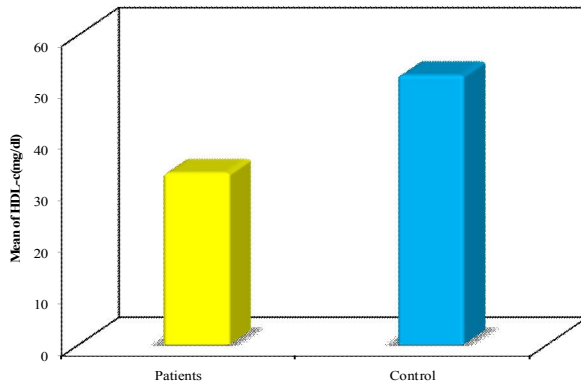


Figure (2): Comparison between the two studied groups according to HDL-c

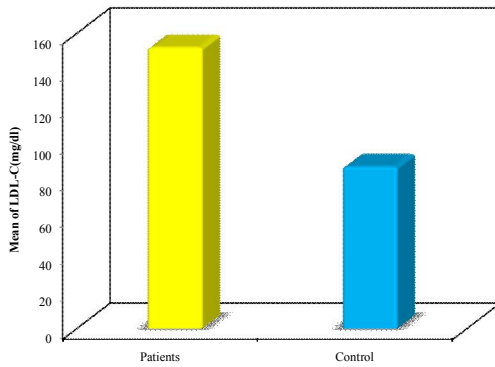


Figure (3): Comparison between the two studied groups according to LDL-C

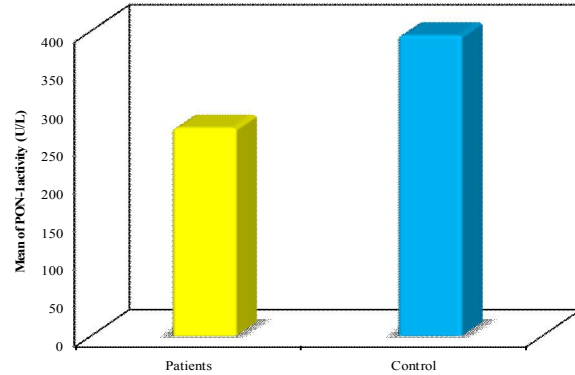


Figure (4): Comparison between the two studied groups according to PON-1 activity

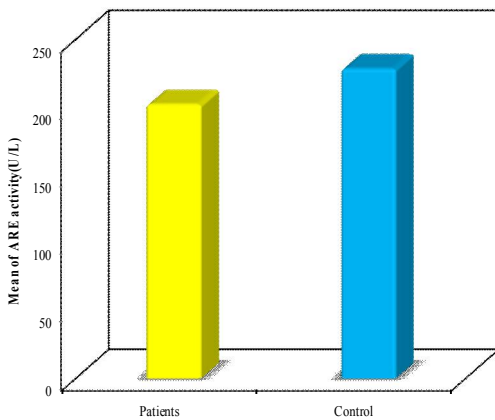


Figure (5): Comparison between the two studied groups according to ARE activity

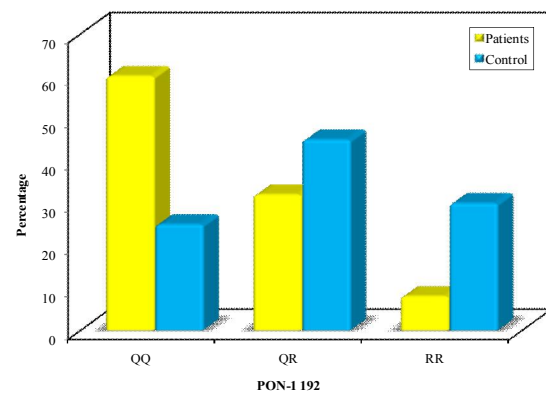


Figure (6): Distribution of PON1 (Q192R) genotype frequency and alleles frequency in the CRC patients and controls

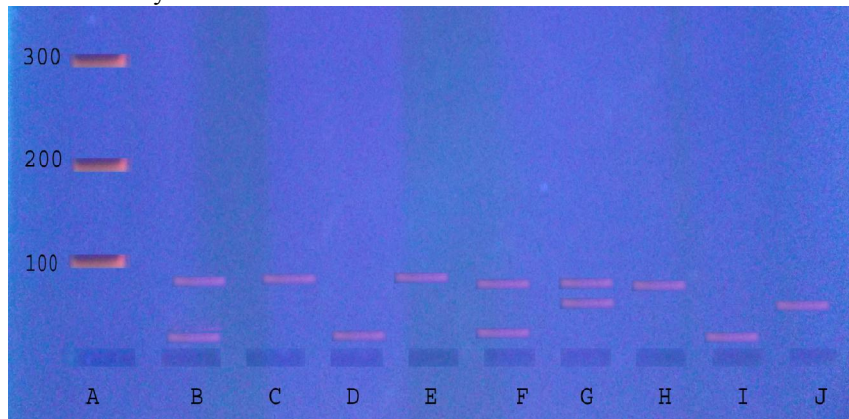


Figure (7): Agarose gel electrophoretic analysis PON1 (Q192R) polymorphism after (AIWI) BspPI digestion analysis:

Lan A represent the molecular (DNA molecular weight marker).

