RESEARCH ARTICLE

Roles for Paraoxonase but not Ceruloplasmin in Peritoneal Washing Fluid in Differential Diagnosis of Gynecologic Pathologies

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Abstract

Background: Intraperitoneal spread of gynecologic cancers is a major cause of mortality and morbidity and often presents with malignant ascites. Microscopic tumor spread can be demonstrated by a peritoneal wash cytology and help assess the prognosis of the disease. In our study, the roles of paraoxonase and ceruloplasmin, measured in peritoneal washing fluid of patients operated for gynecologic pathologies in differential diagnosis was investigated. Materials and Methods: Patients operated for malign or benign gynecologic pathologies in Antalya Education and Research Hospital Gynecology Clinic between 2010-2012 were included in the study. Samples were obtained during surgery. Results: A statistically significant difference was detected between patients with benign and malign diseases with regards to PON1 levels measured in peritoneal washing fluid (p:0.044), the average values being 64.2±30.8 (Range 10.8-187.2) and 41.4±21.4 (Range 10.4-95.5), respectively. No significant variation was evident for ceruloplasmin. Conclusions: Paraoxonase levels measured in peritoneal washing fluid may contribute to the differentiation of malign-benign diseases in gynecologic pathologies.

Keywords: Gynecologic cancer - paraoxonase - ceruloplasmin - peritoneal wash cytology

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Introduction

Gynecologic cancers are a group of diseases including vulvar, vaginal, cervical, endometrial and ovarian cancers as well as gestational trophoblastic neoplasia. Gynecologic cancers remain as a substantial problem in developing countries and constitute 19% of new cancer cases (Sankaranarayanan et al., 2006; Sarkar et al., 2012).

Intraperitoneal spread of the gynecologic cancers is a major cause of mortality and morbidity and often presents with malignant ascites. Without a malignant ascites formation, the microscopic tumor spread can be demonstrated by peritoneal wash cytology (PWC) and the demonstration of these malignant cells establishing the prognosis of the diseases (Shield, 2004). Although at a lower rate, PWC can also demonstrate the occult spread of the benign diseases. Many studies demonstrated the PWC positivity during the course of benign diseases (Sharifi, 2009).

Oxidative stress and free oxygen radicals are the factors which have a role in the development of cancer and they are associated with increase in risk of cancer. Lipid peroxidation products are also considered to have a role during oncogenesis (Ray et al., 2000).

Lipid hydroperoxide (LOOH) products may be formed from unsaturated fatty acids, glycopeptides, and cholesterol as a result of the peroxidative reactions with oxidative stress. Oxidized LDL is the main form of the LOOH products responsible for the development of carcinogenesis associated with oxidative stress. On the contrary, HDL inhibits both enzymatic and non-enzymatic formation of the reactive oxygen types and thus, acts as an anti-carcinogen and an antioxidant (Delimaris et al., 2007).

Paraoxonase is a multifunctional antioxidant enzyme. PON family has three members, PON1, PON2, and PON3, and the genes which control the expression of these enzymes are localized on the 7th chromosome (Macharia et al., 2012). PON3 are expressed in the liver and kidneys, the liver expression of PON1 is limited. PON3 is present free in the blood, in association with HDL (La Du et al., 1999). PON2 does not exist in blood but is expressed in various tissues including mainly heart, kidneys, liver, lungs, placenta, small intestine, spleen, stomach and testicles (Ng et al., 2005).

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HDL-associated PON1 enzyme has three activities, as paraoxonase, arylesterase and lactonase, playing roles in prevention of LDL and HDL from oxidation by hydrolyzing active phospholipids and lipid peroxide products (Canales et al., 2003; Khersonsky and Tawfik, 2005). PON1 activity has been demonstrated to be reduced in patients with coronary artery diseases, hypercholesterolemia, type-2 diabetes and iron deficiency. In clinical studies, a lower serum PON1 activity has been observed in patients with lung, pancreas, gastric and prostate cancer compared to the control groups (Ahn et al., 2013).

PON1 catalyses the hydrolysis of many organophosphates and aromatic carboxylic acid esters and plays an important role in the metabolization of many xenobiotic compounds such as insecticides (paraoxonase and diasoxone) and nerve agents (Sarin, soman and Tabun) (Kosaka et al., 2005). In epidemiological studies, a polymorphism was demonstrated in serum PON1 activity of the individuals (La Du et al., 1986).

Development of cancer and pesticide use was investigated in many studies. Pesticides can expose their carcinogenic effects through mechanisms such as genotoxicity, hormonal effect and immunotoxicity (Yildirim et al., 2013). Gynecologic cancers were demonstrated that they might be associated with pesticide use (Mathur et al., 2008; Sathiakumar et al., 2011).

Ceruloplasmin is a positive acute phase protein with still uncertain functions in inflammatory processes and with both antioxidant and pro-oxidant activities. Ceruloplasmin is copper-dependant. It has a role in iron homeostasis and in the protection against cell damage initiated by free radicals. The level of ceruloplasmin increases in case of physical exercises, 3rd trimestre of pregnancy, ovarian hyperfunction, atherosclerosis, epilepsy and chronic inflammatory diseases as well as with malignities such as cancer of lung, prostate, and stomach and Hodgkin's lymphoma (Senra Varela et al., 1997).

