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Evaluation of oxidative stress markers and intra-extracellular antioxidant activities in patients with endometriosis

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ABSTRACT

Objective: The aim of the study is to evaluate alterations in intracellular and extracellular antioxidant enzymes activities and serum oxidative stress markers in patients with endometriosis.
Study design: The current prospective study consisted of 31 female patients with endometriosis and 27 healthy controls. Serum total thiol, native thiol, disulphide, catalase, myeloperoxidase, and ceruloplasmin concentrations were measured. Laboratory and clinical data of all participants were recorded to compare the differences between the study and the control groups.
Results: Serum native thiol and total thiol levels in the study group were significantly lower than those in the control group [($p = 0.009$, $p = 0.03$, respectively)]. Serum catalase levels are significantly higher in patients with endometriosis comparing to the control group ($p = 0.009$).
Conclusions: The finding that significant differences in serum total thiol, native thiol, and catalase levels observed in endometriotic patients supports that oxidative stress carries weigh in the pathophysiological aspects of endometriosis. Also significantly low levels of extracellular antioxidants and significantly high levels of intracellular antioxidants in endometriotic patients may arise from differences of free radicals in endometriosis and the activity levels of endometriosis. These non-invasive serum markers might give us an opportunity to monitor the disease's progress during the treatment.

Introduction

Endometriosis, the presence of endometrial-like tissue outside the uterus, is one of the most common gynecological pathology in reproductive aged women [1]. Endometriosis is a multifactorial disease associated with inflammatory response of peritoneal cavity. Although etiology and pathogenesis of endometriosis is not well known, there are many studies investigating the relationship between endometriosis and oxidative stress which is considered to play a pivotal role in formation and progression of the disease [2–5]. Reactive oxygen metabolites are produced as metabolic intermediate product from the apoptotic endometrial tissues in endometrial implants [6]. The increase of the production of reactive oxygen and nitrogen species (RONS) known as free radicals or the reduction of antioxidants bioavailability trigger the oxidative stress leaving organisms more susceptible to oxidative damage [7]. The defense system which is regulated by various enzymatic and non-enzymatic processes transforms the derived oxygen and nitrogen species into harmless products in the organisms. In case of an imbalance between oxidant and antioxidant molecules, oxidative stress is induced and the balance has shifted to the oxidative side in the organism.

Catalase is accepted as an intracellular anti-oxidant enzyme which is specifically found in a well-known intracellular organelle called peroxisome [8]. During oxidative stress, the production of this enzyme is induced in order to balance redox reactions. Myeloperoxidase (MPO) is also one of the intracellular antioxidant enzymes typically found in the granules of neutrophils. It plays an important role in killing phagocytosed bacteria [9]. Extracellular environment comprises an effective defense system against oxidant injury. Ceruloplasmin constitutes a significant part of extracellular antioxidant defense system with having both antioxidant and pro-oxidant effects in tissues [10,11].

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Thiols are organic compounds containing carbon-bonded sulfhydryl and hydrogen groups. Under oxidative stress, thiols can react with oxidizing agents and mediate the formation of reversible disulphide bonds (RSSR) between them [12,13]. Dynamic thiol-disulphide homeostasis is maintained in the human body through this reversible disulphide bonds and oxidation reduction reactions [14]. While only one part of this two-sided balance has been measured since 1979, a newly developed method by Erel & Neselioglu has allowed both separate and combined measurements of the thiols and disulphides in the plasma [15]. In addition to its effect on the antioxidant protection, dynamic thiol disulphide homeostasis involves in many crucial biochemical activities in the body [16,17].

Serum CA-125 level can be used to support the diagnose, to assess response to treatment and risk of disease recurrence in endometriotic patients [18–20].

The aim of this study is to evaluate the serum oxidative stress markers and intra-extracellular antioxidant activities and to detect the relation between Ca-125 and these serum markers in patients with endometriosis.

Materials and methods

This prospective controlled study was carried out at the Gynecology Clinic of Ankara Ataturk Training and Research Hospital and Gynecology Clinic of Sincan Nafiz Korez State Hospital. The study protocol was approved by the ethics committee of Yildirim Beyazit University and a written informed consent was obtained from all subjects in the study. The study group involved 34 women who underwent laparoscopy or laparotomy for the treatment of a suspected endometrioma and the diagnosis was confirmed by the pathological results. All of the women with endometriosis have ovarian endometriomas. Of 34 patients, three were excluded from the study due to their different pathological results (serous cystadenoma, hemorrhagic cyst, and mucinous cystadenoma). The control group consisted of 27 healthy women who desired permanent sterilization and underwent laparoscopic tubal ligation. These healthy individuals without any detectable pelvic pathology or visible endometriotic implants during the laparoscopic tubal ligation enrolled in the control group. The patients' characteristics (age, gravidity, parity, body mass index (BMI) (body weight (kg)/(height (m))^2), preoperative serum cancer antigen-125 (CA-125) values, ovarian endometrioma sizes which are measured intraoperatively, and the stage of the endometriosis were recorded. American Fertility Society revised criteria was used for staging of endometriosis [21].

