The role of oxidative-stress related genes in endometriosis-associated infertility

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ABSTRACT

INTRODUCTION

Endometriosis is a complex disease of controversial aetiology defined by the presence of endometrial-like tissue outside the uterus. The general socioeconomic, affecting not only women with the disease but also their partners may be similar to Crohn's disease, diabetes and rheumatoid arthritis, mostly because of associated infertility and affected quality of life:work, education, social and intimate life and general wellbeing.

Despite all of this, there still exists a large diagnostic void between the onset of symptoms and a reliable diagnosis averaging between 8-12 years. There are currently no accurate non-invasive diagnostic tests or biomarkers for endometriosis.

Overall, evidence suggests that currently there are no biological markers that can reliably aid the diagnosis of endometriosis. Therefore, the authors of the 2022 ESHRE guideline concluded that "clinicians should not use measurement of biomarkers in endometrial tissue, blood, menstrual or uterine fluids to diagnose endometriosis."
This makes genetic testing linked to the pathogenic process of endometriosis an intriguing area of study.

During this doctoral research we sought to determine whether there was a relationship between endometriosis-related infertility and four genetic variants of antioxidant enzymes involved in oxidative stress. In this case-control study, the first of this kind in Eastern European women, we investigated the genetic polymorphism of four genes and selected those that encode antioxidant enzymes involved in oxidative stress: glutathione peroxidase 1, GPX1 198Pro>Leu, catalase CAT-262C > T, glutathione S-transferase M1, and T1 null genotype.

We investigated the association between these polymorphisms and endometriosis-related infertility in 103 patients with endometriosis-associated infertility and a control group of 102 post-partum women in an attempt to assess the performance of a non-invasive, dependable and affordable method to replace laparoscopy for the diagnosis of endometriosis, with good sensitivity and specificity.

Material and method

The aim of this study was to investigate four genetic polymorphisms of the antioxidant enzymes involved in oxidative stress (glutathione peroxidase Pro198Leu, catalase C−262T, glutathione peroxidase S-transferase M1 null allele, and glutathione peroxidase S-transferase T1 null allele) and their association with endometriosis-related infertility. We prospectively assessed 103 patients with endometriosis-associated infertility. All subjects underwent laparoscopic surgery at one academic hospital in Romania between 2015–2019.

The inclusion criteria were:

- infertile females aged 24 to 40 years,
- BMI ranging from 19 to 27,
- with visual and microscopic confirmation of endometriosis.

We excluded patients with chronic treatments that may interfere with fertility, and we also ruled out women whose partners were not tested for fertility disorders. We selected 102 consecutive post-partum women with no history of obstetrical diseases and no fertility treatment before pregnancy for the control group.

Surgery with a laparoscopy approach was performed for the endometriosis group (*n* = 103) to confirm the diagnosis and treat the lesions. The indications were either symptoms or infertility. The endometriotic lesions were treated with ablation for peritoneal lesions, cystectomy, or cyst drainage for ovarian endometrioma. In order to exclude other factors causing infertility, tubal chromopertubation was routinely performed perioperatively.

Blood samples were taken on days 2–4 postoperatively. For the control group (*n* = 102), blood samples were collected day 2–4 post-partum, after vaginal or cesarean delivery, during the same period.

Genotyping of glutathione peroxidase (GPX) Pro198Leu and catalase (CAT)-262C > T, glutathione S-transferases (GST) GSTM1, GSTT1 gene polymorphisms were performed by using genomic DNA (gDNA). Genomic DNA of the patients and controls was extracted from peripheral blood collected in EDTA tubes using Genomic DNA Mini Kit (Thermo Fisher Scientific, Norcross, GA, USA), following the manufacturer's recommendations.

GPX1 198Pro > Leu and CAT-262C > T were genotyped by applying the PCR-RFLP (polymerase chain reaction and restriction fragment length polymorphism) technique. In contrast, the multiplex PCR method established GSTM1 and GSTT1 null genotypes, as previously described.

