

No association of GSTM1 null polymorphism with endometriosis in women from central and southern Iran

Seyed Morteza Seifati¹ Ph.D., Kazem Parivar¹ Ph.D., Abbas Aflatoonian² M.D., Razieh Dehghani Firouzabadi² M.D., Mohammad Hasan Sheikhha² M.D., Ph.D.

1 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

2 Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Received: 20 April 2011; accepted: 15 June 2011

Abstract

Background: Endometriosis is one of the most common gynecologic disorders. It is a complex trait and both genetic and environmental factors have been implicated in its pathogenesis. There is growing evidence indicating that exposure to environmental contaminants is a risk factor for endometriosis. Glutathione-S-Transferase M1 (GSTM1) is one of the genes involved in detoxification of endogenous and exogenous compounds.

Objective: Several studies have indicated an association between GSTM1 null mutation and endometriosis. In this study, the possible association between the GSTM1 gene null genotype and susceptibility to endometriosis in woman from central and southern Iran was investigated.

Materials and Methods: One hundred and one unrelated premenopausal women with endometriosis and 142 unrelated healthy premenopausal women without endometriosis were enrolled in the study. Genomic DNA was extracted from Peripheral blood in all subjects. GSTM1 null genotyping was performed by polymerase chain reaction (PCR).

Results: There was no significant difference between frequencies of GSTM1 null genotype in case and control groups (50.5% Vs. 52.1%, $p=0.804$). Furthermore, this genotype was not associated with severity of endometriosis in our sample ($p=0.77$).

Conclusion: further studies involving gene-environment and gene-gene interactions, particularly combination of GSTM1 and other GST gene family polymorphisms are needed.

Key words: Endometriosis, Glutathione-S-Transferase M1, Null genotype.

This article was extracted from Ph.D. thesis.

Introduction

Endometriosis (MIM 131200) is one of the most common gynecologic disorders and a cause of infertility in women. It is characterized by ectopic growth of endometrial tissue outside uterus cavity (1). The disease has been reported in 2-22% of women of reproductive age (2) and in 5-50% of

infertile women (3).

In spite of decades of clinical and basic research on endometriosis, little is known about its etiology and pathogenesis (4). The most widely held theory postulates that viable endometrial cells are carried to peritoneal cavity through Fallopian tubes by retrograde menstruation and are implanted there, giving rise to endometriosis (5). The fact that menstrual debris are found in peritoneal cavity of up to 90% of menstruating women casts some doubt on this theory (6). Endometriosis is a complex trait and both genetic and environmental factors have been implicated in this disease (7).

Corresponding Author:

Seyed Morteza Seifati, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Email: seifati@yahoo.com

The role of genetic factors in endometriosis has been assessed and confirmed through numerous studies (8-13). These studies show a higher risk of endometriosis in first-degree relatives of endometriosis patients compared to general population (10, 11, 14). Twin studies provide further evidence of genetic propensity to endometriosis. These studies show a higher risk of endometriosis in monozygotic twins compared to dizygotic twins (15-17). Several studies have attempted to identify endometriosis susceptibility genes. Candidate genes studied thus far include genes involved in inflammation, steroid synthesis, and detoxification. Prominent among the latter are glutathione-S-transferases (GSTs) (7, 18).

Numerous studies indicate a relation between environmental pollutants and endometriosis (19-21). It has been postulated that variants of genes involved in detoxification of these pollutants may play a role in endometriosis (22). GSTs are phase II detoxifying enzymes, catalyzing conjugation of electrophilic and hydrophobic compounds to reduced glutathione, thereby eliminating their toxicity (23). These enzymes are involved in detoxification of a wide range of toxic and carcinogenic substrates (24).

Many of the known substrates of GSTs are xenobiotic substances and environmental pollutants. Different classes of these enzymes are specific for different substrates (25). Compounds like dioxins, which are ubiquitously present as known environmental pollutants throughout the world and impinge upon the food chain, are among GST's substrates (22, 26). In humans, cytosolic GSTs comprise six classes of enzymes including Mu (μ), Omega (ω), Pi (π), Theta (θ), and Zeta (ζ). Each of these classes is coded by one gene or gene subfamily (27). Glutathione-S-transferase M (GSTM) exists in 5 isoforms (GSTM1-5) and belongs to μ class.

