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Ya Association of Paraoxonase-1(Q192R and L55M) gene polymorphisms and activity with colorectal cancer and effect of surgical intervention

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Background: Colorectal cancer (CRC) is the leading cause of cancer-related deaths. Oxidative DNA damage may contribute to the cancer risk. The antioxidant paraoxonase is an endogenous free radical scavenger in the human body. The aim of this study was to find association between preoperative and postoperative serum paraoxonase-1 (PON1) and arylesterase (ARE) activities in patients with newly diagnosed CRC and to determine (PON1) Q192R and L55M gene polymorphisms. Patients and Methods: this study involved a total of 50 patients with newly diagnosed CRC and 80 healthy controls. PON1 and ARE activities were determined using an enzymatic spectrophotometric method. PON1 Q192R and L55M gene polymorphism was determined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based restriction fragment analysis. The restriction enzyme AlwI was used to examine the Q192R polymorphism and Hsp92II for the L55M polymorphism. Significant differences in the PON1 Q192R polymorphism were found between patients and controls. The Q allele was more frequent in the patient group than in controls, while the R allele was more frequent in the control group. No significant differences were found in the L55M polymorphism. Additionally, there were no significant differences in L and M allele frequencies. The activities of PON1 and ARE lower in QQ and MM genotype, Conclusion: serum PON1 and ARE activities were significantly lower in CRC Patients compared to healthy subjects. The R allele may protect against colorectal cancer.

Keywords: colorectal cancer, Paraoxonase-1, Q192R and L55M gene polymorphisms

INTRODUCTION

Colorectal cancer (CRC) is the term for colon or rectum malignant epithelial tumor. CRC is an important global health problem. CRC is the third most common type worldwide, making it the fourth most common cause of cancer-related death (Aiello et al., 2014). The etiology of CRC remains elusive. It is estimated that up to 10% of CRC cases may be due to hereditary factors (Houlston and Peto 1996), while the other of 90% of CRC cases (sporadic cases) may be attributable to various environmental and lifestyle factors, such as dietary habits,
obesity and physical inactivity. It was found that the human colorectal have increased levels of different markers of oxidative stress, such as increased levels of reactive oxygen species (ROS) which may also play a role in developing CRC. Furthermore, genetic predisposition to CRC may involve polymorphic variations in genes encoding for antioxidant enzymes. Recent studies have found an association between a genetic variant in some genes of antioxidative protective mechanisms and CRC risk. Genetic variations in these enzymes may affect pattern of colonic expression may modulate the ability of gut epithelial cells to cope with damaging metabolic challenges. Possibly altering the risk for CRC.

Human serum Paraoxonase (PON) and arylesterase (ARE) are antioxidant enzymes (Ellidag et al., 2014) synthesized in the liver. PON genes are located in the q21.3 region of the long arm of chromosome 7 (Connolly et al., 2003). The human paraoxonase -1 (PON1) enzyme is a polymorphic, found in plasma together with high density lipoprotein cholesterol (HDL –c high density and it plays important role in preventing the oxidation of plasma lipoproteins (Mackness et al., 1991). It is an esterase enzyme metabolizes many different substrates including organophosphorus (OPs) compounds and (Geldmacher-von Mallinckrodt and Diepgen 1988; Davies et al. 1996), drugs. There are two polymorphisms in the PON1 coding region at positions Gln192→Arg (Q192R) and Leu55→Met (L55M). Q192R have been more widely studied, because the two alleles enzymes have different affinities and catalytic activities towards a number of substrates. Studies showed that polymorphisms of PON1 gene may change PON1 activity. In a study by Eckerson et al (1983) the PON1 activity of PON1 192 Q allele carriers was reported to be lower than that of the R carriers. Reduced PON1 activities have been reported in some malignancy such as gastric and pancreatic carcinoma. Although the association of PON1 polymorphisms and its serum activity were elucidated in several types of malignant tumors such as cancer ovary lung and liver . However there is only one research paper published on the relationship between PON1-ARE activity and PON1 Q192R, L55M polymorphism in colorectal was only in published single paper. Therefore the aim of the present study was to shed light on the role of PON1-ARE activity and assess the role of PON1 Q192R, L55M polymorphisms in colorectal cancer patients.

