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Original Research Article

Association of glutathione s-transferase pi (GSTP1) gene polymorphism in unexplained infertile women

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ABSTRACT

Background : 15–20 million people in India are affected by infertility. Among them, 34% of the couples are shown to have unexplained infertility (UEI). Both genetic and environmental factors influence UEI. Data from various studies show that oxidative stress plays an important role in unexplained infertility. The genes of the phase II detoxification enzyme, Glutathione s-transferase family are upregulated in humans as a defense mechanism opposing the adverse effects of oxidative stress and play important role during pregnancy. Since all investigations for infertility work-up are normal evaluating the causes for UEI will have impact on the treatment protocol. In this study the association between GSTP1 variations and unexplained infertility are discussed.

Aim: To compare the association of GSTP1 polymorphism in women with unexplained infertility and a control group.

Materials and Methods: This is a case control study with 70 normal ovulatory women who conceived within 12 months of contraceptive free intercourse, and with no history of miscarriage were recruited in the control group and 70 women with unexplained infertility were recruited as study group. All participants included in the study were between 28 and 38 years of age. The association of GSTP1 polymorphism were studied using real-time PCR with the Light Cycler instrument (Roche Applied Science).

Results: GSTP1 variant allele frequencies were 0.39 and 0.41for control and cases respectively. The results of this study indicate GSTP1 genetic polymorphisms did not show a significant association with unexplained infertile group.

1. Introduction

Glutathione s-transferase P1 isoform is involved in the detoxification of electrophilic compounds by glutathione conjugation like other forms GSTM and GSTT.[1] GST phase II enzymes are upregulated in response to oxidative stress.[2,3] GSTs act as an defensive system against oxidative stress by reducing ROS to harmless metabolites.[4] GST polymorphisms decreases its protection against oxidative stress and lead to the development of many diseases.[5]

Excess ROS in the follicular fluid overpowers the antioxidant defense capacity and damages the oocytes and fertilization process. Susana Meijide found GST activity higher in small oocyte compare to mature suggesting GST may play a role in the follicle maturation by detoxifying xenobiotics and leads to the normal development of the oocyte.[6] Many researchers have investigated the role of

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GSTM1 and GSTT1 gene polymorphisms in endometriosis. Although GSTP1was found to be one of the most abundant form of GST in reproductive system, there is insufficient data on the role of GSTP1 gene polymorphism in endometriosis.[7] It has been reported that GST enzymes play a important role in female reproduction because of their presence in placenta and ovarian follicles in excessive amounts. Zusterzeel et al stated that among the assorted GST enzymes, GSTP1 is reported to be the predominant isoform in placenta, suggesting a possible role for this enzyme in pregnancy.[8] Studies on polymorphism of genes for detoxification enzymes are associated in the development of miscarriage and negative impact on the development of pregnancy.[9,10] Suryanarayana et al (2004) found an association of idiopathic recurrent pregnancy loss (IRPL) and polymorphism of CYP450 and GSTs genes (2004) in Indian women.[11]

Presence of polymorphism in GST leads to low functional activity of this enzyme and risk for preeclampsia. Allele variants of 1st and 2nd detoxification phase enzymes showed the increased risk of early pregnancy loss.[12]

In exon 5 and exon 6 of GSTP1 gene, two polymorphisms have been reported. Both of them are involved in amino acid substitutions. But the exon 5 transition was linked to enzymatic activity and located within the coding region of the active site of the enzyme. The exon 5 polymorphism at codon 105, leads to Ile-to-Val substitution.[13]

Xenobiotics like phenolic compounds leads to uterine inflammatory pathology. Polymorphism of GST genes involved in detoxification can have negative impact in this situation. There is an increasing awareness of potential links between reproductive health and environmental factors.

2. Material and Methods

2.1. Study design

Case - control study. Study approved by IRB and consent was obtained from all participants.

2.2. Control group

Pregnant women with normal ovulation who attended the antenatal clinic in Obstetrics unit. Samples were collected from them at first-trimester of their pregnancy 70 of these participants who had an uneventful pregnancy were included as control group.

2.3. Inclusion criteria

Women with normal ovulation, aged between 28 and 38 years who conceived within 12 months of contraceptive free intercourse, and who had an uneventful pregnancy.

2.4. Exclusion criteria

Abnormal glycemic status and thyroid function (TSH and Hba1c were measured.) History of adverse effect during pregnancy.

2.5. Study group

70 married women diagnosed with unexplained infertility.

2.6. Inclusion criteria for cases

1. Women with unexplained infertility and aged between 28 and 38 years.
2. Women with normal results for the following tests
   (a) Tubal patency (hysterosalpingogram and/or laparoscopy documents at least one fallopian tube patent.
   (b) Normal ovulatory function (Regular menstrual history /ultrasound documented ovulatory cycle or mid-luteal Progesterone.
   (c) Normal semen analysis for their partners.

2.7. Exclusion criteria

Women in whom one or more of the above test results were abnormal.

2.8. Statistical analysis

Frequency and percentage were reported for Allele and genotype by study and control group. In order to assess the association between GSTP1 and unexplained infertility, logistic regression was used. P-values less than or equal to 0.05 were considered significant.