A gene cluster on chromosome 1 encodes these isoforms (28). GSTM1 gene is located on chromosome 1p13.3 and has four different alleles. GSTM1*A and GSTM1*B alleles are functionally similar and differ in only one amino acid (p.K172N). GSTM1*1 \times 2 allele comprises two tandemly located genes, both of which are active (29). GSTM1*0 allele contains a deletional mutation.

Due to the extent of this deletion, no mRNA or protein product is coded by the allele (30). A/A, A/O, B/B, and B/O genotype are all active and produce functional enzyme but O/O genotype

causes a complete lack of enzymatic activity and is therefore called the null genotype (30, 31).

Frequency of the null genotype differs in different ethnicities. It is estimated at 50% among Caucasians, 67% in Australians and 22% in Nigerians (32). GSTM1 enzyme metabolizes and detoxifies many exogenous and endogenous substrates, including dioxins, and also oxidative stress products produced during repair of ovarian epithelium after follicle rupture such as lipid hydro-peroxides, alkanes, and DNA hydro-peroxides (22, 26). Several studies have indicated an association between GSTM1 null mutation and endometriosis.

GSTM1 null mutation has been associated with endometriosis in Russian, French, Indian, Chinese, Taiwanese, and Turkish women (33-40). No such association has been observed in English, Japanese, and Korean women (23, 26, 41, 42). To investigate the possible association between the GSTM1 gene null genotype and susceptibility to endometriosis and also its severity, we performed a case-control study in woman of central and southern Iran.

Materials and methods

Study population

This case-control study was conducted between February 2009 and January 2011. On the whole, 101 unrelated premenopausal women with endometriosis were recruited at Yazd Mothers' Hospital and the Yazd Research and Clinical Center for Infertility, which are referral points for central and southern regions of Iran.

Endometriosis was confirmed in subjects by laparoscopy after clinical examination. Laparoscopy was performed by an experienced gynecologist. Patients were staged (I-V) according to the guidelines of American Society for Reproductive Medicine (ASRM) classification system for endometriosis (1997).

The control group consisted of 142 unrelated healthy premenopausal women undergoing Cesarean section or hysterectomy at the same centers with no history of endometriosis and without any lesion suggesting endometriosis during Cesarean section or hysterectomy. Age ranged from 20 to 43 years in endometriosis group and from 17 to 52 years in control group.

The study was approved by the Ethics Committee and Research Committee of Yazd Research and Clinical Center for Infertility.

Written informed consent was obtained from all participants.

DNA analysis

Peripheral blood from patients and controls was collected in EDTA-containing tubes. Genomic DNA extraction was performed using AccuPrep® Genomic DNA Extraction Kit (Bioneer, South Korea) based on manufacturer's manual. GSTM1 genotyping for gene deletion was carried out by PCR. Part of the GSTM1 gene inside deletion area was amplified using primers 5'-GAACTCCCTGAAAGCTAAAGC-3' (forward) and 5'-CTTGGGCTCAAATATACGGTGG-3' (reverse) (Cinnagen, Iran).

To check for the success of amplification, a set of β-globin gene primers [5'-GAAGAGCCAAGGACAGGTAC-3' (forward) and 5'-CCACTTCATCACGTTTACC-3' (reverse)] was added to each PCR tube which amplified a 268 base pair segment of this gene. Each amplification reaction contained 100ng genomic DNA, 1× PCR buffer [50 mM KCl, 10 mM tris-HCl (pH 8.4)], 1.5mM MgCl2, 200 μM dNTP, 10 pmol of each primers, and 1.5 U Taq DNA polymerase (Cinnagen, Iran) in a final volume of 25 μl.