PATIENTS AND METHODS

Patients

Fifty newly diagnosed CRC patients (14 females, 36 males; mean age 58.44±3.74 years) admitted to the Outpatient Clinic of Surgery Sohag University Hospital, were included in the present study. The diagnosis of patient was done clinically and confirmed by the microscopic evaluation of colonoscopic biopsy samples, followed by total excision of tumors. The following pathologic findings were assessed: according to modified Dukes classification (stage A=6cases, stage B=24 cases, stage, C=14cases and stage D=6cases). Eighty healthy control subjects of corresponding gender and age (24 females and 56 males; mean age: 44.45±2.88 years) were also enrolled for comparison. The clinico pathological features of the patients and controls were summarized in Table 1 Any patient under antioxidant drugs was excluded. Written informed consent was obtained from patients was taken from patient and controls to be enrolled in the study after explanation of study details. The study was approved by the Ethical Committee Faculty of Medicine.

METHODS

Venous blood was collected from all subjects preoperative after 12 hours fast. Each sample was divided into two halves, one half for serum preparation and the other half was put into tubes prepared with EDTA. All blood samples were stored at -20°C. The postoperative blood samples were collected from 44 patients one month postoperative (there were 6 cases missed to follow up) and the sera are stored until assay.

1-Determination of Plasma Lipids and tumor markers

Total cholesterol, high density lipoprotein cholesterol (HDL-c), was determined as routine parameters by using commercially available assay kits. Low density lipoprotein cholesterol (LDL-c) was estimated using Friedwald formula: LDL-C = total cholesterol – (HDL cholesterol + triglycerides/5) mg/dl. The levels of serum alpha fetoprotein (AFP) and carcino embryonic antigen (CEA) were measured by ELISA before and after one month from the operation.

2-Determination of PON1 activity

PON1 activity was measured by adding 20µl of serum to Tris buffer (100 mmol/l, pH 8.0) containing 2 mmol/l CaCl2 and 1 mmol/l paraoxon (O, O-diethyl-O-nitrophenylphosphate (Sigma) The rate of generation of P-nitrophenol was determined at 405 nm, 37°C over 50 second after 1 minute lag time with the use of continuously recording spectrophotometer as described previously by Eckerson et al.,(1983) and Mackness et al., (1991). 

3-Determination of Arylesterase Activity

Arylesterase activity was measured using phenylacetate as a substrate as previously described by Kilic et al (2005) The reaction mixture contained 750 µl of 0.1 mol/l Tris-HCl
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Table 1. Clinico pathological characteristic of CRC patients (N: 50)

<table>
<thead>
<tr>
<th>Character</th>
<th>Variable</th>
<th>NO of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>females, males</td>
<td>14(28%) 36(72%)</td>
</tr>
<tr>
<td>Age (mean ±SD year)</td>
<td>58.44±3.74 years</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>Well differentiated</td>
<td>46(92%) 4(8%)</td>
</tr>
<tr>
<td></td>
<td>Poor differentiated</td>
<td></td>
</tr>
<tr>
<td>Dukes classification</td>
<td>A 6(12%) B 24(48%) C 14(28%) D 6(12%)</td>
<td></td>
</tr>
</tbody>
</table>

(pH 8.5), 1 mmol/l CaCl2, 125 µl of 12 mmol/l phenylacetate 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). An international unit is the amount of hydrolyzed substrate in mmol/minute.

