Serum Paraoxonase and Arylesterase Activities in Esophageal Cancer:
A Controlled Study

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ABSTRACT

Aim: Upper gastrointestinal tract carcinomas are major health problem around the world. Esophageal cancer (EC) is usually diagnosed at an advanced stage; therefore most therapeutic approaches are palliative. The aim of the study was to investigate the possible relationship between serum activities of paraoxonase (PON1) and arylesterase (ARE), and clinicopathological characteristics in EC.

Method: Forty patients with EC and twenty seven healthy subjects were included in the study. The diagnosis of esophageal cancer was established by endoscopic examination of the esophagus and by biopsy confirmation. PON and ARE activities were determined by or with spectrophotometrically using paraoxon and phenyl acetate as substrates, respectively. Mann-Whitney-U test was used for statistical analysis.

Result: The mean serum PON and ARE activities were significantly higher in the cancer group compared to healthy controls. Besides, mean values of serum PON and ARE activities decreased in stage 3 and stage 4 EC patients compared with stage 2 EC patients. This decrease was statistically significant. There were no statistically correlation between other clinicopathological characteristics and serum PON and ARE activities in this EC patient group.

Conclusion: This is the first report on serum PON and ARE activities in patients with EC. Our results indicate that low serum PON and ARE activities may be an important indicator for advanced stage in EC. But these preliminary results need to be verified by further prospective studies for the early diagnosis of the tumor, for the detection of clinical relapse and for the monitoring of follow up treatment.

Key words: Esophagus cancer, paraoxonase, arylesterase
**INTRODUCTION**

Upper gastrointestinal tract carcinomas are a major health problem all around the world (1). Esophageal cancer (EC) is the eighth most common malignant neoplasm worldwide (2). It is endemic in many parts of the world, particularly in the developing nations (3). Unfortunately EC is often lately diagnosed; therefore most therapeutic approaches are palliative. Reactive oxygen species (ROS), superoxide anion ($\text{O}_2^-$), hydrogen peroxide ($\text{H}_2\text{O}_2$) and hydroxyl radical (HO·) are by-products of physiological cellular functions. When ROS are generated in large amounts they lead to damage of macromolecules such as DNA, proteins and lipids. Thus ROS activate mutagenic events associated with carcinogenesis. The action of ROS in pathological mechanisms and their central role in various fields of biomedical research including neurobiology, cardiology and cancer are more increasing day by day (4,5).

Paraoxonase (PON) is a Ca$^{2+}$-dependent glycoprotein that is associated with high density lipoprotein (HDL). Isoforms of PON are widely available in many tissues of animals such as kidney, liver, small intestine and also serum (6). Although serum PON can contribute to the elimination of organophosphorus compounds and carcinogenic lipid soluble radicals from lipid peroxidation, its physiological role is still not entirely known (7,8). Several non-genetic factors, including diet, acute phase reactions, pregnancy and hormonal factors, cigarette smoking and simvastatin therapy seems to contribute to the modulation of serum PON levels (9). Reduced PON activities have been reported in some clinical conditions such as diabetes mellitus, myocardial infarction and familial hypercholesterolemia (6,9). Oxidative stress and inflammation are believed to be important in carcinogenesis but there were only a few studies on changes in PON activity in cancer patients. Serum levels of PON were reported to be lower in patients with pancreatic and gastric cancer than healthy controls in two case control studies (10,11). PON activity is significantly decreased in lung cancer patients compared to healthy controls (12). Despite these data, the interaction between serum PON and cancer is not clearly known.

In the light of these findings, the aim of the present study was to investigate the possible relationships between serum activities of PON and ARE, and the clinicopathological characteristics of EC.

**MATERIALS AND METHODS**

The study was carried out in a total of forty patients with EC, admitted to the Department of Medical Oncology, Faculty of Medicine, Atatürk University. Pathological conditions leading to secondary lipid disorders, diabetes mellitus, cardiovascular diseases, renal failure, chronic infection and inflammation, alcohol abuse, antilipidemic and antioxidant drug use were the exclusion criteria. The control group was comprised of 27 healthy volunteers. Informed consent was obtained from all participating subjects before the initiation of the study which was carried out according to the rules of the Declaration of Helsinki. All patients were newly diagnosed and none of them were given anti-cancer therapy and radiotherapy before collection of blood samples for biochemical analysis. The diagnosis of esophageal cancer was estab-
lished by endoscopic examination of the esophagus and by biopsy confirmation. Tumors were classified into two types based on histological characteristics: squamous cell carcinoma and adenocarcinoma. Staging of the cancer patients were performed according to the latest pTNM criteria for carcinoma of the esophagus, established by the American Joint Committee on Cancer in 2002 (AJCC cancer staging manual, 6th ed., New York: Springer-Verlag, 2002).