Amplification was performed with initial denaturation at 94°C for 5 min, followed by 35 cycles of amplification which was performed at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min, using Mastercycler (Eppendorf, Germany). PCR products were detected by 1.5% (w/v) agarose gel electrophoresis.

In homozygote non-null and heterozygote subjects a 219 base pair band is observed in agarose gel. The absence of this band (in the presence of β-globin PCR product) indicates the homozygote null genotype.

Statistical analysis

Fisher's exact test was used to compare genotype distributions between endometriosis and controls. Two-sample t-test was performed to compare age, Body Mass Index (BMI), age at menarche, parity and infertility between the endometriosis and control groups.

P-value<0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for Social Science version 11.5 (SPSS Inc., Chicago, IL).

Results

Of the total of 101 patients, 15 (14.8%) had stage I (minimal), 39 (38.6%) had stage II (mild), 24 (23.8%) had stage III (moderate) and 23 (22.8%) had stage IV (severe) endometriosis. 76 patients (75%) suffered from dysmenorrhea, 28 (27.7%) from dyspareunia and 9 (8.9%) from pelvic pain. In 25 (24.8%) patients neither of these symptoms was observed.

No statistically significant difference was observed in the age and infertility of patient and control groups but there were significant differences in BMI, age at menarche, and parity between the two groups (Table I). GSTM1 null genotyping was carried out successfully for all patients and controls. In table 2, frequency of null genotype in GSTM1 gene has been shown for 101 endometriosis patients and 142 controls. There was no statistically significant difference in the frequency of GSTM1 null genotype between patient and control groups (p=0.804). The frequency of GSTM1 null genotype was 45% in patients with advanced stages of the diseases (stages III and IV) and 55% in patients with the early stages (stages I and II). This difference was not statistically significant (p=0.77).

Table I. General characteristics of endometriosis patients and control group.

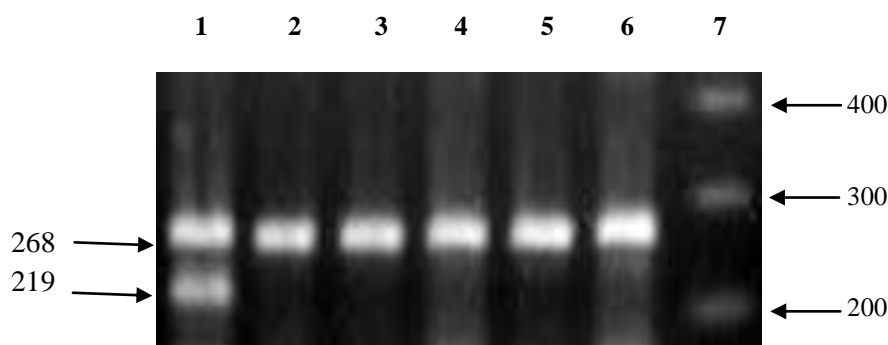
Characteristic	endometriosis	control	p value
Age (yr) (mean±SD)	28.95 ± 5.115	29.82 ± 8.716	0.372 ^a
BMI (kg/m ²) (mean±SD)	24.09 ± 3.725	26.18 ± 4.788	0.001 ^a
Age at menarche(yr) (mean±SD)	13.48 ± 1.299	13.84 ± 0.919	0.019 ^a
Parity (n) (mean±SD)	0.356 ± 0.769	2.458 ± 1.447	<0.05 ^a
Primary infertility [n (%)]	70 (76.9)	7 (63.6)	
Secondary infertility [n (%)]	21 (23.1)	4 (36.4)	0.457 ^b

^a t test

^b Fisher's exact test

Table 2. Frequencies of GSTM1 null and non-null genotypes in endometriosis patients and control group.

GSTM1 genotype	Endometriosis				Total	Controls	p-value ^a
	Stage I	Stage II	Stage III	Stage VI			
Null (%)	6 (40)	22 (56)	13 (54)	10 (43)	51 (50.5)	74 (52.1)	0.804
Non-null (%)	9 (60)	17 (44)	11 (46)	13 (57)	50 (49.5)	68 (47.9)	

^a Fisher's exact test**Figure 1.** Agarose gel electrophoresis of the products of PCR. The 219 bp band shows presence of GSTM1 gene (lane 1). The 268 bp band is a fragment of β -globin gene, which gives a positive PCR control. Lane 7 is 100bp DNA size marker.