2.9. Analysis of GSTP1

Gstp1Adenosine-to-guanidine (A ! G) at codon 105 in exon 5 were measured using HRM based on HybProbe format fluorescence resonance energy transfer (FRET). Two sequence-specific oligonucleotide probes were labeled with different dyes (donor and acceptor), and were added to the reaction mix along with the PCR primers.

2.10. Primers used

2.10.1. PCR primers

1. (F:5′-ACCCCAGGGCTCTATGGGAA-3′
2. R:5′-TGAGGGCACAAGAAGCC CCT-3′)

2.10.2. Hybridization probes

1. (5′-LCR640-TGTGAGCATCTGCACCAGGGTTGGGCG-3′and
2. 5′-TGCAAATACATCTCCCTCATCTACACCAAC-FL-3′)

2.10.3. Analysis of GSTP1 polymorphism

1. PCR reactions total volume of 20μl
2. 4 mM MgCl2,
3. 0.5 pmol of each hybridization probe,
4. 10 pmol of each PCR primer,
5. 2μl of Probe Fast Start Master
6. 100 ng genomic DNA.

2.11. The amplification programs

Initial denaturation step at 95 °C for 3 min, followed by45 cycles of denaturation (95 °C for 5 s).

1. Annealing (55 °C for 10 s), and extension (72 °C for 25 s).
2. Melting curve analysis was one cycle de-naturation at 95 °C for 2 min,
3. Followed by an increase in temperature from 50 to 80 °C cooling-down steps of one cycle at 40 °C for 30 s followed.

3. Results

DNA samples obtained from 140 participants both study group and control were analyzed for the variant single nucleotide polymorphisms in GSTP1. The case and control groups were similar with respect to age (Table 1). None of the women included in present study were either smokers or alcohol consumers.

There was no significant association between GSTp1 genotype or the GSTP1 variant allele and unexplained infertile group. The val/val homozygous allele frequency was increased in cases compared to control group. The frequencies of GSTP1 variants (combined ile/val and val/val) were 58.14% and 60.56% in unexplained infertile group and control. But this difference did not reach statistical significance. (Table 2)

GSTP1 variant allele frequencies were 0.39 and 0.41 for control and cases. The frequency of G allele was found to be higher in cases. But this difference was not statistically significant (Table 3)

4. Discussion

Studies showed that alleles of GSTP1 IIe105Val polymorphisms were not associated with gestational Diabetes.[14,15] Detoxification enzymes influences the pregnancy outcome and its effect on oxidative stress influences embryonic growth and development.[16] This draws attention to future studies on GSTP1 polymorphism on various reproductive disorders. Because of the importance of this enzyme in human reproductive systems[17,18] the present study was undertaken to study GSTP1 polymorphism in the specific group of unexplained infertile women from Indian subcontinent. Studies on GSTP1 variant was not found to be associated with recurrent

Table 1: Baseline characteristics of study subjects

	Groups		
	Control (n=70)	Case (n=70)	P value
	Mean ± SD / Median (IQR)	Mean ± SD / Median (IQR)	
Age (yrs)	26.70 ± 4.40	30.39 ± 4.36	< 0.001
Body Mass Index (kg/m2)	23.14 ± 2.41	24.54 ± 4.27	0.02
HBA1C (%)	5.27 ± 0.43	5.28 ± 0.37	0.888
Height (cm)	155.55 ± 5.48	154.53 ± 6.66	0.323
Weight (kg)	55.89 ± 5.49	58.74 ± 10.56	0.049
TSH*	1.56 (1.11, 2.10)	2.08 (1.60, 2.85)	< 0.001

Note: * represents the variables which are reported by Median (IQR)

Table 2: Distribution of GSTp1 allele frequency between study and control group

Variables	Groups		Odds Ratio	P value
	Control	Case		
GSTp1	n (%)	n (%)		
AA (ile/ile)	28 (39.43)	30 (42.86)	Ref	-
AG (Ile/Val)	31 (43.66)	23 (33.86)	0.69(0.33,1.46)	0.334
GG (Val/Val)	12 (16.90)	17 (24.28)	1.32(0.54,3.25)	0.543

Table 3: Allele frequency of GSTP1

GSTP1	Control N=71 (%)	Case N=70 (%)	P value
A	87 (61.3)	83 (59.28)	0.734
G	55 (38.7)	57 (40.7)	

pregnancy loss (RPL) in the South Indian population. GSTP1 variant allele when present in combination with GSTM1 null variant did not show any association with recurrent miscarriages (RM). Assessment related to the expression of these genes in unexplained infertility and environmental stress may help in the maintenance of a successful pregnancy.

5. Conclusion

An association between unexplained infertility with GSTP1 polymorphisms did not exist. The genotypes and allele frequencies of this polymorphisms may not be useful marker for the prediction of unexplained infertility risk. The main limitation of our study was the small sample size. Further studies with larger sample size are needed to confirm the association between the GST gene polymorphisms and the risk of developing UEI.

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7. Ethics Approval and Consent to Participate

This Study has confirmed by the Ethical committee (IRB) of Christian Medical College and Hospital under number of IRB Min. No 9216 – and the signed informed consent was obtained from all participants

8. Competing Interests

The authors declare that they have no competing interests.

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