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Original Research Article

Serum and peritoneal fluid levels of ischemia modified albumin in moderate/severe endometriosis

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ABSTRACT

Background: Recently, the role of oxidative stress in progression of endometriosis has been reported. Ischemia-modified albumin (IMA) is a marker of protein oxidation and very limited number of studies has evaluated the role of IMA in endometriosis. This study was designed to evaluate the serum and peritoneal fluid IMA levels in moderate/severe endometriosis as a marker for oxidative stress.
Methods: This study was designed as a prospective controlled clinical trial. The study group consisted of 35 cases who underwent laparoscopy and with a diagnosis of moderate/severe endometriosis. The control group (n=35) was cases without endometriosis that underwent laparoscopy for tubal sterilization. The serum and peritoneal fluid IMA levels were measured spectrophotometrically by colorimetric method with complex of albumin non-binding cobalt and dithioerthreitol.
Results: Although the median serum IMA levels in study and control groups were similar (p=0.553), the levels of peritoneal fluid IMA were significantly higher in study group (p=0.044). In endometriosis cases with dysmenorrhea peritoneal fluid IMA levels were much higher than cases without dysmenorrhea (p=0.018).
Conclusions: The increased levels of IMA in peritoneal fluid of endometriosis support the possible role of oxidative stress in endometriosis. With this study, peritoneal fluid IMA levels are initially documented in endometriosis cases.

Keywords: Endometriosis, Ischemia-modified albumin, Laparoscopy, Oxidative stress, Peritoneal fluid

INTRODUCTION

Endometriosis is a benign, estrogen-dependent, chronic gynecological disorder characterized by the presence of endometrial tissue outside the uterus. It affects 6%-10% of women of reproductive age and is known to be associated with pelvic pain and infertility.[1] Despite a large number of studies on endometriosis, its etiology has not been clearly defined yet. Recent data suggest that a combination of factors (hormonal, genetic, environmental, anatomical and immunological) plays a role in the pathogenesis of this disorder.[2,3]

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio can be altered by increased levels of reactive oxygen species (ROS) and /or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms.[4,5]

Although, the current literature suggest that endometriosis induce local inflammatory processes and women with endometriosis experience a greater degree of oxidative stress than healthy fertile women[6], the results of the previous studies documenting the role of augmented oxidative stress in pathogenesis and progression of endometriosis are conflicting.[6-9] While some studies failed to observe increased OS in systemic circulation or peritoneal fluid of patients with endometriosis, others have reported increased levels of OS markers in those with the disease.[10-13]

Ischemia-modified albumin (IMA) measured by the albumin cobalt binding test was used originally as a biomarker for evaluating patients with cardiac ischemia.[14] The accurate mechanism of how IMA is produced in presence of ischemia is unclear but appears to be related to the production of ROS that modify the metal binding sites.[15] For IMA, N-terminal modifications results in reduction to cobalt binding capacity of serum albumin and the albumin cobalt binding test determines the level of serum IMA. As elevated IMA levels may indicate the deterioration of oxidative balance, this study was designed to evaluate the systemic circulation and peritoneal fluid IMA levels in moderate/severe endometriosis. There is no study that have measured IMA in the peritoneal fluid which is the important player in the etiology of endometriosis.

METHODS

A controlled, prospective, clinical trial was carried out following approval from the Human Research Ethics Committee of the Hospital and written informed consent was obtained from all participants. The patients with dysmenorrhea, dyspareunia and/or pelvic pain and an ovarian mass compatible with endometrioma at transvaginal ultrasonography were asked to participate to the study performed during January 2013 and April 2013. During the study period, 35 fulfilled the inclusion criteria and accepted to participate in the study. All the cases were between 30 and 45 years of age and the diagnosis of endometriosis was confirmed histopathologically by laparoscopy. All the participants of the study group were stage 3-4 endometriosis according American Fertility Society (r-ASRM) scoring system.[16] The control group was healthy women aged 30-45 years with normal pelvic anatomy without endometriosis that underwent laparoscopy for tubal sterilization (n=35).

The exclusion criteria were history of any drug use known to interfere the results for at least 6 months before the study (antioxidants, vitamin C and E, hormone preparations), stage 1-2 (minimal/mild) endometriosis at laparoscopy, current smokers, cases with high C reactive protein and/or white blood cell count levels (normal value 4 to 10 $x10^3/\mu L$), active pelvic inflammatory disease, presence of any acute or chronic disease (hyperprolactinemia, thyroid dysfunction, adrenal dysfunction, diabetes mellitus, hypertension) and pregnancy.

Information on medical, gynecologic and obstetric history, sociodemographic parameters, and personal habits (i.e. smoking and alcohol intake) was obtained from each participant. After overnight fasting, venous blood samples (5 mL) were collected from the antecubital vein from all patients before any intervention. At laparoscopy, a lavage of the lower peritoneal cavity with 20 mL normal saline was performed and peritoneal fluid samples were collected from the pouch of Douglas into a sterile syringe from all the participants. Samples were immediately centrifuged, and serum was separated and frozen at -80ºC until assay. IMA concentrations were analyzed by measuring the complex composed of dithioerthreitol (DTT) and cobalt unbind to albumin by colorimetric method in spectrophotometer. The analyses in spectrophotometer (Human Humalyzer 2000) was performed at 470 nm for detection of absorbance of the specimens and the results were given as absorbance units (ABSU).

Statistical analysis

Data analysis was performed by using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, United States). The mean differences between groups were compared by Student's t test. Mann Whitney U test was applied for the comparisons of the median values. Nominal data were analyzed by Chi-square or Fisher's exact test, where appropriate. A p value less than 0.05 was considered statistically significant.