**Biochemical Assays**

Fasting blood samples of 3-5 ml were collected in the morning by venipuncture in vacutainer tubes containing no additives, after 8-12 hours of fast. Sera were obtained after centrifugation at 3500×g for 5 minutes. Serum samples were stored at -20°C until analyses. Serum levels of total cholesterol, low density lipoprotein (LDL) and HDL-cholesterol were measured by an Olympus AU 600 automatic analyzer (Olympus Optical Co, Japan) using commercially available assay kits.

**Determination of paraoxonase (PON) and arylesterase (ARE) activities**

PON activity was measured using diethyl-p-nitrophenylphosphate as substrate, as previously described [13, 14]. Assays were made either without additional NaCl (baseline activity) or with 1 M NaCl included in the assay buffer (salt-stimulated activity), following the formation of p-nitrophenol by its absorbance at 405 nm for 3 min. Enzymatic activity was calculated using the molar extinction coefficient 18 000 M⁻¹ cm⁻¹. One unit of paraoxonase activity was defined as the enzyme quantity that disintegrates 1 μmol paraoxon substrate in one minute. Arylesterase activity was measured spectrophotometrically using phenylacetate as previously described [13,14]. The molar extinction coefficient of 1310 M⁻¹ cm⁻¹ was used for calculation of activity. One unit of arylesterase activity equaled 1 mmol of phenylacetate hydrolyzed per min, and activity was expressed as units per ml of serum.

**Statistical analysis**

Statistical analyses were performed with the SPSS 11.5 statistical package (SPSS Inc., Chicago, IL, USA). Data were expressed as median (range) for age and mean ± standard deviation for the other parameters. Mann-Whitney U non-parametric test were used as statistical analysis in comparing the data between the groups. A p value less than 0.05 was considered as statistically significant.

**RESULTS**

Demographical and laboratory parameters of the patients and healthy control subjects are shown in Table 1. No significant differences were observed between patients and controls (age, total cholesterol, HDL-cholesterol and LDL-cholesterol). Tumor characteristics (histological type, stage, location and type of esophageal obstruction) of the patients with esophageal cancer are shown in Table 2. The median age was 63.59±8.4 years (range 19-80). Fifty per cent of the patients with esophageal cancer had stage III tumors and there were no cases in stage I. Among the 40 patients with EC, 8 cases (20%) had adenocarcinoma and 32 cases (80%) had squamous cell carcinoma. As shown in Table 2, most of our study population presented with esophageal ob-

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### Table 1. Clinical characteristics and serum PON and ARE activities of patients and healthy controls.

| Characteristics                      | Patients (n:40) | Controls (n:27) | p value |
|--------------------------------------|----------------|----------------|---------|
| Age (year), median (range)           | 63.59 (19-80)  | 61.86 (26-75)  | >0.05   |
| Sex, n (%)                           |                |                |         |
| Female                               | 20(50)         | 11(40)         |         |
| Male                                 | 20(50)         | 16(60)         |         |
| Smoker / nonsmoker, n (%)            | 17(42)/23(58)  | 10(37)/17(63)  |         |
| Total cholesterol (mg/dl)            | 168.8±20.4     | 189.2 ± 28.4   | >0.05   |
| HDL-cholesterol (mg/dl)              | 38.3±8.6       | 44.9±6.5       | >0.05   |
| LDL-cholesterol (mg/dl)              | 121.7±43.0     | 112±13.5       | >0.05   |
| Basal PON (U/ml) mean±SD             | 59.1±35.2      | 38.6±36.5      | <0.005  |
| Salt stimulated PON (U/ml) mean±SD   | 89.2±56.8      | 68.4±61.4      | <0.005  |
| ARE (U/L) mean±SD                   | 37.1±14.4      | 20.2±13.3      | <0.001  |
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Table 2. Tumor characteristics of patients with esophageal cancer.

