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Original Article

Correlation between serum and peritoneal fluid glutathione S-transferases T1 concentration with different stages of endometriosis

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ABSTRACT

Endometriosis is a gynecological disease defined by the histological presence of endometrial glands and stroma outside the uterine cavity. Ectopic endometrial cell proliferation and chronic inflammation in endometriosis were shown to be associated with oxidative stress (OS) induction. OS is a condition in which reactive oxygen species (ROS) overproduction and antioxidant deficiency cause a shift in oxidant/antioxidant balance. Glutathione S-transferases (GSTs) comprise a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. The aim of this project was to study the concentrations of GSTT1 in the serum and peritoneal fluid (PF) of patients with different stages of endometriosis. Frothy two PF and serum from normal and 152 from different stages of patients with endometriosis (stage I: n = 30, stage II: n = 39, stage III: n = 43 and stage IV: n = 40) were included in this study. The level of GSTT1 in the serum was determined by enzyme linked immunosorbent assay (ELISA). The results showed the presence of GSTT1 in all serum and peritoneal fluid samples, while, starting from stages I to IV endometriosis, a significant decrease in GSTT1 concentration was seen as compared to controls. It is concluded that levels of GSTT1 is negatively correlated with advanced stages of endometriosis. It is also suggested that the detection of serum and/or peritoneal fluid GSTT1 concentration may be valuable in the classifying of endometriosis.

1. Introduction

Endometriosis is a gynecological disease that affects up to 25–40% of fertile women of reproductive age [1]. It was first described in 1960 and it is a common, benign, estrogen dependent gynecological disorder related to pelvic pain and infertility. It is described by the presence of endometrial tissue outside its normal location [2]. Age, body mass index, race, alcohol use and cigarette smoking are related to the frequency of the disease [3]. Retrograde menstruation remains the dominant theory for the development of pelvic endometriosis, [4] but the main molecular mechanisms responsible for the disease are unknown.

Many studies were shown the association of endometriosis and oxidative stress [5]. Antioxidant treatment is one of the most important therapeutic pathways for endometriosis. Antioxidants can modify the development of endometrial cells in endometriosis [6]. Oxidative stress occurs when there is a disruption between reactive oxygen species (ROS) production and the antioxidant defense. Cellular targets of ROS are macromolecules. To protect themselves from the deleterious effects of ROS, cells have developed a wide range of antioxidant systems to limit production of ROS, inactivate them and repair cell damage [7]. The impact of oxidative stress has been shown in endometriosis, tubal infertility and recurrent pregnancy loss [5,8,9].

Glutathione s-transferases (GSTs) constitute a superfamily that play a key role in phase II cellular detoxification and are generally considered as antioxidant enzymes [10]. Based on the similarity of the amino acid sequence, GSTs have been grouped into at least seven classes known as alpha (a), mi (m), pi (p), theta (q), zeta (z), sigma (s), and omega (W). The GSTs catalyze the conjugation of the glutathione (GSH) to a number of exogenous and endogenous substances with electrophilic functional groups (e.g. products of oxidative stress, environmental pollutants, and carcinogens),

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thus neutralizing their electrophilic sites, and rendering the products more water-soluble [11].

GSTs are important because of their role in removing hyperoxides or by balancing H_2O_2 homostasis in signaling cascades [12]. It was shown that GST Pi levels in the cerebrospinal fluid of patients with Parkinson's disease decreased compared to normal controls [13]. It was also demonstrated that GST P knockout mice were more susceptible to the neurotoxic effects and showed that GST P may act as an endogenous regulator of stress by controlling JNK activity [14]. The association of GSTT1 gene polymorphisms and male infertility has been reported [15]. It has been also shown that GSTM1 null genotype is associated with higher risk of endometriosis in an Iranian population [16]. In this study we aimed to analyze the levels of GSTT1 in the peritoneal fluid and serum of patients with different stages of endometriosis.

2. Materials and methods

2.1. Subjects

After ethic committee's approval and informed consent the samples of serum and peritoneal fluid (PF) from normal subjects and patients with endometriosis were collected. Peritoneal fluid (PF) and serum samples from normal controls (n = 42) and different stages of endometriotic patients (n = 152) (stage I: n = 30, stage II: n = 39, stage III: n = 43 and stage IV: n = 40) were enrolled in this study. The controls contained of women undergoing laparoscopic tubal ligation or diagnostic laparoscopy with no pelvic findings of endometriosis, inflammatory disease, or uterine fibroids. Patients that received endocrine therapy during the last six months and women with other causes of chronic pelvic pain including infectious, gastrointestinal, musculoskeletal, neurologic or psychiatric were excluded. Samples of all cases and controls were matched on age. None of the patients suffered from known diabetes mellitus or infection. All participants were asked to fill out a questionnaire on their medical history, family history of disease, infertility, surgical history, and prescribed. This research project has been approved by the university ethics committee and has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The serum and PF samples were kept at −70 °C for further analysis.

