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Serum paraoxonase and arylesterase activities in patients with epithelial ovarian cancer

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ABSTRACT

Objective. The HDL-associated paraoxonase and arylesterase activities play a role in decreasing oxidative stress, which is known to contribute to cancer development. The aim of this study was to evaluate serum paraoxonase and arylesterase activities and lipid hydroperoxide (LOOH) levels in patients with newly-diagnosed epithelial ovarian cancer.

Materials. Serum paraoxonase and arylesterase activities, total free sulfhydryl (−SH) levels and LOOH levels were measured in patients with epithelial ovarian cancer ($n=24$) and controls ($n=29$).

Results. Serum paraoxonase activity and arylesterase activity, as well as −SH levels were significantly lower ($p=0.003$, $p<0.001$ and $p<0.001$, respectively) in patients with epithelial ovarian cancer compared to controls, while LOOH levels were significantly higher ($p<0.001$). A significant inverse correlation was found between the stage, grade and CA-125 level of ovarian cancer and paraoxonase activity ($rho=-0.630$, $p=0.001$ and $rho=-0.601$, $p=0.002$ and $rho=-0.436$, $p=0.033$, respectively), arylesterase activity ($rho=-0.601$, $p=0.002$ and $rho=-0.713$, $p<0.001$ and $rho=-0.580$, $p=0.003$, respectively), and −SH levels ($rho=-0.642$, $p=0.001$ and $rho=-0.637$, $p=0.001$ and $rho=-0.530$, $p=0.008$, respectively). In contrast, there was a positive correlation between LOOH and the stage, grade and CA-125 level of ovarian cancer ($rho=0.565$, $p=0.004$ and $rho=0.479$, $p=0.018$ and $rho=0.642$, $p=0.001$).

Conclusion. Our results suggest that diminished paraoxonase and arylesterase activity, −SH levels and increased LOOH levels are associated with particular stage, grade and CA-125 level of ovarian cancer.

Introduction

Ovarian carcinoma is the leading cause of death among patients with gynecologic malignancies [1]. Most ovarian cancers originate from the ovarian surface epithelium. However, the etiology and pathogenesis of ovarian cancer are still largely unknown. Risk factors for ovarian carcinoma include inflammation, excessive number of lifetime ovulations, increases in steroid hormone levels, heredity, infertility, age, asbestos, talc, and reproductive factors such as nulliparity [2,3]. Recent molecular studies have shown that ovarian cancer displays acquired genetic alterations of oncogenes and tumor suppressor genes such as BRCA1, p53, nm23 and K-ras, which may be due to inflammation and oxidative stress [4]. Moreover, oxidative stress may be involved in cell proliferation and malignant conversion during the development of ovarian cancer [5]. Additionally, elevated oxidative stress and free oxygen radicals have been associated with the increased risks of various cancers. End products of lipid peroxidation have also been thought to play a role in oncogenesis [6,7].

Lipid hydroperoxide (LOOH) is a well-known marker of oxidative stress formed from unsaturated phospholipids, glycolipids, and cholesterol by peroxidative reactions under oxidative stress [8]. Oxidized low-density lipoprotein (LDL) is the main form of LOOH responsible for the development of oxidative stress-related carcinogenesis [8–10]. Conversely, high-density lipoprotein (HDL) prevents both enzymatic and nonenzymatic generation of reactive oxygen species, and thus, acts as an anticarcinogen and a powerful antioxidant [11].

Paraoxonase-1 (PON1) was first detected in immunoprecipitates of high-density lipoprotein (HDL) after electrophoresis of human serum in 1961 [12]. PON1 is an HDL-associated enzyme with three activities: paraoxonase, arylesterase and dyazoxonase [13]. It is also a Ca^{2+}-dependent serum esterase that is synthesized in the liver [14]. PON1 plays a key role in the protection of LDL and HDL from oxidation by hydrolyzing activated phospholipids and lipid peroxide products [15,16]. Interestingly, PON1 activity is decreased in subjects who have had coronary artery disease [17], hypercholesterolemia, type 2

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diabetes [18], and in subjects with iron-deficiency anemia [19]. Furthermore, it has been demonstrated that serum PON1 activity is lower in patients with lung, pancreatic, gastric, gastroesophageal and prostate cancer, when compared to control groups [20–24].

To the best of our knowledge, paraoxonase and arylesterase activities in patients with epithelial ovarian cancer have not been evaluated. In addition, it is still unknown whether there is any relationship between paraoxonase and arylesterase activities in patients with epithelial ovarian cancer. The purpose of this study was to evaluate the association between epithelial ovarian cancer and paraoxonase activity, arylesterase activity and total free sulfhydryl (−SH) levels. We sought to identify a role for these proteins as antioxidants and to elucidate the possible function of serum levels of, for example, lipid hydroperoxide (LOOH) as an oxidative stress marker.