4- Polymorphism analysis

Genomic DNA was extracted from whole heparinized blood samples, using (CinnaPure DNA Cat No. PR881612. Tehran). A previously reported by Adkins et al (1993) PCR-RFLP method was used to determine the PON1 192 and PON1 55 gene polymorphisms. PCR was used to amplify the gene with 5'-TAT TGT TGC TGT GGG ACC TGA G-3' and 5'-CAC GGT AAA CCC AAA TAC ATC TC-3' primers for determination of the PON1 192 gene polymorphism and 5'-GAA GAG TGA TGT ATA GCC CCA G-3' and 5'-TTT AAT CCA GAG CTA ATG AAA GCC-3' for the PON1 55 polymorphism. The PCR mixture (total, 25 µl) contained 1-2 µl DNA, 1 µl of each primer, 5 µl dNTP, 1.5 µl of MgCl2 and 0.3 µl Taq polymerase. For the PON1 192 gene polymorphism the mixture was incubated at 95˚C for 2 minutes, then 35 cycles of 94˚C for 1 minute to denature, 61˚C for 1 minute to anneal the primers and 72˚C for 1 minute to elongate the strand PCR. Amplification kit was obtained from (CinnaGen Co.Cat No. PR8252C, Tehran). After PCR process, the Alw1 (BspI) (Biolabs Cat. No. R0513S- England) restriction enzyme was used and 2% agarose gel electrophoresis was performed to identify the possible polymorphism. Alw1 (BspI) digestion generated the following fragments: PON1 192 R allele, fragments of 66 bp and 33 bp; PON1 192 Q allele, a single fragment of 99 bp. For the determination of the PON1 55 locus polymorphism, the PCR reactions started with incubation at 95˚C for 5 minutes and 30 cycles of denaturation for 1 minute at 92˚C, followed by annealing for 45 seconds at 52˚C and elongation for 45 seconds at 72˚C. The restriction enzyme was N1a111 (Hsp92I) (Biolabs Cat. No. R0125S- England) for determination of the PON1 55. N1a111 (Hsp92I) digestion generated the following fragments: for PON1 55 M allele, fragments of 126 bp and 44 bp; for PON1 55 L allele, a single fragment of 170 bp. The digested PCR products of the two PON1 polymorphisms were separated on 3% agarose and visualized using ethidium bromide.

Statistical analyses

The data were statistically analyzed Using SPSS software version 16, 0 (SPSS Inc, Chicago, IL, USA). 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 and by the Mann-Whitney U-test in abnormal distribution. The association between PON-1 192, 55 genotypes between CRC patient and control was examined by chi-square, Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to risk factor for CRC. The distributions of genotypes were tested for Hardy-Weinberg equilibrium (HWE) (with df = N-2). P value of 0.05 was considered statistically significant. The Pearson’s correlation analysis was used to assess the relationship between different parameter.

RESULTS

1: Clinical characters

Table 1 showed the mean age of CRC patients and it was 58.44± 3.74 years. Males in our study were more than females and the ratio of M/F was 2.57: 1. As regarding histopathological character, 92% of tumor was well differentiated and 4% was poorly differentiated. Dukes’
Comparing the lipid profile in CRC patients and control, The serum total cholesterol, TG, LDL levels in patients with CRC were significantly higher compared to control \((P < 0.01)\) (Table 1). Serum HDL cholesterol was significantly lower \((P < 0.001)\) in the cancer patients as compared with controls. However the serum activity of PON1 and ARE were lower in CRC patients compared to controls which was statistically significant \((242.6 \pm 120.0 \text{ and } 197.3 \pm 72.15 \text{ activity (U/L) respectively versus } 394.1 \pm 83.44 \text{ and } 228.4 \pm 83.43 \text{ activity (U/L) respectively in control. } P \text{ value } <0.001\) (Table 2). There was significant correlation between PON1 and ARE activities in patient group. The PON1 activity was standardized with HDL concentrations \((\text{PON1}/\text{HDL ratio})\). It was found that PON1/HDL is low in CRC patients \((7.469 \pm 1.8 \text{ versus } 8.42 \pm 2.6 \text{ P value NS})\).It is obvious from table 2 the PON1/ARE ratio was low in patients as compared with control\((1.229 \pm 0.3 \text{ and } 1.725 \pm 0.4 \text{ respectively} \) Furthermore serum levels of AFP and CEA were statistically significant higher in the CRC patient compared with control \((33.41 \pm 4.65 \text{ and } 17.96 \pm 2.3 \text{ (ng/ml) versus } 1.65 \pm 0.19 \text{ and } 1.5 \pm 0.11 \text{ (ng/ml) } P \text{ value } <0.0001\).