Serum sampling and biochemical analysis

Venous blood samples for measurement of thiols, disulphides, myeloperoxidase, ceruloplasmin, and catalase values were drawn from the participating patients in the morning of the operation day and after overnight fasting. The blood samples were centrifuged immediately; sera were separated after centrifugation at 1600 × *g* for 10 min and stored at −80 C° until the analyzing time. Some of the collected blood samples which belonged to patients whose pathological investigation yielded different results rather than endometriosis and patients with visible pelvic pathologies during the laporoscopic tubal sterilization were excluded. The sample recollected to measure disulphides was treated with N-ethilmaleimide protecting the SH group from changing to S–S. Thiol/disulphide homeostasis tests were measured by automated spectrophotometric method described by Erel & Neselioglu. For short, disulphide bonds were first reduced to form free functional thiol groups with sodium borohydride. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent reduction of DTNB (5,5′-dithiobis-(2-nitrobenzoic) acid), and all of the thiol groups including reduced and native thiol groups were determined after the reaction with DTNB. Half of the difference between the total thiols and native thiols provide the dynamic disulphide amount [15]. Catalase activity was measured by Goth's method [22]. Sample (0.2 ml) was incubated in 1.0 ml substrate (65 μmol per H_2O_2 in 60 mmol/L sodium–potassium phosphate buffer, pH 7.4) at 37 °C for 60 s. The enzymatic reaction was stopped with 1.0 ml of 32.4 Mm ammonium molybdate, and the yellow complex of molybdate and H_2O_2 was measured at 405 nm against a blank. One unit of catalase decomposes 1 μmol of H_2O_2 min^{-1} under these conditions. Results were expressed as kU/L. Serum MPO activity was measured by a modification of the o-dianisidine method [23] based on kinetic measurement at 460 nm with the rate of the yellowish orange product formation from the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide (H_2O_2). One unit of MPO was defined as that degrading 1 μmol of H_2O_2 min^{-1} at 25 °C. MPO activity was expressed in units per liter serum. Ceruloplasmin levels were measured by method described by Erel O [24]. This method is automated, colorimetric, and based on the enzymatic oxidation of ferrous ion to ferric ion. The results were expressed in units per liter serum.

Sample size was calculated based from previous study with 0.05 alpha error end 80% power [3]. 15 of the subjects were needed for both groups by using serum mean of Total Oxidative Stress levels. Statistical analysis and calculations were carried out by using IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) And MS-Excel 2007 programmes. Data's compliance with the normal distribution was evaluated by Shapiro Wilk test. Normally distributed data were expressed as mean ± STD but not normally distributed data were expressed as median ± IQR. Differences between the values of patient and control groups were evaluated by independent t test. Spearman correlation test was used for correlation of data. Statistical significance was accepted as $p < 0.05$.

Results

Preoperative indications, types of surgical approaches and the size of the endometrial cyst were listed in Table 1. While all of the control group participants underwent laparoscopy (100%), case group participants underwent either laparoscopy (58.1%) or laparotomy (41.9%). Table 2 shows patients' characteristics andserum thiol, disulphide and catalase levels in both study and the control groups. The mean age did not differ between study (35.7 ± 7.6 years) and the control groups (37.1 ± 4.7 years) ($p = 0.43$) (Table 2). There was no significant difference of BMI between endometriotic (26.3 ± 1.7 kg/m^2) and healthy women (26.5 ± 3.9 kg/m^2) ($p = 0.84$) (Table 2). The

Table 1
Preoperative indications, surgical approaches and size of the endometriotic cysts.

Parameters Subjects ($n = 31$)	Distribution of endometriosis
Preoperative indications	
Infertility	7 (22.6%)
Pelvic mass	14 (45.2%)
Pelvic pain	10 (32.3%)
Surgical approaches	
Laparoscopy	18 (58.1%)
Laparotomy	13 (41.9%)
Size of the cyst	
≤4 cm	10 (32.3%)
>4 cm	21 (67.7%)