The statistical analysis was performed using STATA version 16.1. Descriptive statistics assessed the similarity between the two groups. The chi-square test was used to evaluate the genotype distribution and frequencies between the two groups having discrete data sets. When necessary, Yates's and Fisher's corrections were applied. Alpha was set at 0.05. Odds ratios (ORs) and confidence intervals (95% CI) were calculated to assess the risk of developing the disease for each genotype.

The Ethics Committee of the University of Medicine, Pharmacy, Science and Technology, "George Emil Palade" Targu Mures, Romania, approved the study (No 6253/4 March 2022).

Results

The CAT-262C > T variant homozygous genotype (TT) and heterozygous genotype (CT) represent risk factors in endometriosis development (p = 0.013 for CT and p = 0.019 for TT). Moreover, we found significant associations between variant genotypes (CT, TT) of GPX1 198Pro > Leu and the risk of developing endometriosis (p = 0.040 for CT and p = 0.019 for TT).

The null genotype of GSTM1 was significantly higher in the endometriosis group (p < 0.0001). No significant differences were found in the frequency of GSTT1 between the two groups.

The other investigated genotypes did not show an association between gene polymorphisms and the risk of developing endometriosis.

Discusion

Both groups of patients were selected to have a similar number of observations as well as age distribution and no previous medical diagnoses that might interfere with that of endometriosis- associated infertility, thus reducing possible unforeseen biases.

The literature's interest has shifted in the last decade and there has been more research on the involvement of a genetic predisposition in the development of certain diseases.

Our study suggested that polymorphisms of GPX1 198Pro > Leu and CAT-262C > T are both associated with the incidence of symptomatic endometriosis. Very few articles address the association between aberrant expression of GPX1 and CAT genes and endometriosis.

Authors suggest that the implication of GPX1 and CAT gene polymorphisms can cause endometriosis but request more extensive studies to confirm their findings.

According to our results, CAT-262C > T is a risk factor for endometriosis development. The relatively small number of patients is a limitation of this study, and therefore the results must be interpreted cautiously.

GST is a family of antioxidant enzymes, divided into eight classes. Due to the frequent allelic changes, GSTT1 and GSTM1 are the most intensively studied variants. The meta-analysis by Sun-Wei Guo suggests that only GSTT1 is associated with developing endometriosis. Almost a decade later, two other meta-analysis studies conclude that both GSTT1 and GSTM1 null genotypes could be risk factors for endometriosis but suggest that further studies are needed for confirmation. In comparison, our findings indicate that only GSTM1 is correlated with endometriosis. This two meta-analyses also concluded that the association of both null genotypes for GSTT1-GSTM1 is related to endometriosis. Our study confirms this theory, as our findings show a powerful association in this case (p = 0.0004).

Our results also reveal that GPX1 198Pro > Leu, CAT-262C > T, and GSTM1 null genotype may be risk factors. The association between GSTM1-GSTT1 null genotype may play a significant

role in endometriosis-associated infertility. However, GSTT1 null genotype does not influence the disease.

Compared to the existing literature, this discrepancy in our findings might be explained by demographic differences, as ethnicity and environmental factors play an important role in developing the disease.

Conclusions

Visual identification of endometriotic lesions with microscopic confirmation remains the accepted gold standard for endometriosis diagnosis, but general anesthesia and laparoscopy are required. In this regard, a panel of genetic or laboratory markers is needed for the early diagnosis of this prevalent disease, especially in the case of young patients with future pregnancy intentions. Polymorphism of GPX1 198Pro > Leu and CAT-262C > T are both associated with symptomatic endometriosis, and CAT-262C > T and GSTM1 are risk factors for disease's development.

Our study suggested that polymorphisms of GPX1 198Pro > Leu and CAT-262C > T are both associated with the incidence of symptomatic endometriosis.According to our results, CAT-262C > T is a risk factor for endometriosis development.The association of both null genotypes for GSTT1-GSTM1 is related to endometriosis. Our study confirms this theory, as our findings show a powerful association in this case (p = 0.0004). Our results also reveal that GPX1 198Pro > Leu, CAT-262C > T, and GSTM1 null genotype may be risk factors. The association between GSTM1-GSTT1 null genotype may play a significant role in endometriosis-associated infertility.