Serum Paraoxonase and Arylesterase Activities in Esophageal Cancer:

A Controlled Study

Kerim Çayır[1], Mehmet Bilici[1], Salim Başol Tekin[1], Fatih Kara[2], Atilla Turkyılmaz[3], Abdülkadir Yıldırım[2]

Atatürk University, School of Medicine, Department of Internal Medicine, Division of Medical Oncology[1], Departments of Biochemistry,[2] Thoracic Surgery[3], Erzurum, Turkey

Eur J Gen Med 2010;7(4):398-403

Received: 28.08.2010

Accepted: 15.12.2010

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*

Correspondence: *Dr. Kerim Cayir, Terminal cad. Polat Sitesi C Blok Kat: 4 Erzurum - Turkey*
Phone : 904422317231, Fax: 04422361301
E-mail : drkcayir@hotmail.com

Özefagus kanserli hastalarda serum paraoksonaz ve arilesteraz aktiviteleri: Vaka-kontrol çalışması
Amaç: *Üst gastrointestinal sistem kanserleri tüm dünyada önemli bir sağlık problemidir. Özefagus kanseri genellikle geç evrelerde tanı konulur. Bu sebeple bir çok tedavi yaklaşımı palyatif olarak yapılır. Bu çalışmanın amacı özefagus kanserli hastalarda paraoksonaz (PON) ve arilesteraz (ARE) aktiviteleri ile bu hastaların klinikopatolojik özellikleri arasındaki muhtemel ilişkiyi araştırmaktır.*
Metod: *Bu çalışmaya 40 tane özefagus kanser tanısı konulan hasta ve 27 tane de sağlıklı gönüllü dahil edildi. Özefagus kanser tanısı endoskopik olarak ve biyopsi ile konuldu. PON ve ARE aktiviteleri spektrofotometrik yöntemle paraoksan ve phenylacetat substrat olarak kullanılarak ölçüldü. İstatistiki analiz Mann-Whitney-U testiyle yapıldı.*
Bulgular: *Kontrol grubu ile karşılaştırıldığında serum PON ve ARE aktiviteleri ortalama değerleri kanserli grupta belirgin olarak yüksekti. Ayrıca Evre 3-4 özefagus kanserli hastaların PON ve ARE aktiviteleri ortalama değerleri, evre 2 özefagus kanserli hastalara göre azalmıştı. Bu azalma istatistiki olarak anlamlı idi. Özefagus kanserli hastaların diğer klinik ve patolojik özellikleri ile PON ve ARE aktiviteleri arasında istatistiki öneme sahip bir ilişki bulunamadı.* ***Sonuç:*** *Bu araştırma özefagus kanserli hastalarda yapılan serum PON ve ARE aktiviteleri ile ilgili ilk çalışmadır. İleri evre özefagus kanserli hastalarda düşük serum PON ve ARE aktiviteleri önemli bir belirteç olabilir. Fakat bu ön sonuçlar tümörün erken tanısı, klinik olarak nüksün tespiti ve tedavi sonrası izlem açısından prospektif çalışmalarla doğrulanmalıdır.*
Anahtar kelimeler: *Paraoksonaz, kanser, özefagus*

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 (O_{2^-}), hydrogen peroxide (H_2O_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-

***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*

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-

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

struction, 38% of the cases had complete and 52% had partial obstruction.

Serum PON, ARE activities, total cholesterol, HDL- and LDL-cholesterol levels were determined in patients and in healthy control subjects. Serum total cholesterol, HDL- and LDL-cholesterol levels were not significantly different between patients and controls group. The mean serum basal PON, salt stimulated PON and ARE activities of the patients were significantly higher than controls ($p<0.05$, $p<0.05$, $p<0.001$, respectively, Table 1). In the present study, The tumor was localized in the lower third in 63% of the patients, in 32% of the patients in the middle part and in 5% of the patients in the upper part of the esophagus. There were not statistically significantly between Basal PON, salt stimulated PON and ARE activities and tumor localizations such as upper, middle and lower esophagus ($p>0.05$) (Data not shown).

The patients were divided into 2 groups according to the histological type of tumor as squamous cell carcinoma and adenocarcinoma. Serum basal PON, salt stimulated PON and ARE activities were compared between the two groups. We didn't detect a significant difference in serum basal PON, salt stimulated PON and ARE activities between histological types of esophageal cancer ($p>0.05$).

