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CYP1A1 and GSTM1 genes polymorphism and its association with endometriosis : A pilot study

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ABSTRACT

Objective: To study the genetic association between Cytochrome P450 family 1 (CYP1A1) T6235C polymorphism and glutathione S-transferase M1 (GSTM1) null mutations and endometriosis. **Methods:** A total of 121 unrelated women having complaints of pelvic pain, dysmenorrhea, dysuria, dyschezia, dyspareunia and infertility were enrolled. Out of these 71 consented for laparoscopy, 66 were diagnosed as endometriosis as per operative. Genomic DNA isolated from endometriosis patients and controls were subjected to polymerase chain reactions to determine the GSTM1 null genotypes whereas polymorphism of CYP1A1 T6235C was determined through PCR-RFLP. **Results:** The GSTM1 null genotype was found to be associated with endometriosis however there was no significant difference in the frequencies of the CYP1A1 6235 CC genotype between endometriosis patients and controls. The homozygous mutant and allele frequency of CYP1A1 T6235C differed significantly between patients having endometriosis and healthy control. **Conclusion:** The data of the present study clearly suggests that GSTM1 null allele and CYP1A1 C allele is a genetic risk factor for endometriosis in North Indian population.

1. Introduction

Endometriosis an enigmatic disease, better a syndrome that starts around the prepubertal age and flourishing after menarche with symptoms progressing in intensity throughout years. Endometriosis can cause significant pelvic pain, pelvic masses which can result in multiple operations and infertility, having a significant detrimental impact on patients quality of life and substantial morbidity. Studies suggests relationship between exposure to dioxins and polychlorinated biphenyls (PCBs), severe endometriosis, and altered immune responses [1, 2]. Increase level of serum dioxins is found in women with peritoneal endometriosis and deep endometriotic lesions compared to fertile women without disease [3].

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Recent human studies indicate that environmental dioxins, including PCBs, play a role in the pathophysiology of endometriosis. Studies suggest that dioxins and certain PCBs exposure promotes endometriosis via stimulation of chronic inflammation potentially leading to enhanced estrogen synthesis and disruption of progesterone-dependent remodeling responses that normally limit the development of endometriosis [1]. Hence, polymorphisms in detoxification pathway leading to impaired biotransformation functions may increase susceptibility to endometriosis.

The gene encoding the enzyme CYP1A1 gene (15q22-q24) has a polymorphism which is 264 bases downstream from the polyadenylation site at the 3′ end of exon 7. It has been demonstrated that high-inducibility of the Cytocrome P450 family 1 (CYP1A1) gene is related to the presence of the MspI polymorphism [4]. The glutathione S-transferases (GST) catalyse the conjugation of reactive hydrophobic and electrophilic compounds to reduced glutathione thereby deactivating their toxicity. Many of the known GST substrates are xenobiotic, and different classes of enzymes are specific for different substrates [5]. GSTM1 (1p13) deletions result in null alleles, for which homozygosity confers a complete lack of enzyme activity. The enzymes

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appear to be important in the detoxification of products of oxidative stress, for example lipid hydroperoxides, alkenals and DNA hydroperoxides, as well as potential carcinogens such as methyl halides, benzo[a]pyrene epoxides and polycyclic aromatic hydrocarbons (PAH) present in diet and in tobacco smoke[6]. The null mutation of GSTM1 has been associated with colon cancer[7], chronic bronchitis in heavy smokers[8] and prostate cancer[9].

Looking at the importance of CYP1A1 and GSTM1 in reproductive health, we hypothesized that polymorphism in this gene may increase the susceptibility to endometriosis. Therefore, we recruited patients with endometriosis to find if allelic variant of CYP1A1 and null genotype of GSTM1 gene affects the risk of endometriosis.

2. Materials and methods

This was the prospective study conducted in Gynaecological OPD of Sir Sunderlal Hospital, BHU in which 121 patients who came with presenting complaints of pelvic pain, dysmenorrhea, dysuria, dyschezia, dysparenuia and infertility. Out of these 71 consented for laparoscopy, 66 were diagnosed as endometriosis as per operative finding. Cases were selected on the basis of history of infertility, pelvic pain and dysmenorrhea, dysuria, dyschezia, dysparenuia with the symptoms and signs suggestive of endometriosis. Patients with inflammatory bowel disease, pelvic inflammatory disease, urinary tract infection, functional cyst, irritable bowel syndrome, genital tuberculosis were excluded from the study. 100 control with no history of gynecological complication and having regular cycle were enrolled for the study.

2.1. Specimens

Blood samples of all the cases and controls were drawn from the antecubital vein and stored at 4 ℃ till genomic DNA isolation was performed.

