Research Article

Glutathione Peroxidase-1 Gene Polymorphism (GPx-1 Pro198Leu) in Association with Blighted Ovum

Hubungan Polimorfisme Gen Glutation Peroksidase-1 (GPx-1 Pro198Leu) dengan Kehamilan Kosong

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Abstract

Objective: To evaluate salivary GPx-1 gene polymorphism in pregnant women suffering from blighted ovum.

Method: In this case-control study, 34 blighted ovum patients and 34 healthy controls were studied. Genomic DNA was extracted from the saliva. The genotypes were determined by restriction fragment length polymorphism (RFLP-PCR) technique. Mad Calc (version 12.1) was used for statistical analysis.

Result: The frequency of CC, CT, and TT genotypes of GPx-1 gene were 41%, 44% and 14%, respectively in blighted ovum patients and in healthy volunteers were 44%, 47%, and 8.82-9%, respectively. After statistical analysis, the study showed no significant association between this polymorphism and blighted ovum (with p = 0.63).

Conclusion: These results indicated no significant association between GPx-1 (Pro198Leu) polymorphism and blighted ovum. However, further research is required to clarify the role of gene polymorphism in blighted ovum.

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Keywords: abortion, blighted ovum, glutathione peroxidase-1, GPx-1, RFLP-PCR

Abstrak

***Tujuan**: Untuk mengevaluasi polimorfisme gen GPx-1 dari ludah perempuan hamil yang menderita kehamilan kosong (blighted ovum).*

***Metode**: Dalam studi kasus - kontrol ini, 34 pasien dengan kehamilan kosong dan 34 kontrol yang sehat dipelajari. Genomik DNA diekstraksi dari air liur pasien tersebut. Genotipe ditentukan dengan teknik RFLP-PCR. Mad Calc (versi 12.1) digunakan untuk analisis statistik.*

***Hasil**: Frekuensi genotype CC, CT dan TT dari gen GPx-1 berturut-turut adalah 41%, 44% dan 14% pada pasien kehamilan kosong dan pada pasien yang sehat: 44%, 47% dan 8,82-9%. Setelah dianalisis secara statistik, tidak terdapat hubungan yang signifikan antara polimorfisme gen tersebut dengan kehamilan kosong (p = 0,63).*

***Kesimpulan**: Tidak terdapat hubungan yang signifikan antara polimorfisme gen GPx-1 (Pro198Leu) dengan kehamilan kosong. Bagaimanapun, penelitian lebih lanjut diperlukan untuk mengklarifikasi pengaruh dari polimorfisme ini pada kehamilan kosong.*

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***Kata kunci**: aborsi, GPx-1, glutation peroksidase-1, kehamilan kosong, RFLP-PCR*

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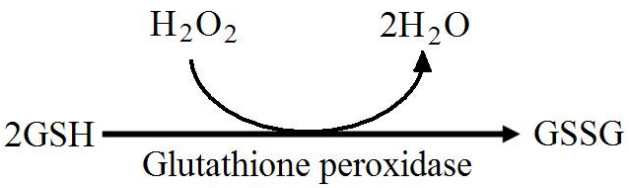
INTRODUCTION

One of the most common causes of abortion in the first trimester of pregnancy is blighted ovum. Once the egg is fertilized by sperm, the fertilized egg is implanted in the uterus and cell division begins. Cell divisions in succession to the formation of the placenta and the pregnancy sac continue, but the embryonic division is likely to be stopped and the pregnancy sac is formed, the embryo is not observed.

This condition is also called blighted ovum or anembrionic pregnancy. The cause is unknown, but the researchers generally consider the cause of disease to be genetic and chromosomal abnormalities. The cause of 50% of first trimester abortion is due to pregnancy without an embryo.[1,2] The symptoms of this disorder are mild retardation, menstrual or abdominal pain, swelling in the breasts, brown vaginal discharge, a positive pregnancy test and a subjective impression of a natural pregnancy. Although there is no fetus, the placenta continues to grow and pregnancy hormones from the placenta secrete into the mother's blood. This disease can be diagnosed before the second month of pregnancy by ultrasound testing, showing a bag with a

diameter of 20 mm or more.[3,4] But a fetus does not exist. The most common cause of this condition is chromosomal abnormalities. It caused less fertilization and decreases the number of chromosomes that can stop cell division of the zygote. Other factors can be poor quality of egg and sperm. It has been reported that the cause of one-third of spontaneous abortion before eight weeks of pregnancy is blighted ovum.[5,6] In general, it is believed that chromosomal abnormalities cause 50% of the first trimester abortion.[1]