The false positiveness of PWC in benign diseases and false negative results in early-stage diseases are its important restrictions. In recent disease staging guidelines, although the PWC positiveness does not alter the disease stage, it's a bad prognostic factor and is effective in determination of the treatment intensity (Selvaggi et al., 2003). In our study, the role of paraoxonase and ceruloplasmin, which were measured in peritoneal washing fluid of the patients operated for gynecologic pathologies and diseases in differential diagnosis of the gynecologic pathologies was investigated.

Materials and Methods

Selection of patients

Patients operated in Antalya Education and Research Hospital Gynecology Clinic between 2010-2012 due to malign or benign gynecologic pathology was included in the study. The exclusion criteria: inoperable advanced stage and metastatic patients, patients without sufficient sample for evaluation and patients with clinically determined acid were not included in the study. All the patients were evaluated by standard pre-operative assessment for disease staging and the determination of the prognosis. The approval of Ethics Committee was obtained. Demographical information, such as age, gender, disease stage, was obtained by screening the patient files. Patients were allocated into four groups in accordance with the results of the cytological examinations; benign histopathology with benign cytology, malign histopathology with benign cytology, benign histopathology with malign cytology and malign histopathology with malign cytology.

Obtaining samples

Samples were obtained during the operation from the patients for whom an operation was planned due to gynecologic pathology. After penetrating into peritoneum during operation, peritoneal cavity was flushed with 100 mL of sterile at first. Right after that, the fluid in peritoneal cavity was aspirated and sent to the cytology lab for cytological examination. After the cytological examination, the remaining samples were stored at -80o C. These samples were used for PON1 and ceruloplasmin measurements.

Measurement of activity levels of ceruloplasmin and PON1

The level of ceruloplasmin was measured by nephelometric method (Image 800, Beckman Coulter, Fullerton, CA). The level of paraoxonase was measured spectrophotometrically (Architect c4000, Abbott, US) by modified Eckerson method. During the measurement of PON1 enzyme activity by modified Eckerson method, paraoxonase (0.0 diethyl-0-p-nitrophenylphosphate, Sigma Chemical Co, London, UK) was used as a substrate.

The measurement of paraoxonase is based on the spectrophotometrical measurement of free p-nitrophenol formed as a result of the enzymatic hydrolysis of paraoxonase at 37°C and 412 nm wavelengths (Eckerson et al., 1983). The values of PON1 activity below 59 U/L were defined as low activity and the values above 59 U/L were defined as non-low activity.

Statistical analyses

The statistical analyses were performed by using SPSS for Windows 15.0 software. The appropriateness of the variables to normal distribution was examined by using visual (histogram and probability graphics) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). In Kolmogorov-Smirnov test, the situations where p value was over 0.05 were considered as normal distribution. Since ceruloplasmin, PON1 did not exhibit a normal distribution; the differences between patient group and control group were investigated by Mann-Whitney U test, a non-parametrical test.

For the relations between variables at least one of which do not distribute normally or is ordinal, the correlation efficient and the statistical significances were calculated by Spearman test. For the statistical significance, the total type 1 error level was accepted as 5%.

Results

67 women operated due to gynecologic pathology were included in the study. The ages of the patients were

Table 1. Patient Characteristics

	Bening	p value
N (%)	46 (68.7%)	
Age	45.3±12.3 (Range 15-78)	p=0.142
Serviks	3 (6.5%)	
Uterus	18 (39.1%)	
Over	25 (54.3%)	
Ceruloplasmin (mg/dL)	28±12.7 (Range 0-61.8)	p=0.361
PON1 (U/L)	64.2±30.8 (Range 10.8-187.2)	p=0.044

Table 2. Patient Groups Based Cytology and Histopathologic Examination

		Histopathology		
		Malign	Benign	Total
Cytology	Malign	7	0	7
	Benign	14	46	60
Total		21	46	67

46.9±12.9 (Range 14-78). Histopathological diagnosis was benign in 46 (68.7%) patients and malignant in 21 (31.3%) patients. No differences were detected in terms of age between the patients with malignant or benign disease (p:0.142) (Table 1).

The tissue from which the benign disease originated was found as ovarium (54.3%) whereas it was found as uterus (57.1%) in malignant disease. Of the benign ovarium diseases, the most frequently detected one was simple ovarium cysts. In patients evaluated histopathologically as malign, T1 lesion was detected in 65% of the patients, T2 lesion in 5% and T3 lesion in 30%.

PCW was reported as benign in 60 patients (89.6%) and as malign in 7 patients (10.4%). When the cytological and histopathological examinations were evaluated together, all the patients evaluated as malign by cytology were diagnosed with malign disease histopathologically whereas 14 of the patients with a benign cytology evaluation were diagnosed with malign disease histopathologically (Table 2). PCW sensitivity was detected as 33.3% and specificity as 100%. There was a significant difference between patient groups for the low and non-low PON1 activity (p:0.013). PON1 activity was detected as low in all of the patients evaluated cytologically benign and diagnosed with malign disease histopathologically.