2.2. Analysis of GSTT1 concentration by ELISA

GSTT1 concentrations in PF and serum were measured using the sensitive two sited enzyme linked immunosorbent assay (ELISA) and antiserum against human GSTT1. Microtiter plates (Human GSTT1 ELISA Kit (Sandwich ELISA) - LS-F21079, LifeSpan BioSciences, Inc.). All reagents, samples and standards were prepared. 100 µl of Sample, Standard, or Blank was added to each well and incubate for 90 min at 37 °C. 100 µl of 1x Biotinylated detection Antibody was added and incubated for 1 h at 37 °C. After that they were washed three times. 100 µl of 1x HRP conjugate was added and incubated for 30 min at 37 °C. They were washed and 90 µl of TMB substrate solution was added and incubate for 15 min at 37 °C. 50 µl of stop solution was added and was read immediately at 450 nm.

2.3. Statistical analysis

All data presented are expressed as means ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) and only values with $P \leq 0.05$ were considered as significant.

3. Results

3.1. Characteristics of subjects

Demographic and clinical characteristics of patients and controls are shown in Table 1. The endometriosis patients were classified according to the American Society of Reproductive Medicine classification (stage I-IV). Of the 152 patients, 30 (19.7%) had stage I, 39 (25.4%) had stage II, 43 (28.2%) had stage III, whereas 40 (26.3%) patients had stage IV (Table 1).

3.2. Analysis of GSTT1 in the serum and PF by ELISA

Using ELISA, it was shown that the concentration of GSTT1 in the serum and PF samples of patients with endometriosis was lower than in control group. The results showed that all PF and serum samples, presented GSTT1 expression, whereas, starting from stages I to IV endometriosis, a decrease of protein expression was observed (from stages I to IV, serum levels of 2.76 ± 0.22, 2.58 ± 0.22, 2.31 ± 0.16 and 2.21 ± 0.18 ng/ml as compared to controls (3.02 ± 0.32) and peritoneal fluid levels of 1.06 ± 0.34, 0.97 ± 0.33, 0.72 ± 0.16, 0.47 ± 0.21 ng/ml respectively, as compared to controls (1.43 ± 0.27) ($P < 0.001$) (Figs. 1 and 2). A decreased concentration of GSTT1 is shown to be associated with advanced stages of endometriosis.

4. Discussion

Endometriosis is a chronic inflammatory disease that is characterized by the growth of endometrial tissue outside the uterine cavity. Reduced implantation seen in patients with endometriosis is still a matter of debate [17]. Many studies on unexplained fertility show that oxidative stress may be important in the pathophysiology of endometriosis [5]. Changes in the levels of soluble CD44 and soluble cMET in the serum and peritoneal fluid of patients with different stages of endometriosis were demonstrated [18,19]. It was demonstrated that there is a decreased serum levels of paraoxonase-1, a lipoprotein that prevent oxidative modification

Table 1
Characteristics of the patients with endometriosis and controls.

No. of patients	152
Average age (range) (years)	29.6 (24–39)
Stage of disease[a]	
Stage I	30
Stage II	39
Stage III	43
Stage IV	40
Number of controls	42
Average age (range) (years)	29.1 (23–39)

[a] Revised American Fertility Society (AFS) staging system: Stage I = minimal disease, Stage II = mild disease, Stage III = moderate disease, Stage IV = severe disease.

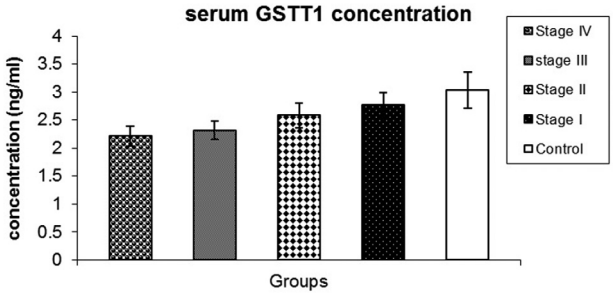

Fig. 1. GSTT1 concentration in the serum from controls and patients with different stages of endometriosis (ng/ml). A decreased concentration of GSTT1 was seen to be associated with advanced stages of endometriosis ($P < 0.001$).

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