### 3: Effect of surgery in antioxidants enzymes activity and tumor markers

It is clear from table 6 that surgery has effect on the antioxidants enzymes activity and tumor marker. Mean serum activity of PON1 and ARE was reduced significantly after one month from surgery and the activity of these enzymes almost reached normal level\((272.60 \pm 118.82 \text{ and } 197.3 \pm 72.15 \text{ versus } 394.09 \pm 81.84 \text{ and } 228.40 \pm 13.17 \text{ respectively})\). Also serum levels of AFP and CEA decreased significantly after one month from surgery (table3).
Table 4. Distribution of PON1 (Q192R and L55M) genotype frequency and alleles frequency in the CRC patients and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CRC patients (n = 50)</th>
<th>Control (n = 80)</th>
<th>Odds ratio</th>
<th>Confidence interval 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PON-1 55</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>38 (76%)</td>
<td>40 (50%)</td>
<td>3.167</td>
<td>1.447 – 6.930</td>
<td>&lt;0.003*</td>
</tr>
<tr>
<td>LM</td>
<td>10(20%)</td>
<td>24 (30%)</td>
<td>0.583</td>
<td>0.251 – 1.354</td>
<td>0.207</td>
</tr>
<tr>
<td>MM</td>
<td>2 (4%)</td>
<td>16(20%)</td>
<td>0.167</td>
<td>0.0366 – 0.760</td>
<td>&lt;0.010*</td>
</tr>
<tr>
<td>L Allele</td>
<td>86(86%)</td>
<td>104(65%)</td>
<td>3.308</td>
<td>1.724 – 6.346</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>M Allele</td>
<td>14(14%)</td>
<td>56(35%)</td>
<td>0.302</td>
<td>0.158 – 0.580</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>PON-1 192</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QQ</td>
<td>30(60%)</td>
<td>20(25%)</td>
<td>4.500</td>
<td>2.106 – 9.613</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>QR</td>
<td>16(32%)</td>
<td>36(45%)</td>
<td>0.575</td>
<td>0.275 – 1.205</td>
<td>0.141</td>
</tr>
<tr>
<td>RR</td>
<td>4(8%)</td>
<td>24(30%)</td>
<td>0.203</td>
<td>0.066 – 0.627</td>
<td>0.003</td>
</tr>
<tr>
<td>Q Allele</td>
<td>76(76%)</td>
<td>76(47.5%)</td>
<td>3.500</td>
<td>2.011 – 6.091</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>R Allele</td>
<td>24(24%)</td>
<td>84(52.5%)</td>
<td>0.286</td>
<td>0.164 – 0.497</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

χ²: Chi square test
*: Statistically significant at p ≤ 0.05

Table 4: Distribution of PON1 Gene polymorphisms

The distribution of PON1 L55M genotypes was consistent with Hardy–Weinberg equilibrium in cases and control. In our study for the PON1 gene L55M polymorphism, the LL genotype was most common genotype and it was found in 38 (76%) patients, whereas the LM genotype was found in 10 (20%) patients and the MM genotype was present in 2 (4%) patients. In the control group, LL, LM and MM genotypes were found in 40 (50%), 24 (30%), and 16 (20%) subjects, respectively. As regard the risk development of CRC, our data indicated that both LL homozygous (OR 3.167, 95% CI 1.447–6.93. P = <0.003) and MM homozygous (OR, 0.1667 95% CI 0.03–0.766, P = <0.010) significantly increased the risk of CRC. No significant association observed between (LM) genotype and CRC risk (OR 0.58, 95% CI 0.23–1.3 54, P = 0.207). The frequency of L and M alleles showed increased risk for CRC. Frequency of L and M in patients versus control is 86(86%) , 104(65%), 14(14%), 56(35%) respectively. The data for L and M alleles revealed (OR 3.3, 95% CI 0.23–1.3 54, P = 0.207). The frequency of L and M alleles showed increased risk for CRC. Frequency of L and M in patients versus control is 86(86%) , 104(65%), 14(14%), 56(35%) respectively. The analysis of our data showed that there was an association between QQ and RR genotypes and risk of CRC. (OR: 4.500, 95% CI 2.106 – 9.613, P<0.001 and OR: 0.203, CI 95% 0.066 – 0.6270, P <0.003) However there was not an association found between QR heterozygous genotype and developing risk for CRC. The frequency of Q and R alleles showed increased risk for CRC Frequency of Q and R in patients versus control is 76(76%) , 76(47.5%) , 24(24%) and 84(52.5%) respectively . Analysis the data for Q and R alleles revealed (OR 3.5, 95% CI 2.011–6.091 P =< 0.001 and OR 0. 286, 95% CI 0.164–0.497, P = 0.001). The distributions of the genotype and allele frequencies for PON1 L55M polymorphism in patient and controls are represented in table 4.