2.2. Genotyping for CYP1A1 and GSTM1 null polymorphisms

Genotype analyses of CYP1A1 and GSTT1 were performed by previously used forward and reverse primers [10]. In each reaction, 50 ng of genomic DNA was amplified in 1X of PCR buffer containing 200 μM dNTPs and their respective forward and reverse primers containing 0.5 U of Taq DNA polymerase. All reactions were conducted in an oil-free thermal cycler (Thermal Cyclers, BioRad Inc.) The amplified products were electrophoresed on 2% agarose gels containing ethidium bromide and the product bands were visualized under ultraviolet light. Fifteen microliters of PCR product was then digested with 1U of *Msp*I enzyme as specified by the manufacturer protocol (Fermentas Inc.). Separation of the products was performed on a 2% agarose gel stained with ethidium bromide. If there was a T > C transition the 340 bp product of CYP1A1 gene fragment generated two subfragments of 200 and 140 bp. The presence of functional GSTM1 gene was determined by amplification of the band of the expected size. Individuals were determined null for GSTM1 gene when a band of the expected size was absent in the presence of the positive internal control band (an exon fragment of CYP1A1).

2.3. Statistical analysis

Hardy-Weinberg equilibrium was tested using the goodness-of-fit *chi* square test with two degrees of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Statistical significance of the differences in the frequency of genotypes using the *chi*-square test, odds ratio at 95% confidence interval (95 % CI) were calculated to assess the relative risk conferred by a particular allele and genotype. Power of study was calculated using G* power. Yates' correction was done to prevent the overestimation of statistical significance for small data. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant.

3. Results

The genotype frequencies in the control group for all the polymorphisms fitted well in the Hardy-Weinberg equilibrium ($P>0.05$). The genotype and allele frequencies for T6235C polymorphism of CYP1A1 gene were compared between the laparoscopicaly positive endometriosis cases and control group (Table 1). For T6235C polymorphism, C allele frequency was found to be 33.3% (44/132) and 22.5% (45/200) in cases and controls respectively, T allele frequency was found to be 66.7% (88/132) and 77.5% (155/200) in cases and controls respectively. Comparison of allele frequencies showed statistically significant difference of the T6235C polymorphism between the laparoscopic positive endometriosis cases versus control group (*OR*= 1.73 and 95% *CI*=1.05-2.84, $P=0.02$, Yates *P* value=0.03). The genotype frequencies of SNP T6235C however did not differed significantly between two groups ($P=0.05$, Yates *P* value=0.10). The genotype frequencies for GSTM1 gene were also compared between the in laparoscopicaly positive endometriosis cases and control group (Table 2). For GSTM1, null genotype frequency in cases and controls was 40.90% and 16% respectively. The difference of genotype frequencies was found to be statistically significant between

Table 1

Genotype and allele frequencies of CYP1A1 T6235C single nucleotide polymorphism (SNP) among endometriosis and control groups.

Genotype	Cases (n=66)	Controls (n=100)	Yates corrected *P* value	Odds ratio	95% *CI*
TT	31(46.97)	59(59)	-	-	-
TC	26(39.39)	37(37)	0.49	1.33	0.68-2.59
CC	9(13.64)	4(4)	0.03	4.26	1.30-13.98
TC + CC	35(53.03)	41(41)	0.17	1.61	0.86-3.01

Table 2

Genotype frequencies of GSTM1 null genotype among endometriosis and control groups (n,%).

Genotype	Cases (n=66)	Control (n=100)	Yates corrected *P* value	Odds ratio	95% *CI*
GSTM -/-	27(40.90)	16(16)	P=0.0006	3.63	1.79-7.36
GSTM +/+	39(59.09)	84(84)			

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the laparoscopic positive endometriosis cases versus control group (*OR*=3.63, 95% *CI*=1.79-7.36, *P*=0.0003, Yates *P* value=0.0006).

4. Discussion

Endometriosis is a common gynecological condition in which endometrial tissue from the uterus is found in abnormal locations in the body, commonly on nearby pelvic organs such as the ovaries and fallopian tubes. This misplaced endometrial tissue responds to normal hormonal signaling from estrogen and progesterone causing cyclic growth and bleeding, which results in tissue accumulation, inflammation, and adhesion formation.

A certain group of women develop endometriosis implies that there is increased susceptibility to development of disease in certain cases. Individual's susceptibility is influenced not only by genetic background but also by the interaction of genes with environmental factors. Dioxin-TCDD, dioxin like PCBs and phthalate esters have been implicated as factors involved in the development of endometriosis[11-12]. The lack of detoxification, which is genetically determined, might be a risk factor for endometriosis development[13]. Reactive oxygen species (ROS), which are highly reactive oxygen free radicals generated as a result of interaction with these chemicals cause cellular damage, and the growth of endometrial tissue[14].

Metabolism of environmental toxins is typically performed sequentially by phase I (functionalization) and phase II (conjugation) reactions. Phase I drug-metabolizing enzymes (*e.g.*, CYP1A1) act by introducing a functional group into their endogenous and exogenous substrates. A procarcinogenic compound thus is metabolically activated by a (phase II) conjugating enzyme that renders the compound inactive, and, hence, is no longer carcinogenic or procarcinogenic. CYP1A1 and GSTM1 are one of the most important parts of phase I and phase II enzymes respectively. Moreover the CYP1A1 T6235C polymorphism and GSTM1 null mutation influence the enzyme activity. Based on these backgrounds, we investigated the association and GSTM1 polymorphisms and endometriosis. In the present study, we found null genotype of GSTM1 increase the endometriosis risk.