Discussion

The role of genetic factors in endometriosis has been assessed and confirmed through numerous familial and twin studies in different populations (10, 11, 14-17) and several studies have implicated GSTM1 gene as a possible candidate gene for susceptibility to endometriosis (18).

We performed a case-control study in woman of central and southern Iran to test the hypothesis that the GSTM1 gene null genotype is associated with susceptibility to endometriosis or severity of it. Our study showed no significant difference between the frequencies of GSTM1 null genotype in case and control groups (50.5% versus 52.1%, $p=0.804$). Also, this genotype was not associated with severity of endometriosis in our sample ($p=0.77$). This is in accordance with the OXEGENE Collaborative study in England (2001) (26), and studies performed by Baxter *et al* (2001) in Southern England (41), Morizane *et al* (2004) in Japanese women (23), and Hur *et al* (2005) in Korean women (42). These studies found no association between GSTM1 null genotype and endometriosis. On the other hand, Baranov *et al* (1996) and Ivashchenko *et al* (2003) reported such an association among Russian women (33, 43).

Subsequent studies by Baranova *et al* (1999) in French women (44), Lin *et al* (2003) and Peng *et al* (2003) in Chinese women (37, 38), Hsieh *et al* (2004) in Taiwanese women (39), Babu *et al*

(2005) and Roya *et al* (2009) in Indian women (35, 36), and Aban *et al* in Turkish women (40) yielded similar results. Recently, a study performed in Rasht in northern Iran also showed such an association (45). These different results in different populations are to be expected. Association between a gene variant and a disease might only be manifest in populations with a particular genetic and environment background.

Circumstances like linkage disequilibrium, gene-gene interactions, and gene-environment interactions can lead to failure of replication of results in association studies in populations with different genetic and environment backgrounds (46). There is growing evidence indicating that interaction of xenobiotic compounds present in different environments with variants of genes involved in their detoxification may change the risk of endometriosis (47). For example, Roya *et al* (2009) studied polychlorinated biphenyls (PCBs) and GSTM1 polymorphism and their possible role in the pathogenesis of endometriosis in Indian women. They found that women with GSTM1 null phenotype and higher concentrations of PCBs might have an increased susceptibility to endometriosis (36).

Taking these considerations into account, further studies in a variety of populations, involving gene-environment and gene-gene interactions, particularly combination of polymorphisms of GSTM1 and other types of GST

gene family are needed to elucidate the relation of GSTM1 polymorphisms and endometriosis.

Acknowledgements

This study was supported by a grant from Yazd Research and Clinical Center for Infertility.