| pTNM Stage | n (%) | Serum PON activity | Serum ARE activity |
|------------|-------|--------------------|--------------------|
| II         | 7 (18) | 96.971             | 48.085             |
| III        | 20 (50) | 50.565             | 35.370             |
| IV         | 13 (32) | 51.984             | 33.715             |
| Histologic type |       |                    |                    |
| Squamous cell | 32 (80) | 21.610             | 20.890             |
| Adenocarcinoma | 8 (20)  | 16.060             | 18.940             |
| Tumor location |       |                    |                    |
| Upper part  | 2 (5)  | 79.100             | 45.000             |
| Middle part | 13 (32) | 62.684             | 36.207             |
| Lower part  | 25 (63) | 55.712             | 36.864             |
| Obstruction |       |                    |                    |
| No          | 4 (10)  | 41.625             | 26.775             |
| Partial     | 21 (52) | 64.295             | 42.042             |
| Complete    | 15 (38) | 56.613             | 32.820             |

Discussion

ROS, O2•-, H2O2, and HO are constantly generated in vivo during normal cell metabolism, particularly including mitochondrial respiration processes, fatty acid degradation in peroxisomes, biotransformation of various xenobiotics and drugs, and as a result of extracellular events, including virus or bacteria-infected cell phagocytosis, inflammation, UV and ionic irradiation (4). Increased amounts of ROS production or decreased ROS scavenging lead to oxidative stress. Oxidative stress may be harmful for cellular macromolecules such as DNA, lipids and proteins. ROS have been considered as DNA-damaging agents that increase cellular mutation rate and thus promote oncogenic transformation (15,16).

PON is a glycoprotein enzyme with 354 amino acids. It is encoded by the PON gene which is localized in the Q21-22 regions on the 7 chromosome. PON family of genes consists of 3 members: PON1, PON2 and PON3. It was suggested that PON2 and PON3 do not hydrolyze paraoxon due to a missing lysine residue in the 105. Besides, these two isoforms are not found in plasma (6,17). Carcinogenic lipid soluble radicals are formed as a result of lipid peroxidation, and PON1 binds to the these resultant (7,8,18). Serum PON1 activity was suggested to be inversely associated with oxidative stress in serum and macrophages and that PON1 deficiency results in increased oxidative stress (19).

Serum basal PON1, salt stimulated PON1 and ARE activities of our patients with esophageal cancer were higher compared to healthy controls and this difference was statistically significant. The activities of these enzymes decreased in patients with stage 3 and 4 esophageal cancer, compared to patients in stage 2 and this decrease was also statistically significant. Although the activities of these enzymes are assessed in some types...
of cancer, to our knowledge, this is the first study in the medical literature written in English, that focused on the association between serum PON and ARE activities and disease activity in esophageal cancer. The results of recent studies investigating the association between serum PON activity and various forms of cancer are highly variable. In a study conducted by Kafadar et al., serum PON1 activities of patients with high grade glioma and menegioma were significantly lower than controls (20). Similarly, Aкcay et al. reported decreased serum PON activities in patients with gastric and pancreatic cancers (10, 11). In another study conducted with lung cancer patients selected from a Turkish population, serum PON1 and ARE activities were found to be decreased (12). The results of the present study contradict the results of the above mentioned studies from the literature. One of the reasons that may account for this discrepancy may be the specific tumor biology of esophageal cancer. Genetic differences may also be one of the probable explanations for this discrepancy. Although we could not perform genotyping analyses in the present study due to technical inconveniences, in a study performed in patients with lung cancer, subjects with the PON genotype Q/Q were reported as cases with a significantly increased risk for the development of lung cancer. In the same study, a similar correlation between the R/R and Q/R genotypes and cancer development was not observed (22). While Marchesoni et al. could not find an association between PON1 polymorphism and prostatic cancer (22), Antognelli and colleagues reported an increased risk for prostatic cancer in patients with PON192/QQ genotype compared to those with the PON192/RR genotype (23). Finally, Van Der Logt et al. could not detect a significant difference in PON1 genotype between patients with colorectal cancer and healthy control subjects (24). We could not compare our results with the results of other studies in the literature since we were not able to find a study focusing on the association between esophageal cancer and PON activity. This is the first study in that aspect.

This study demonstrated that serum basal PON1, salt stimulated PON1 and ARE activities accompanying the increase in the stage of the tumors may be interpreted as the sign of a defect in the antioxidant defense system in these patients with advanced stages of esophageal cancer. But these preliminary results need to be verified by further prospective studies prior to making any comment on the use of these enzymes for the early diagnosis of the tumor, for the detection of clinical relapse and for the monitoring of follow up treatment.

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