The most common of these chromosomal abnormalities can be reciprocal displacement (62%), Robertsonian translocation (16%), inversion (16%), deletion (3%) and duplication (8%).[5,1] The purpose of the study was to investigate the GPx-1 (glutathione peroxides-1) gene polymorphism, in pregnant women with blighted ovum. GPx-1 gene produces glutathione peroxides-1, an important cytosolic peroxidase. This is the most important antioxidant enzyme derived from the cells of the body in cytosol.[6] The enzyme is a selenoprotein which reduces free radicals in cells.



The Original Reaction GPx-1

Glutathione peroxidase-1 has a tetramer structure which each monomer weighing 22 KDa and is a part of selenocysteine in the active site. Selenocysteine works directly to substrate peroxide electrons and it is oxidized during this process.[7,8] Five isoforms of glutathione peroxidase-1 are encoded by different genes. The most frequent type in the cytoplasm of all mammalian glutathione peroxidase-1 is GPx-1 and its preferred substrate is hydrogen peroxide (H_2O_2). Glutathione peroxidase-1 with the original reaction reduces free radicals and oxygen species in the cells. As a result, the cellular DNA is protected from damage. One of the properties of this protein cytosolic tail of a polialanine (ALA) in the N-terminal region, which consists of three, five, six, or seven alanine repeats in this region. Since during pregnancy women are under severe stress, keeping the body stable is very important, so the study of body fluids antioxidants and antioxidant activity of these gene is most important. GPx-1 somatic cells are also an important antioxidant, which express nearly in all our cells in the cytosol. In this study, the relationship between polymorphisms of this gene in women with blighted ovum is investigated. Intracellular antioxidant glutathione peroxidase-1, one of the most toxic substances that prevent the accumulation of intracellular catalase monomers, is even more efficient. GPx-1 gene expression pattern in different conditions, in men and women, in normal circumstances and stressful, in resting cells or growing together. GPx-1 genes are located in chromosome 3P21.3.[7] This gene includes two exons and gene Pro198Leu, which is one of the most important of these, is the polymorphism genes GPx-1 plays an important role in cancer, rheumatoid arthritis, diabetes and arthrosclerosis.[10,11]

METHODS

In this case-control study, 34 patients and 34 controls were involved. An informed consent letter was obtained from all participants approved by University of Guilan's institutional organization.

For collecting saliva sampling, the subjects rinsed their mouth with distilled water and collected about 5 ml of their unstimulated saliva samples in a sterile falcon tube. The samples were immediately centrifuged to remove cell derbies and kept at -20°C until examination. Then DNA was extracted from saliva being check for the correct extraction by horizontal electrophoresis method using 1% agarose gel with ethidium bromide. The DNA molecules from extraction were made transparent in order to observed the band. Genotype Pro198Leu by RFLP-PCR method was then determined, i.e. the PCR reaction product for this polymorphism in the gene for GPx-1. Therefore, GPx-1 gene polymorphism in codon 198 is reproduced there. The position for identification of the enzyme can be created when that piece Apa1 is duplicated containing the C allele (Pro). In this case the cutting enzyme reasonable for cutting the pieces is 77 and 237 bp. On the other hand, if the fragment amplified with allele T (Leu) is the enzyme, it disappears and 314 bp fragments remain intact. So, based on the 3 genotype, there are 3 possible outcomes. The homozygous genotype Leu/Leu reveals only one band of 314 bp. Heterozygous genotype Pro/Leu

creates three bands of 314, 77, 237 bp and thus homozygous genotype Pro/Pro creates two bands, 77 and 237 bp.