Table 5: Serum PON1 and ARE activity in different genotypes of PON 1 192 and PON1 55.

Serum PON1 activity was statistically significantly lower in PON1 Q192R and PON1 L55M genotypes carrier in CRC patient compared the control group as shown in Table 5. As regard ARE enzyme activity in PON1192/55 genotypes no significantly difference was observed between patients and control. PON1 activity was significantly higher in PON1 RR and PON1 55 LL genotype carriers in controls compared to that of the other genotypes (QQ, QR and MM, LM, respectively) (p<0.001) . Although there was difference in enzyme activities of PON1 and ARE among different genotypes and alleles of PON1 192/55, in patients group, this difference did not reached significant level.
Table 5. PON1 and ARE activity in the control and CRC patients according to PON1 55/192 genotype

<table>
<thead>
<tr>
<th>PON192</th>
<th>PON1 (U/L)</th>
<th>ARE (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control</td>
</tr>
<tr>
<td>QQ</td>
<td>159.9±30.65</td>
<td>319.1±87.48</td>
</tr>
<tr>
<td>QR</td>
<td>129.7±29.76</td>
<td>395.1±111.44</td>
</tr>
<tr>
<td>RR</td>
<td>95.6±20.43</td>
<td>485.1±83.44</td>
</tr>
<tr>
<td>F</td>
<td>2.153</td>
<td>15.854</td>
</tr>
<tr>
<td>p</td>
<td>0.127</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Different superscripts are significant
Data are expressed as mean ± standard deviation
F: F test (ANOVA)
*: Statistically significant at \( p \leq 0.05 \)

Table 6. Serum level of AFP and CEA according to PON1 55/192 genotype in CRC Patients

<table>
<thead>
<tr>
<th></th>
<th>LL (n = 38)</th>
<th>LM (n = 10)</th>
<th>MM (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum level of AFP (ng/ml)</td>
<td>33.8±5.06</td>
<td>32.0±2.96</td>
<td>32.7±2.40</td>
</tr>
<tr>
<td>Serum level of CEA (ng/ml)</td>
<td>17.03±0.93</td>
<td>16.4±1.40</td>
<td>17.6±0.070</td>
</tr>
</tbody>
</table>

Table 6 Tumor markers in different genotypes of PON 1 192 and PON1 55.

No significant difference was found among serum level of AFP and CEA in different genotypes of PON1 192 or PON1 55 (Table 6).

Table7. Correlation between tumor markers and antioxidant enzymes in CRC patients

Table 7 showed that there positive significantly correlation between PON1 enzyme activity and ARE enzyme activity,
Table 7. Correlation between tumor markers and antioxidant enzymes in CRC patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>PON-1</th>
<th>ARE</th>
<th>AFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARE activity (U/L)</td>
<td>r</td>
<td>-0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.433</td>
<td>-0.56</td>
</tr>
<tr>
<td>Serum level of AFP (ng/ml)</td>
<td>r</td>
<td>0.16</td>
<td>-2.55*</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.880</td>
<td>0.013</td>
</tr>
</tbody>
</table>

*Correlation is significant at the p 0.05 level

meanwhile there was negative correlation between serum level of CEA and ARE serum enzyme activity.