References

- Ozkan S, Murk W, Arici A. Endometriosis and infertility: epidemiology and evidence-based treatments. *Ann N Y Acad Sci* 2008; 1127: 92-100.
- Mahmood TA, Templeton AA, Thomson L, Fraser C. Menstrual symptoms in women with pelvic endometriosis. *Br J Obstet Gynaecol* 1991; 98: 558-563.
- Missmer SA, Cramer DW. The epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 2003; 30: 1-19.
- D'Hooghe TM, Debrock S, Hill JA, Meuleman C. Endometriosis and subfertility: is the relationship resolved? *Semin Reprod Med* 2003; 21: 243-254.
- Sampson JA. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. *Am J Pathol* 1927; 3: 93-110.
- Seli E, Arici A. Endometriosis: interaction of immune and endocrine systems. *Semin Reprod Med* 2003; 21: 135-144.
- Montgomery GW, Nyholt DR, Zhao ZZ, Treloar SA, Painter JN, Missmer SA, et al. The search for genes contributing to endometriosis risk. *Hum Reprod Update* 2008; 14: 447-457.
- Kennedy S. The genetics of endometriosis. *J Reprod Med* 1998; 43 (Suppl.): 263-268.
- Zondervan KT, Cardon LR, Kennedy SH. The genetic basis of endometriosis. *Curr Opin Obstet gynecol* 2001; 13: 309-314.
- Simpson JL, Bischoff FZ. Heritability and molecular genetic studies of endometriosis. *Ann N Y Acad Sci* 2002; 955: 239-251.
- Stefansson H, Geirsson RT, Steinthorsdottir V, Jonsson H, Manolescu A, Kong A, et al. Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod* 2002; 17: 555-559.
- Treloar S, Hadfield R, Montgomery G, Lambert A, Wicks J, Barlow DH, et al. The International Endogene Study: a collection of families for genetic research in endometriosis. *Fertil Steril* 2002; 78: 679-685.
- Vigano P, Infantino M, Lattuada D, Lauletta R, Ponti E, Somigliana E, et al. Intercellular adhesion molecule-1 (ICAM-1) gene polymorphisms in endometriosis. *Mol Hum Reprod* 2003; 9: 47-52.
- Kennedy S, Mardon H, Barlow D. Familial endometriosis. *J Assist Reprod Genet* 1995; 12: 32-34.
- Moen MH. Endometriosis in monozygotic twins. *Acta Obstet Gynecol Scand* 1994; 73: 59-62.
- Hadfield RM, Mardon HJ, Barlow DH, Kennedy SH. Endometriosis in monozygotic twins. *Fertil Steril* 1997; 68: 941-942.
- Treloar SA, O'Connor DT, O'Connor VM, Martin NG. Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril* 1999; 71: 701-710.
- Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II-endometriosis. *Hum Reprod Update* 2009; 15: 97-118.
- Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fundam Appl Toxicol* 1993; 21: 433-441.
- Gibbons A. Dioxin tied to endometriosis. *Science* 1993; 262: 1373.
- Holloway M. An epidemic ignored. Endometriosis linked to dioxin and immunologic dysfunction. *Sci Am* 1994; 270: 24-26.
- Kim SH, Choi YM, Lee GH, Hong MA, Lee KS, Lee BS, et al. Association between susceptibility to advanced stage endometriosis and the genetic polymorphisms of aryl hydrocarbon receptor repressor and glutathione-S-transferase T1 genes. *Hum Reprod* 2007; 22: 1866-1870.
- Morizane M, Yoshida S, Nakago S, Hamana S, Maruo T, Kennedy S. No association of endometriosis with glutathione S-transferase M1 and T1 null mutations in a Japanese population. *J Soc Gynecol Invest* 2004; 11: 118-121.
- Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; 30: 445-600.
- Board P, Coggan M, Johnston P, Ross V, Suzuki T, Webb G. Genetic heterogeneity of the human glutathione transferases: a complex of gene families. *Pharmacol Ther* 1990; 48: 357-369.
- Hadfield RM, Manek S, Weeks DE, Mardon HJ, Barlow DH, Kennedy SH. Linkage and association studies of the relationship between endometriosis and genes encoding the detoxification enzymes GSTM1, GSTT1 and CYP1A1. *Mol Hum Reprod* 2001; 7: 1073-1078.
- Nebert DW, Vasiliou V. Analysis of the glutathione S-transferase (GST) gene family. *Hum Genomics* 2004; 1: 460-464.
- DeJong JL, Mohandas T, Tu CP. The human Hb (mu) class glutathione S-transferases are encoded by a dispersed gene family. *Biochem Biophys Res Commun* 1991; 180: 15-22.
- McLellan RA, Oscarson M, Alexandrie AK, Seidegard J, Evans DA, Rannug A, et al. Characterization of a human glutathione S-transferase mu cluster containing a duplicated GSTM1 gene that causes ultrarapid enzyme activity. *Mol Pharmacol* 1997; 52: 958-965.
- Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 1988; 85: 7293-7297.
- Smith CM, Kelsey KT, Wiencke JK, Leyden K, Levin S, Christiani DC. Inherited glutathione-S-transferase deficiency is a risk factor for pulmonary asbestosis. *Cancer Epidemiol Biomarkers Prev* 1994; 3: 471-477.
- Smith G, Stanley LA, Sim E, Strange RC, Wolf CR. Metabolic polymorphisms and cancer susceptibility. *Cancer Surveys* 1995; 25: 27-65.
- Baranov VS, Ivaschenko T, Bakay B, Aseev M, Belotserkovskaya R, Baranova H, et al. Proportion of the GSTM1 0/0 genotype in some Slavic populations and its correlation with cystic fibrosis and some multifactorial diseases. *Hum Genet* 1996; 97: 516-520.
- Baranova H, Bothorishvilli R, Canis M, Albuissou E, Perriot S, Glowaczower E, et al. Glutathione S-transferase M1 gene polymorphism and susceptibility to endometriosis in a French population. *Mol Hum Reprod* 1997; 3: 775-780.