The average value of ceruloplasmin was detected as 26.6±13.6 (Range 0-61.8). No differences were detected between patients with benign and malign diseases with levels measured in peritoneal washing fluid (p:0.361). The average value of ceruloplasmin was measured as 28±12.7 (Range 0-61.8) in patients with benign disease and as 23.6±15.2 (Range 2-53) in patients with malign disease.

The average value of PON1 was detected as 57.1±10.4 (Range 10.4-187.2). A statistically significant difference was detected between patients with benign and malign diseases with regards to PON1 levels measured in peritoneal washing fluid (p:0.044). The average value of PON1 was measured as 64.2±30.8 (Range 10.8-187.2) in patients with benign disease and as 41.4±21.4 (Range 10.4-95.5) in patients with malign disease. A significant correlation between PON1 level and ceruloplasmin level was detected (p:0.452 r:0.452).

Discussion

In our study, we determined that PON1 value measured in peritoneal washing fluid obtained intraoperatively was at a lower value in patient with malign disease compared to patients with benign disease. We couldn't demonstrate the benefit of ceruloplasmin value in the differentiation of malign-benign diseases.

Kokouva et al. suggested that, based on a study on patients with lymphohematopoietic cancer from a region of Greece where intensive agricultural activities were performed, PON1 polymorphism could increase the risk of development of lymphohematopoietic malignancies in susceptible individuals exposed to pesticides (Kokouva et al., 2013).

PON1 polymorphism both increases the sensitivity to pesticides and decreases the PON1 catalytic activity with pesticides. As a consequence, the metabolization of many chemical agents can be changed (La Du et al., 2001; Zhou et al., 2007). In our study, we determined that PON1 levels decreased in patients with a malign disease. We couldn't measure the level of pesticides in these patients. This is one of the weak aspects of our study. In gynecologic cancers, pesticides may cause carcinogenesis by reducing the PON1 activity. We think that studies investigating the correlation between PON1 levels and pesticide levels in gynecologic cancers are needed.

PON1 activity was studied in gynecologic cancers. Camuzcuoglu et al. demonstrated the reduction of PON1 activity in epithelial ovarian carcinoma. In this study, the relationship between stage and CA-125 levels and PON1 activity was detected (Camuzcuoglu et al., 2009). Arpaci et al. determined that ovarian cancer was observed earlier in PON1 192 AB genotype individuals compared to PON1 BB genotype individuals and suggested that this might be due to the protective effect of PON1 BB genotype (Arpaci et al., 2009).

Low PON1 activity was demonstrated in gastroesophageal junction, lung, prostate, pancreas and stomach cancers (Akcay et al., 2003a; 2003b; Elkiran et al., 2007; Krzystek-Korpacka et al., 2008; Stevens et al., 2008). Although the statistical significance was not achieved in their study, Bobin-Dubigeon et al. suggested that the reduction of PON1 activity in breast cancer could be associated with the short survey (Bobin-Dubigeon et al., 2012).

Vaidya et al. determined that ceruloplasmin level was higher in patients with cervical cancer compared to healthy controls. In this study, it was suggested that the low ratio of copper/ceruloplasmin could be associated with advanced stage (Vaidya et al., 1998). In patients with breast cancer, ceruloplasmin level was found to be high and associated with bad prognosis (Ozyilkan et al., 1992; Arumanayagam et al., 1993). In our study, ceruloplasmin levels were not found to be different with regards to benign-malign pathologies. The reason for that can be associated with the low number of patients and/or with the distrubition of ceruloplasmin levels in a narrow range.

Generally, ceruloplasmin levels were determined to be increased during an inflammation whereas PON1 activity was demonstrated to be reduced (Nowak et al., 2010). In

our study, on the contrary, a positive significant correlation between ceruloplasmin and PON1 was detected. Much to our knowledge, there is no study performed investigating ceruloplasmin level and PON1 activity level in peritoneal washing fluid before.

PWC is used for the determination of microscopic tumor focuses which cannot be observed macroscopically. Although the existence of malign cells in peritoneal cavity does not alter the disease stage, it is of prognostic importance (Anastasiadis et al., 2011). In our study, we determined that PON1 activity measured in peritoneal fluid along with PWC had an additional diagnostic benefit.

The specificity and sensitivity of PWC in gynecologic pathologies were investigated in many studies. In a study where the role of PWC was investigated in gynecologic malignities, Zuna et al. determined a positive cytology result in 80.4% of 90 patients with ovarian cancer, in 31.2% of the 16 patients with borderline ovarian cancer, in 12.6% of the patients with endometrial cancer and in 8.7% of the patients with cervical cancer. They suggested that PWC was considerably specific (98.1%) but less sensitive (82.9%) (Zuna et al., 1996). In our study, a less sensitivity was detected when compared to these ratios. We think that the reason for that was the inclusion of patients with benign pathology as well as patients with malign pathology.

Paraoxonase levels measured in peritoneal washing fluids contribute to the differentiation of malign-benign diseases in gynecologic pathologies. In addition, we think that the role of PON1 in gynecologic cancers and its relation with pesticide levels should be investigated.

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