DISCUSSION

Colorectal cancer defined as the cancerous growths in the colon and rectum (Sameer; 2013). It is accepted that individual differences in genetic factors of low penetrance and environmental exposure may influence the risk for CRC (Kemp et al., 2004). Polymorphism in genes encoding for detoxification enzymes may be importance in susceptibility to toxic or carcinogenic environmental chemicals (Nebert., 2000; De Jong et al., 2002). 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. When an increase in the oxidants and a decrease in the antioxidant defense system cannot be prevented, the oxidative/antioxidative balance eventually shifts toward the oxidative status. Proteins, lipids and DNA are significant targets for oxidative attack, and modification of these molecules can increase the risk of somatic mutations and neoplastic transformation. 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 (Valko et al., 2006; Ellidag et al., 2014). 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 not fully understood, but it protects LDL from oxidation via hydrolyzing lipid peroxides. These enzymes contribute to the detoxification of (Ops) compounds and carcinogenic lipid-soluble radicals from lipid peroxidation.

To our knowledge, there is only two reports demonstrating lower serum PON1, ARE activities in CRC patients (Gouedard et al., 2003, Deakin et al., 2003). It has been shown that there is inversely relationship between serums PON1 and ARE activity and oxidative stress. Moreover it has been indicated that PON1 deficiency induced an increase in oxidative stress (Kumon et al., 2003) . In our study, serum PON1 and ARE activities were found to be significantly lower in patients with CRC compared to controls. Also PON1 / ARE ratio was significant low in patient compared to control. Many studies have indicated decreased arylesterase and/or paraoxonase activities in different types of cancer. Furthermore the serum lipid profile of CRC patients were more than control, and statistically significant. To test whether the decrease in the serum activity of PON1 was caused by reduction in HDL level, we calculated the PON1/HDL ratio which was low in patients compared to control. Positive correlation was determined between serum PON1 activity and serum levels of HDL. These results revealed that serum PON1 activity reduction did not dependent on HDL concentration in patients group. Our results support and confirm results of two recent reports done by Balci et al (2012) and Bulbuller et al (2013) who demonstrate Low plasma paraoxonase/arylesterase activities in CRC Turkish patients. The mechanism of the reduction of serum PON1 and ARE activities in CRC patients is not clearly understood. This reduction could be related to increased lipid peroxidation, since oxidized lipids are reported to inhibit PON1 and ARE activities. Furthermore the decrease in the activity of PON1 and ARE in CRC patients
may be due to inhibition of PON1 and ARE by generated carcinogenic lipids in cancer patients. Lower levels of PON1 may increase the CRC vulnerability to genomic damage by reducing the ability to detoxify inflammatory oxidants as well as dietary carcinogens. In addition, the activity of PON1 has been reported to be significantly reduced in some conditions accompanied the oxidative stress such as malignant tumors. Similarly PON1 activity in patients with CRC may be a result of elevated activity of ROS in CRC. PON1 is highly localized in colon and associated with the development of CRC is consistent with the anti-inflammatory role of this enzyme. PON1 activity is also reduced among patients with chronic colitis and possibly CRC cancer.

In our study we get step further by determination PON1 and ARE enzyme activity one month after removal of tumor by surgery. Our results indicated that the activities of these enzymes and serum levels of CEA and AFP returned almost two normal levels. Our result may be explained by removal of tumor decrease generation of free radicals and carcinogenic product of oxidative stress. The change occurred in serum PON1 and is activity before and after surgery is similar to changed in serum level of CEA and AFP. Therefore serum PON1 and ARE may be used as markers for evaluation tumor diagnosis and prognosis .This observation was not recorded before and required further investigation in different kind of cancer. Several studies have shown that there an association between PON1 (Q192R and L55M) gene polymorphisms and different types of cancer including cancer breast, bladder, prostate, ovarian cancer, pancreatic cancer and gastrointestinal tumor. However data are conflicting and other studies failed to find any association with cancer and PON1 (Q192R and L55M) gene polymorphisms. However as regard colorectal cancer, only one paper has so far been published about the association between PON1 gene polymorphism and CRC. To the best of our knowledge, we present here the first study that investigates the association of PON1 gene polymorphisms and activity of PON1 and ARE in CRC patients.