35. Babu KA, Reddy NG, Deendayal M, Kennedy S, Shivaji S. GSTM1, GSTT1 and CYP1A1 detoxification gene polymorphisms and their relationship with advanced stages of endometriosis in South Indian women. *Pharmacogenet Genomics* 2005; 15: 167-172.
36. Roya R, Baludu GS, Reddy BS. Possible aggravating impact of gene polymorphism in women with endometriosis. *Ind J Med Res* 2009; 129: 395-400.
37. Lin J, Zhang X, Qian Y, Ye Y, Shi Y, Xu K, et al. Glutathione S-transferase M1 and T1 genotypes and endometriosis risk: a case-controlled study. *Chin Med J (Engl)* 2003; 116: 777-780.
38. Peng DX, He YL, Qiu LW, Yang F, Lin JM. Association between glutathione S-transferase M1 gene deletion and genetic susceptibility to endometriosis. *Di 1 Jun Yi Da Xue Xue* 2003; 23: 458-459.
39. Hsieh YY, Chang CC, Tsai FJ, Lin CC, Chen JM, Tsai CH. Glutathione S-transferase M1*null genotype but not myeloperoxidase promoter G-463A polymorphism is associated with higher susceptibility to endometriosis. *Mol Hum Reprod* 2004; 10: 713-717.
40. Aban M, Ertunc D, Tok EC, Tamer L, Arslan M, Dilek S. Modulating interaction of glutathione-S-transferase polymorphisms with smoking in endometriosis. *J Reprod Med* 2007; 52: 715-721.
41. Baxter SW, Thomas EJ, Campbell IG. GSTM1 null polymorphism and susceptibility to endometriosis and ovarian cancer. *Carcinogenesis* 2001; 22: 63-65.
42. Hur SE, Lee JY, Moon HS, Chung HW. Polymorphisms of the genes encoding the GSTM1, GSTT1 and GSTP1 in Korean women: no association with endometriosis. *Mol Hum Reprod* 2005; 11: 15-19.
43. Ivashchenko TE, Shved N, Kramareva NA, Ailamazian EK, Baranov VS. Analysis of the polymorphic alleles of genes encoding phase 1 and phase 2 detoxication enzymes in patients with endometriosis. *Genetika* 2003; 39: 525-529.
44. Baranova H, Canis M, Ivaschenko T, Albuisson E, Bothorishvilli R, Baranov V, et al. Possible involvement of arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1 genes in the development of endometriosis. *Mol Hum Reprod* 1999; 5: 636-641.
45. Hosseinzadeh Z, Mashayekhi F, Sorouri ZZ. Association between GSTM1 gene polymorphism in Iranian patients with endometriosis. *Gynecol Endocrinol* 2011; 27: 185-189.
46. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002; 4: 45-61.
47. Guo SW, Simsa P, Kyama CM, Mihalyi A, Fulop V, Othman EE, et al. Reassessing the evidence for the link between dioxin and endometriosis: from molecular biology to clinical epidemiology. *Mol Hum Reprod* 2009; 15: 609-624.