CYP1A1 T6235C polymorphism and GSTM 1 null mutation has studied previously in association with endometriosis in different population (Table 3). The relationship between CYP1A1 T6235C polymorphism and risk of endometriosis studied by several group found no association of disease with respect to controls [15-17]. An association between endometriosis and the GSTM1 null mutation was observed in a Slavic population, French population and in taiwanese han population and other population [13, 15, 18-22]. Some

Table 3

Characteristics of included studies of glutathione S transferase GSTM1 and CYP1A1 and endometriosis.

Reference	Sample number	Inference
Baranova *et al*, 1997[8,13]	50 endometriosis patients and 72 controls.	GSTM1 null deletion is found to be associated with endometriosis.
Baranova *et al*, 1999[19]	36 patients with minimal/mild; group I, 29 patients with moderate/severe endometriosis; group II and 72 controls of French origin	GSTM1 null deletion is found to be associated with endometriosis.
Peng DX *et al*, 2003[20]	76 patients with endometriosis and 80 controls (surgical patients for gynecological problems other than endometriosis	GSTM1 gene deletion might be a risk factor for endometriosis in women of Han nationality who are native residents in Guangdong Province.
Aban M *et al*, 2007[21]	150 women who were diagnosed by means of surgery and histopathology as having endometriosis. The control group consisted of 150 women who displayed no evidence of endometriosis during exploratory laparotomy or laparoscopy.	GSTM1-null allele was associated with a significantly increased risk of endometriosis and smoking with a decreased risk of endometriosis separately.
Roya R *et al*, 2009[22]	Ninety seven women diagnosed by laparoscopy and 102 women without endometriosis as controls.	Women with GSTM 1 null mutation have an increased susceptibility to endometriosis.
Wu *et al*, 2012[15]	121 patients with advanced-stage endometriosis and 171 control subjects	CYP1A1 genetic polymorphism was not associated with development of endometriosis whereas GSTM1 was found to be associated with endometriosis.
Baxter SW *et al*, 2001[23]	84 cases of endometriosis, and 219 controls	GSTM1 null allele is not an endometriosis susceptibility gene.
Hadfield RM *et al*, 2001[24]	148 women each with endometriosis (sporadic cases, *n*=91; familial cases, *n*=57), and a control group of 53 women with a normal pelvis at hysterectomy	No. significant difference in frequency of GSTM1 & GSTT 1 null mutation AND CYP1A1 in cases and control was found. But combination of GSTM1 null genotype and CYP1A1 polymorphism was associated with in risk of endometriosis.
DA *et al*, 2003 [25]	Arvanitis A group of 275 women with sporadic endometriosis was compared with a group of 346 fertile, endometriosis-free women.	CYP19 VNTR (TTTA) (10) allele as well as the combined genotype CYP1A1 polymorphism and GSTM1 null deletion associate with the endometriosis phenotype, whereas the GSTT1 null deletion does not.
Ivashchenko TE *et al*, 2003 [26]	74 patients with extragenital endometriosis and 39 Controls	No. significant difference in frequency of GSTM 1 null mutation in cases and control was found.
Peng DX *et al*, 2002 [17]	76 patients with endometriosis and 80 controls (surgical patients for gynecological problems other than endometriosis	Msp I polymorphisms of cytochrome P4501A1 in itself might not be associated with the susceptibility to endometriosis in women of Han Nationality in Guangdong Province.
Morizane *et al*, 2004[27]	114 unrelated women with endometriosis were enrolled. Samples of umbilical cord blood obtained from 179 female newborn infants were used as population controls	No association with GSTT1 null mutation and GSTM1 null mutation was found with cases.
Ding *et al*, 2004[28]	107 controls and 41 cases of Uygurs Nationality and the 105 controls and 80 cases of Hans Nationality	No evidence was found to suggest an association between GSTM1-null genotype and endometriosis in the Hans and Uygurs.
Hur SE *et al*, 2005[29]	Blood samples were available from 259 controls and 194 patients with advanced endometriosis diagnosed by both pathology and laparoscopic findings.	GSTM1, GSTT1 and GSTP1 genetic polymorphism are not associated with development of endometriosis in Korean women.

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groups however found non association of GSTM1 with Endometriosis [23-29]. The present study however found an association of CYP1A1 'C' allele and GSTM1 null allele with laparoscopicaly positive cases. The discrepancy could be due to the different ethnicities in the populations.

In the present study we tried to establish an association between CYP1A1 T6235C and GSTM1 null genotypes and their possible impact in developing endometriosis in the North Indian women. The lack of detoxification, which is genetically determined, might be a risk factor for endometriosis development and more study from other population and in more number of samples would be valuable to determine it as risk factor for endometriosis.

Declare of interest statement

We declare that we have no conflict of interest.

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