LanB: represent the product of PCR amplification of the gene that is presented by QR at 99 bp and QQ at 30 bp fragment.

Lan C: represent the product of PCR amplification of the gene that is presented by QR at 99 bp fragment.

LanD: represent the product of PCR amplification of the gene that is presented by RR at 30bp fragment.

LanE: represent the product of PCR amplification of the gene that is presented by QQ at 99 bp fragment.

Lan F,G,H,I,J: represent the product of PCR amplification of the gene that is presented by QR at 99,69,30bP fragment.

#### 4. Discussion

Colorectal cancer (CRC) is one of the major causes of mortality and morbidity, and is the third most common cancer in men and the second most common cancer in women worldwide. The incidence of CRC shows considerable variation among racially or ethnically defined populations in multiracial/ethnic countries. The tumor aegnesis of CRC is either due to the chromosomal instability or micro satellite instability or involving various proto oncogenes, tumor suppressor genes, and also epigenetic changes in the DNA (Mackness et al., 1991).

All cells in the human body sustain a condition of homeostasis between the oxidant and antioxidant species. Oxidant-antioxidant balance is very important for normal metabolism, signal transduction and regulation of cellular functions. In fact, the development of cancers and their progression have already been linked to DNA mutations and damage, genome instability, and cell proliferation caused by oxidative stress (Ellidag et al., 2013).

As regard age in the present study as shown in (table 1) there is no significant difference value in CRC patients compared to controls. These results were in accordance to Hyun-Ju Kim et al (2013) who recorded that there is no significant difference in age of CRC patients compared to controls. In contrast Hamit et al (2013) showed significant value of age and increase in incidence of CRC in elderly people.

Lipids are major cell membrane components essential for various biological functions including cell growth and division of both normal and malignant cells. The increased utilization of lipids by the highly proliferating malignant cells for new membrane biogenesis results in the depletion of total cholesterol (TC) levels. (Gilbert et al., 1981). It is also believed that carcinogens induce generation of free radicals and reactive oxygen species that may result in oxidation or peroxidation of polyunsaturated fatty acids which releases peroxide radicals. This affects the essential constituents of the cell membrane and might be involved in carcinogenesis (Patel et al., 2004).

As regard Plasma Lipids (as shown on table 4, fig 1,2 and 3), the present work showed significant reduction of the serum level of HDL and TG concomitant with significant increase in the serum levels of LDL and TC in patients compared with controls Our results confirmed and supported results of Balci et al., (2012) who indicated that the serum total cholesterol and LDL cholesterol levels in controls were significantly lower compared to patients with colorectal cancer and serum HDL cholesterol was significantly lower in the CRC patients as compared with controls. Similarly Harel (2004) indicted that, plasma HDL and TG significantly reduced in patient with ovarian cancer. Furthermore

Valko (2006) recorded reduction in HDL serum level in lung cancer patients than controls. In contrast, Gan et al (1991) showed no significant reduction of plasma lipids level in CRC and bladder cancer patients compared to controls. Also Cejas et al (2010) recorded no significant differences were observed between esophageal cancer patients and controls.

The human body has a number of endogenous free-radical scavenging systems. HDL-associated PON1 and ARE are among the enzymes involved in such systems. The physiological role of PON1 is protection of LDL from oxidation via hydrolyzing lipid peroxides. These enzymes contribute to the detoxification of organophosphorus compounds and carcinogenic lipid-soluble radicals from lipid peroxidation (Krzystek-Korpacka et al., 2013). Some studies have revealed that PON1 expression is alleviated in human lung cancer, pancreatic and gastric cancer. Epidemiologic and molecular studies showed important genetic polymorphism at positions 192 of the PON1 gene which arises from the substitution of amino acids at position 192. Substitution of glutamine (Q genotype) at position 192 of the PON gene by arginine (R genotype) leads to the polymorphism (Q192R) (Elkiran et al., 2007).

The present study as shown in (table 4 and Fig. 4 and 5) showed significant reduction of serum activity of PON1 and ARE in CRC patients compared with control group. These results were in line with Gonenc et al (2013) who reordered a significant reduction of serum level of PON1 and ARE in CRC patient. The reason for this might be an increase in intracellular oxidants that leads to a parallel decrease in antioxidants, finally disrupting the structure of enzymes, in this case PON1 and ARE. Also Emin et al (2007) recorded decrease activities of PON1 and ARE enzymes on patients with lung cancer compared to the control groups and this may be due to decrease HDL concentrations that affect PON1 activity.

Serum PON1 together with ARE have been demonstrated to function as single enzyme. PON1 activity varies widely among individuals, partly related to polymorphisms. The PON1 gene has two common coding region polymorphisms (Aynacioglu et al., 1999). A genetic polymorphism of PON1 activity determines high versus low paraoxon hydrolysis in human populations (Humbert et al., 1993). PON1 also has ARE activity, which doesn't exhibit activity polymorphism and can therefore serves as an estimate of enzyme protein. Reduced serum PON1 activities have been reported in patients with diabetes mellitus, hypercholesterolemia and cardiovascular disease. These diseases are characterized by an increase oxidative stress (Ayub et al., 1999).