Extraction of DNA and PCR

Salivary DNA was extracted by use of an extraction GPPKit (From sinaclon company) to ensure proper extraction by spectroscopy and gel electrophoresis. The replication of DNA was the next experimental step. Primers were designed using the program oligo 7 primer analysis software: F($_5$/GTGTGCCC-CTACGCAGGTA$_3$/) and a primer R($_5$/CACACAGTT-CTGCTGACACC$_3$/). The PCR 314 bp by using agarose gel electrophoresis. The final step was to identify the Pro198Leu GPx-1 polymorphic region using Apa1 restriction enzyme. The PCR product was digested by Apa1 and two 237, 77 bp mutant into two pieces in a homozygous 314 bp fragment. The three carriers in the individual heterozygote pieces 237, 314 and 77 bp were observed and the individual patient's homozygote the two pieces on the electrophoresis gel and 77, 237 bp joint was observed. Taking into account all patient samples and control data analyzed and the following results were obtained.

Genotypic and allelic frequencies of the glutathione peroxidase-1 gene analyzed in the patient and the control group were compared. The results in both tests and controls were compared using Chi-Square test. Data were analyzed by Med Calc (version 12.1).

RESULTS

In this study, 34 female patients of blighted ovum and 34 healthy women were control group the aged interval was 20 to 35 years. Among the 34 women with blighted ovum, 14% have Pro/Pro, 44% of heterozygous genotypes Pro/Leu and 41% homozygous genotype Leu/Leu, respectively. The genotype distribution differs between patient and control test with a not significant Chi-square test (p-value = 0.63).

On this basis, there were not significant differences between the two groups (patients and control persons) from genotype polymorphism GPx-1. The study will continue allelic between the patient and control possible. The frequency of allelic Pro and Leu among patients 63%, 36% and in the control group 68%, 32% respectively. The difference is in the allelic of both distribution and significance level p=0.72 (Table 1). The Pro198 Leu gene, polymorphism in the population GPx-1 is not associated with disease blighted ovum.

DISCUSSION

The results of the research were studied; significant differences between the control and the patient population in the distribution and abundance of allelic genes did not show GPx-1 catheterizations separately. Heterozygote T/C genotype in the population was impressive. It is important to carriers of the mutant allele C. In this study, the research about the disorder in pregnant women before three months with blighted ovum is abortion. One of the most important antioxidants in the body is enzyme GPx-1. This gene in the body of the patient and all healthy individuals varies in expression with a different activity level. Since the cells are always in the face of ROS molecules, these molecules (such as hydrogen peroxide, superoxide) are highly reactive. During a natural process in the cytosol of all cells, antioxidant substances (including antioxidant enzymes) react with these toxins.[10,12,13]

Table 1. Genotype and Allele Distributions of GPx-1 (Pro 198Leu) Polymorphism.

	Controls n (Frequency %)	Patient n (Frequency %)	p-Value
Genotype			p = 0.63
CC	15 (44%)	14 (41%)	
CT	16 (47%)	15 (44%)	
TT	3 (8.82%)	5 (14 %)	
Allele			p = 0.72
C	46 (68%)	43 (63%)	
T	22 (32%)	25 (36%)	

The study and much research have been done in relation to the genes responsible for the production of antioxidants. This is the first study on the relationship between gene polymorphism of glutathione peroxides 593C/T + 1 and blighted ovum. Studies have shown that higher levels of hydrogen peroxide on the cell Leu allele than that of Pro allele. The aminoacid proline in comparison with Leu, is more effective in enzyme activity.[11]

In a healthy person, free radicals, and defense system antioxidants are in a state of balance.[14] This polymorphism is evident other diseases such as bladder cancer,[13] atherosclerosis, rheumatoid arthritis, and Alzheimer's disease.[9,10,15] The role for allelic variation within the gene for the glutathione peroxidase 1 (GPx-1) in the risk or etiology of breast cancer was investigated.[9]

The frequency of the genotype TT GPx-1 gene has been reported to be significantly higher in women with breast cancer. However, no association exists between GPx-1 Pro198Leu and breast cancer risk.[13]

These results indicated that the TT genotype of the GPx-1 gene had no significant association between this polymorphism and blighted ovum. It could be suggested that in a larger patients' population a possible relationship between GPx-1 gene polymorphism and blighted ovum be traced. However, further research is required to clearly comment on the role of gene polymorphism in blighted ovum.

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