Materials and methods

Study population

The study was conducted at the Departments of Obstetrics and Gynecology and Clinical Biochemistry, Harran University, Departments of Obstetrics and Gynecology, Afyonkarahisar Kocatepe University, and Izmir Eagean Maternity and Women's Health Hospital between August 2007 and July 2008. Informed consent for participation in the study was obtained from all women. The study protocol conforms to the principles of the Helsinki Declaration and was approved by the Medical Ethics Committee of Harran University. The study group included 24 patients with epithelial ovarian cancer, previously untreated. The control group was composed of 29 healthy women. All participants have had poor socioeconomic status. Those subjects with concurrent or previous malignant disease (n=4), inadequate renal, hepatic, and cardiac functions (n=9, n=11, and n=9, respectively) and women with low malignant potential, germ cell, stromal, and other non-epithelial ovarian tumors (n=5) were excluded. Patients with previously-performed chemotheraphy, radiotherapy and surgery (n=11) were also excluded from the study. During this period mentioned above, a total of 73 ovarian cancer patients were diagnosed and treated, but only 24 of them (32.9%) met our selection criteria. Patients were analyzed in terms of age at diagnosis, parity, body mass index, International Federation of Gynecologists and Obstetricians (FIGO) stage, grade, histology and pre-operative CA-125 level. Patients were followed for 1 years.

All patients underwent surgical exploration and cytoreduction as the initial treatment, performed by a staff gynecologic oncologist and a fellow. Surgical staging was done according to FIGO criteria. The pathology for all patients was reviewed by a gynecologic pathologist. After surgery, all patients were treated with intravenous platinum and paclitaxel for adjuvant chemotherapy.

Blood sample collection

Blood samples of the study group were acquired just before operation. Blood samples were obtained in the morning from the cubital vein after an overnight fast. Samples were drawn from cubital vein into blood tubes and were immediately separated from the cells by centrifugation at 3000 ×g for 10 min, stored on ice at −80 °C, then analyzed.

Measurements of paraoxonase and arylesterase activities

Paraoxonase and arylesterase activities were measured using commercially available kits (Relassay, Turkey). Paraoxonase activity measurements were performed both in the absence and presence of NaCl (salt-stimulated activity). The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorption at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorption coefficient at pH 8.5, which was 18.290 M^{-1} cm^{-1} [25]. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorption coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated per minute under the above conditions and expressed as U/L [26].

Measurement of lipid hydroperoxide levels

Serum lipid hydroperoxide levels were measured by the ferrous ion oxidation-xylenol orange (FOX-2) method previously described [27].

Measurement of total free sulfhydryl groups

Measurement of total free sulfhydryl groups Serum −SH levels were assayed according to the method of Ellman [28] as modified by Hu et al. [29]. Briefly, 1 ml of buffer containing 0.1 M Tris, 10 mmol/l EDTA, pH 8.2, and 50 ml serum was added to cuvettes, followed by 50 ml of 10 mmol/l 5,5-dithiobis 2-nitrobenzoic acid in methanol. Blanks were run for each sample as a test. After incubation for 15 min at room temperature, sample absorbance was interpreted at 412 nm on a Cecil 3000 spectrophotometer (Cecil Instruments, Cambridge, UK). Sample and reagent blanks were subtracted. The concentration of −SH groups was calculated using reduced glutathione as the free −SH group standard and the results were expressed as millimolars/liter. Coefficients of variation for measurement of serum −SH levels were 3.6%.

Measurement of other biochemical parameters

The levels of triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) were determined using commercially available assay kits (Abbott®, Illinois, USA) with Abbott Aeroset auto-analyzer (Abbott, Illinois, USA). Levels of serum CA 125 were determined using commercially available assay kits (Roche® Diagnostics Modular Analytic E170, Electro-chemiluminescent technology, Basel, Switzerland).

Statistical analysis

All analyses were conducted using SPSS 11.5 (SPSS for Windows 11.5, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (S.D.). Parameter comparisons were performed using the Mann–Whitney U test. Normality of distribution was evaluated with the Kolmogorov–Smirnov test. Spearman's correlation (rho) test was used for group comparison. All statistical tests were two-sided. A p-value <0.05 was considered significant.

Results

Demographic and clinical data on the patients with epithelial ovarian cancer and controls are summarized in Table 1. While LDL-C

Table 1
Demographic and clinical parameters of patients with ovarian cancer and controls

	Cases (n=24)	Controls (n=29)	p value
Age (years)	51±13	50±10	0.552
Parity	4.3±2.6	7.3±2.9	0.003
Body mass index (kg/m^2)	28±6	30±4	0.116
Total cholesterol (mg/dl)	185±43	212±39	0.036
Triglyceride (mg/dl)	142±48	156±93	0.629
LDL (mg/dl)	115±41	130±33	0.174
HDL (mg/dl)	41±8	50±11	0.002

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol. Values are mean±S.D.