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 paraoxone 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, Akçay 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 polymorphiysm 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 were significantly higher in patients with esophageal cancer, compared to healthy controls. Although the effect of defects in the antioxidant defense system in cancer development is clearly established, our results do not support these views at first glance. But, the decrease in 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.

REFERENCES

1. *Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics 2007. CA Cancer J Clin 2007; 57:43-6.*
2. *Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality and prevalence across five continents: Defining priorities to reduce cancer disparities in different geographic response of the world. J Clin Oncol 2006; 24:2137-50.*
3. *Day NE, Varghese C. Oesophageal cancer. Cancer Surv 1994; 19-20:43-54.*
4. *Cejas P, Casado E, Belda-Iniesta C et al. Implications of oxidative stress and cell membrane lipid peroxidation in human cancer (Spain). Cancer Causes Control 2004;15:707-19.*
5. *Behrend L, Henderson G, Zwacka RM. Reactive oxygen species in oncogenic transformation. Biochem Soc Trans 2003; 31(Pt 6):1441-4.*
6. *Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmacol 1998;31:329-36.*
7. *Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 1998; 101:1581-90.*
8. *Li Hl, Liu DP, Liang CC. Paraoxonase gene polymorphisms, oxidative stress, and diseases. J Mol Med 2003; 81:766-79.*
9. *Schiavon R, Battaglia P, De Fanti E et al. HDL3-related decreased serum paraoxonase (PON) activity in uremic patients: comparison with the PON1 allele polymorphism. Clin Chim Acta 2002; 324:39-44.*
10. *Akçay MN, Yilmaz I, Polat MF, Akçay G. Serum paraoxonase levels in gastric cancer. Hepatogastroenterology 2003; 50 (Suppl 2):cclxxiii-cclxxv.*
11. *Akçay MN, Yilmaz I, Polat MF, Akçay G. Serum paraoxonase levels in pancreatic cancer. Hepatogastroenterology 2003; 50 (Suppl 2):ccxxv-ccxxvii.*
12. *Elkiran ET, Mar N, Aygen B, Gursu F, Karaoglu A, Koca S. Serum paraoxonase and arylesterase activities in patients with lung cancer in a Turkish population. BMC Cancer 2007;15:48.*

13. Furlong CE, Richter RJ, Seidel SL, Motulsky AG. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. Am J Hum Genet 1988; 43:230-8.

14. Eckerson HW, Wyte CM, La Du BN . The Human Serum Paraoxonase/Arylesterase Polymorphism. Am J Hum Genet 1983; 35:1126-38.

15. Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat Res 2001; 477:7-21.

16. Demple B, Harrison L. Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 1994; 63:915-48.

17. Cathcart MK, McNally AK, Chisolm GM. Lipoxygenase-mediated transformation of human low density lipoprotein to oxidized and cytotoxic complex. J Lipid Res 1991; 32:63-70.

18. Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE. The effect of teh human serum paraoxonase polymorphism is reserved with diazoxon and sarin. Nat Genet 1996; 14:334-6.

19. Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M. Paraoxonase (PON1) deficiency is associated with increased macrophage oxidative stres: studies in PON1-knockout mice. Free Radic Biol Med 2003; 34:774-84.

20. Kafadar AM, Ergen A, Zeybek U, Agachan B, Kuday C, Isbir T. Paraoxonase 192 gene polymorphism and serum paraoxonase activity in high grade gliomas and menegiomas. Cell Biochem Funct 2006; 24:455-60.

21. Lee CH, Lee KY, Choe KH et al. Effects of oxidative DNA damage induced by polycylic aromatic hydrocarbons and genetic polymorphism of theparaoxonase -1 (PON1) gene on lung cancer. Prev Med Pub Health 2005; 38:345-50.

22. Marchesani M, Hakkarainen A, Tuomainen TP et al. New paraoxonase 1 polymorphism II02V and the risk of prostate cancer in Finnish men. J Natl Cancer Inst 2003; 95:812-8.

23. Antognelli C, Mearini L, Talesa VN, Giannantoni A, Mearini E. Association of CYP 17, GSTPI and PON1 polymorphisms with the risk of prostate cancer. Prostate 2005; 63:240-51.

24. Van Der Logt EM, Janssen CH, Van Hooijdonk Z et al. No association between genetic polymorphisms in NAD(P)H oxidase p22phox and paraoxonase 1 and colorectal cancer risk. Anticancer Res 2005; 25:1465-70.