As regard PON1 L55M polymorphism, our results revealed that the frequencies LL genotypes and L allele were significantly higher in the CRC patients than in controls; moreover, patients having LL genotype significantly increased the risk of CRC up to 3.167 fold. Investigators of PON 1 L55 in relation to cancer ovary reported higher risk associated with the genotype, which is consistent with our results. The result of this study is inconsistent with the previous study of Van der Logt et al (2005) who did not find any differences between distributions of genotype and allele frequencies between patients and controls. The discrepancy in result may be due to selection of cases and different ethnic group. Our results can be explained by that PON1 55 LL genotype may alter the activity of enzyme by certain mechanism lead to develop CRC due to reduction in antioxidant and anti-inflammatory activity. Our study indicated that there an association and reduced risk of CRC in patients carried PON1-55M genotype. The PON1-55M variant is associated with a high enzyme activity which could mean that the conversion rate of carcinogenic compounds is increased and that these compounds may accumulate in the colonic lumen which make the epithelial of colon more vulnerable for production of colorectal carcinoma.

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. Our results showed that PON1 192 QQ genotype and Q allele is a significant risk factor for CRC and the risk of CRC up to 4.5 fold. Also, there was a marked reduction in the frequency of PON1 192 R allele in patients which mean that R allel associated with decrease risk with CRC. Our results does not agree with results of Van der Logt et al (2005) A similar results were reported, by Antognelli et al.(2005), who found that PON192/QQ was associated with a significant increased risk for prostate cancer Antognelli et al.(2005) and Gallicchio et al. (2007) Showed that R allele was associated with decreased risk of bladder cancer. They explained their result by that the Q to R substitution leads to the production of an enzyme with a higher detoxification activity against potentially carcinogenic products of oxidative stress and lipid peroxidation. In another study Lee et al.,(2005) have shown that carriers of the PON1 192 QQ genotype have increased risk of lung cancer. Also our result is in basic agreement of result of Ergen et al (2011) who find that PON1QQ genotype is a significant risk factor for risk factor for osteosarcoma. However the results of Ferre et al.(2003) suggest that in addition to genetic factors other contributors such as nutrition and lifestyle do play an important role in determining PON1 enzyme activity. The conflicting results may be due to ethnic difference, sample size, and selection bias.

The present study demonstrated a significant alteration in the activity of PON1 and 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 fast than alloenzyme coded with PON1 192 Q alleles However Q type isoenzyme allele is more 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.
As regard PON1 L55M genotypes, our results indicated that the MM a homozygous genotype has significant low enzymatic activity when it is compared to ML and LL genotypes, this result is in basic agreement with previous reports of Mackness et al (1997 ). Nevin et al. (1996) reported that the PON1 genotype accounts for 76% of the variation in serum PON1 enzyme activity level. In addition, PON1 serum levels are modulated by disease state, dietary, lifestyle and environmental factors and, therefore, may vary up to 13-fold between individuals (Deakin and James 2004; Draganov and LaDu 2004). L55 isoforms are more stable and resistant to proteolysis; this phenomenon partly explains their association with higher PON1 levels. L55 isoforms are more stable and resistant to proteolysis; this phenomenon partly explains their association with higher PON1 levels. However, human studies characterizing the PON1 polymorphisms have indicated the importance of estimating the PON1 status (i.e. genotype and phenotype taken together) rather than genotyping alone (Costa et al., 2003). 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. In conclusion our results showed low PON1 and ARE enzyme activities in patients compared to control .Lower serum activity of PON1 returned to normal level after removal of the tumor. Also we showed that there is risk between PON1/55 and 192 and CRC. Moreover, we revealed that PON1 192 R has less enzyme activity than PON192 Q. Similarly PON1 192L has less enzyme activity than PON192M.

REFERENCES