An elevated oxidative status has been found in many types of cancer cells, and the introduction of chemical and enzymatic antioxidants can inhibit tumor cell proliferation. High doses and/or inadequate removal of reactive oxygen species (ROS) result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological molecules including DNA (Cejas et al., 2004). Human serum PON1 and ARE are esterase enzymes that have lipophilic antioxidant characteristics. Serum PON1 binds to HDL and contributes to the elimination of organ phosphorus compounds, such as paraoxon, and carcinogenic lipid soluble radicals from lipid peroxidation. PON1 is one of the endogenous free-radical scavenging systems in the human body (Aviram et al., 1998).

Some studies reported that a total of 198 SNPs occur in the non-translated 5-end, exonic regions, intronic regions and the non-translated 3-end of the PON 1 gene (La Du, 2003). Most of these SNPs are of great importance because they affect the activity of the PON 1 enzyme to a large extent, although the PON 1 activity may also be influenced by the disease status of the populations being studied (Nevin et al., 1996). Among the exonic SNPs, the two most common and widely studied are the Q192R and L55M SNPs. The Q192R polymorphism occurs on exon 6 of the paraoxonase gene; and it results in the replacement of the codon CAA which codes for glutamine (Q) by codon CGA which codes for arginine (R) at position 192 of the amino acid sequence (Humbert et al., 1993). The Q192R substitution has been shown to affect the activity of PON 1 in an individual whilst the M55L substitution at position 55 was reported in an earlier study to be a determinant of the serum concentration of the enzyme. A later study, however, revealed that the 55 polymorphism also has a significant effect on the activity of PON1 (Mackness et al., 1991). The triplet codon that codes for glutamine (Q) or arginine (R), thereby rendering a unique polymorphic change at position 192, serves as the basis for classification of PON 1 enzyme into three phenotypes, represented as A, B and AB. The A phenotype (allozyme) corresponds to the polymorph with glutamine (Q) at position 192; whereas the polymorph substituted with arginine (R) gives the B phenotype (Adkins et al., 1993). The AB phenotype has both Q and R substitutions as an allelic pair at the gene loci.

The present study which conducted on Egyptian persons (as shown in table 3 and figures 6 and 7) demonstrated that, the QQ genotype was the most frequent among the CRC patients and the controls (60%), followed by QR genotype (32%). The RR was the least frequent genotype in the two populations (8%). There was significant difference in genotype

distribution of patients and controls. In accordance Kuremoto et al., (2003) recorded that, RR genotype was the most frequent among the diabetics and the controls, followed by QQ genotype. The QR was the least frequent genotype in the two populations.

Studies conducted in many populations have shown varying distributions of the PON 1 Q192R genotypes and alleles. The results indicated that the variations may be dependent on the populations studied, that is their respective continents and countries of origin. It has also been observed that even within a particular country, many variations can be seen at the ethnic level. The R allele has been reported to be more frequent in African-Americans, some Southern, Central and West Africans; whereas, the Q allele is common in the temperate regions of Europe and North America (La Du, 2003). Several studies indicated that there an association between PON1 192 QQ genotype and an increased risk of lung, breast and prostate cancer, and osteosarcoma. Other studies showed that the R allele is associated with an increased risk of ovarian cancer, non-Hodgkin's lymphoma, lung cancer and multiple myeloma.

The present study (as shown in table 3 and figures 6 and 7) also revealed a significant alteration in the activity of PON1 in patient with CRC patients in relation to gene polymorphisms: the QQ genotype has lowest enzyme activity followed by, the QR genotype which has moderate activity, and the RR genotype has the highest enzyme activity. The Q192R polymorphism of the PON1 gene can also modify the affinities and catalytic activities of the enzyme PON1. The alloenzyme coded by 192 R allele hydrolyzes different substrate faster than alloenzyme coded with PON1 192 Q alleles. However Q type isozyme allele is more efficient in protecting against low-density lipoprotein oxidation than the R-type. As regard ARE no significant change was observed among different PON1 Q192R genotypes. The causes are not known and must be investigated further. The importance of PON1 as a predictive risk factor and its role in prognosis must be investigated. The limitation of present work is sample size and grouping of tumor histologically.

We conclude that serum PON1 and ARE activities were significantly lower in CRC Patients compared to healthy subjects concomitant with significant increase in the serum level of LDL and significant reduction of HDL and TG. These observations suggested the hypothesis that defects in the antioxidant system capacity and altered PON 1 activity may be involved in the pathogenesis of CRC. More wide spread studies are recommended to assess the relation between PON1 activity and pathogenesis of CRC.



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**Ethical statement**

This study was carried out at Medical Biochemistry Departments and General Surgery Departments, Sohag University, Alazhar University and during the summer months (May, June and July) of 2016. Written informed consent was taken from each participants and this study was approved from the ethics committee of Faculty of Medicine, Sohag University, Sohag, Egypt.

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