RESULTS

The mean age and body mass index (BMI) of all the participants (n=70) were 34±3.1 years (ranged from 30 to 44) and 25.9±3.7 kg/m^2(ranged from 18 to 36), respectively. Neither the age nor the BMI differed between the endometriosis and control groups (Table 1).

Table 1: The baseline characteristics of cases and controls.

	Endometriosis group (n=35)	Control group (n=35)	*p*
Age (years), mean±sd	33.3±3.0	34.6±3.1	0.08
BMI (kg/m^2), mean±sd	25.2±4.2	26.5±3.1	0.16
Stage (r-ASRM) n (%)			
-Stage 3	12 (34.3)		
-Stage 4	23 (65.7)		
Symptoms n (%)			
Infertility	11 (31.4)		
Dysmenorrhea	26 (74.3)		

BMI: Body mass index

The analyses of IMA levels showed much higher levels in peritoneal fluid when compared with serum levels both in endometriosis and controls (Table 2). However, peritoneal fluid IMA levels were statistically significantly higher among endometriosis cases when compared with controls (Table 2). Regarding the serum IMA levels, no significant difference was found between stage 3 and 4 cases and in cases with or without dysmenorrhea (p>0.05). However, peritoneal fluid IMA levels of endometriosis cases with dysmenorrhea was significantly higher than cases without dysmenorrhea (0.56±0.15 vs 0.40±0.21, respectively; p=0.02).

Table 2: IMA levels in patients with endometriosis and disease-free controls.

	Serum IMA (ABSU), mean±sd	Peritoneal fluid IMA (ABSU) mean±sd	p
Endometriosis group (n=35)	0.39±0.16	0.52±0.18	0.00
Stage 3 (n=12)	0.44±0.17	0.53±0.10	
Stage 4 (n=23)	0.36±0.14	0.51±0.20	NS
p	NS	NS	NS
Dysmenorrhea (n=26)	0.38±0.14	0.56±0.15	NS
Without dysm. (n=9)	0.41±0.20	0.39±0.21	NS
p	NS	0.01	
Infertility (n=11)	0.33±0.10	0.53±0.12	NS
Fertile (n=24)	0.41±0.16	0.51±0.20	NS
p	NS	NS	
Control group (n=35)	0.33±0.23	0.44±0.15	NS
p	NS	0.04	

DISCUSSION

In presented study, peritoneal fluid IMA levels were found to be significantly higher in patients with endometriosis when compared to healthy controls. This result documenting oxidative stress in peritoneal fluid of advanced stage endometrosis supports the data that oxidative stress might play a role in pathogenesis or progression of the disease.

In endometriosis, peritoneal fluid containing ROS-generating iron, macrophages and environmental contaminants is thought to be disrupt the balance between the ROS and antioxidants, resulting in increased proliferation of endometriotic tissues and adhesions.[17,18] In a recent metaanalysis, increased levels of different oxidative stress markers were reported in 11 studies where 19 studies were analysed.[19] Among these studies only one, have evaluated the levels of serum IMA in endometriosis as a marker of protein oxidation.[20] In that study, as in ours, no significant difference was found in serum IMA levels when endometriosis cases were compared with healthy controls.[20] However, that study did not analyse peritoneal fluid IMA levels. In endometriosis, peritoneal fluid contains high concentrations of pro-inflammatory and chemotactic cytokines that activates phagocytic cells and apoptotic endometrial tissue accumulated in endometriotic implants, which are the basic producers of ROS.[12] Therefore, in our study peritoneal fluid IMA levels were analysed as well. Up to day numerous papers reported increased levels of different oxidative stress markers in peritoneal fluid of endometriosis cases.[8,12,13,21] The results of this study suggested that OS, detected as elevated IMA levels, may be confined to the peritoneal cavity in women with advanced stage endometriosis. Moreover, this is the first is study in the literature documenting IMA levels in peritoneal fluid of endometriosis cases.

In several studies, it is also stated that moderate doses of ROS induce endometriotic growth and proliferation, whereas higher doses of ROS show directly cytotoxic and apoptotic effects.[22] Even if accumulating evidence indicates that oxidative stress is related to the progression of endometriosis, the serum and peritoneal fluid IMA levels of cases at stage 3 and 4 endometriosis were found to be similar in the presented study.

According to previous data in the literature endometriosis stage, lesion type or site is not associated with occurrence of endometriosis symptoms.[23] However, mediators of fibrosis and inflammatory changes in the peritoneal fluid environments are likely involved in the development of the symptoms associated with endometriosis.[18] Supporting this data, the results of this study showed higher levels of oxidative stress, in terms of peritoneal fluid IMA levels, in cases with dysmenorrhea when compared with patients without dysmenorrhea.

New assays developed by commercial firms are usually preferred for detection of IMA serum levels. The previous data documenting serum IMA levels in endometriosis cases used an ELISA method by a commercial kit.[20] Therefore, the authors determined serum IMA levels in U/mL which makes it impossible to compare their data with our results (ABSU). The test in the study of Lambrinoudaki is an indirect test for IMA using an antibody where the method in present study is different from the commercial kits, which is a direct measurement of IMA.[20] Therefore, more valid data is accounted by our study.

These data provide further evidence that IMA, as an oxidative stress marker, is significantly increased in peritoneal fluid samples from patients with advanced stage endometriosis compared with controls.

Moreover, a significantly increased level of oxidative stress in patients suffering from dysmenorrhea might be an explanation for the occurring symptomatology. However, further research is needed to explain whether oxidative stress plays a role in the pathogenesis or in the progression of the disease.

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