Original Research Paper

The Association of Early Pregnancy Loss with Polymorphism in Xenobiotics Detoxification Genes

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Abstract: Pregnancy loss and other pregnancy complication can be connected with environmental and lifestyle risk factor, among which effect of chemical compounds is the strongest. Effects of xenobiotics can be modified by allele variants of xenobiotic detoxification enzymes phase I or II. A total of 71 women with early pregnancy loss and 101 control patients were examined by a case-control methodology. The *Ile462Val CYP1A1*, *Arg47His ADH1B*, *Glu487Lys ALDH*, *I105V GSTP1* polymorphisms were genotyped by allele-specific polymerase chain reaction. Our data demonstrated that the heterozygous *Glu487Lys ALDH* genotypes rate were higher in the pregnancy loss patients (12.71%) compared to the control group (2.0%). There was no difference between two groups detected for other polymorphisms. However, presence of polymorphic variants of genes of 1st and 2nd detoxification phases can have additive effect and cause multifactorial pathology risk increase. It is shown, that combination of polymorphic variants of *ALDH2* и *GSTP1* genes in genotype results in 5 fold pregnancy loss risk increase. Combination of polymorphic variants of *ALDH2*, *ADH1B* и *GSTP1* genes in genotype results in 9 fold pregnancy loss risk increase. The results demonstrated that combination of allele variants of 1st and 2nd detoxification phases in woman genotype increases the risk of early pregnancy loss.

Keywords: Early Pregnancy Loss, Gene Polymorphism, Xenobiotics Detoxification System, *ALDH2*, *GSTP1*

Introduction

Etiological factors of human reproduction disorders are numerous and diverse (Cramer and Wise, 2000). Pregnancy loss and other pregnancy complication can be connected with environmental and lifestyle risk factor, among which effect of chemical compounds is the strongest. Xenobiotics can affect genetic system and induce teratogenesis in fetus. Delivery of chemicals into the body causes metabolism disorders, cell and tissue physicochemical structure damage, resulting in pathological changes.

Effects of xenobiotics can be modified by allele variants of xenobiotics detoxification enzymes of phase I or II (Hirvonen, 1995; Wang *et al*., 1998). Depending on genotype person can become resistant or conversely have high sensitivity to xenobiotics (Nebert and Carvan, 1997). Biotransformation system genes encode big group of enzymes involved in detoxification of xenobiotics. Detoxification process consist of xenobiotic activation phase, which results in the formation of intermediate metabolites, detoxification phase when intermediate metabolites convert into water dissolved non-toxic products and excretion phase.

In phase I of detoxification pathway cytochrome P450 family of enzymes and other enzyme families such as oxidases, reductases and dehydrogenases catalyze the conjugation of new or modifying functional groups (–OH, –SH, –NH3) to xenobiotics (Conney, 1982). Unstable electrophilic products of phase I have toxic effect.

Intermediate metabolites conjugate with endogenous ligands during phase II of biotransformation that increases hydrophilicity and promotes product excretion. The second phase includes transferase family of genes, such as Glutathione-S-Transferase (GST), N-Acetyltransferase (NAT), UDF-Glucagon-Sulfotranferase (UGT). Enzymes of second phase provide transformation of electrophilic compounds into water-dissolved non-toxic metabolites which are eliminated from the organism.

Numerous researches stated that effective functioning of biotransformation system is crucial for human reproduction (Baranova *et al.*, 1999; Hadfield *et al.*, 2001). It is known that in the first term of pregnancy placenta expresses cytochrome and glutathione-S-transferase genes (Hakkola *et al.*, 1996; Van Lieshout *et al.*, 1998; Knapen *et al.*, 1999). Effective functioning of biotransformation system in the first term of pregnancy is important for successful completing of critical stages of embryogenesis and organogenesis (Myllynen *et al.*, 2007). Detoxification enzymes inactivate reactive oxygen species which are formed during biotransformation of numerous exogenous substances. It is known that antioxidant enzyme activity in the first term of pregnancy is quite low (Hempstock *et al.*, 2003). Exposure to xenobiotics could increase the risk of oxidative stress development in the forming placenta that causes chromosome aberrations, cell lysis and finally fetus death. Xenobiotics can affect apoptosis level in syncytiotrophoblast (Marana *et al.*, 1998; Vogt Isaksen, 2004).

Optimal level of enzyme activity is very important. Genetic variability of the detoxification system enzymes can disrupt the balance between I and II phases of xenobiotics biotransformation and can result in the individual differences in efficiency of detoxification of endogenous and exogenous substances.

It is stated that *CYP1A1* gene polymorphism causes the synthesis of enzyme with increased activity that results in the accumulation of toxic electrophylic compounds formed after the first detoxification phase in the organism (Crofts *et al.*, 1994). Inactive *GSP1* alleles or alleles with lower functional activity are also associated with increased level of toxic intermediate metabolites of xenobiotics (Watson *et al.*, 1998).

Disturbance of detoxification system functioning caused by genotype can result in the intensification of toxic impact of xenobiotics detoxification products on the fetus that can be the one of the etiologies of early pregnancy loss.

Data on the association of *CYP1A1* and *GSTP1* gene polymorphism with pregnancy loss risk increase are controversial (Zusterzeel *et al.*, 2000a; Suryanarayana *et al.*, 2004). Data on the alcohol dehydrogenase and aldehyde dehydrogenase gene polymorphism effect on pregnancy loss risk are very poor.

The present study was designed to examine the frequencies of gene polymorphism in women with pregnancy loss in the first trimester.

Materials and Methods

Prior to inclusion in the study, all subjects underwent a standard diagnostic work-up. The women were examined using transvaginal ultrasonography for the absence of uterine abnormalities and polycystic ovary syndrome. Women with previously diagnosed arterial hypertension, diabetes, thyroid diseases, autoimmune pathology and infections during pregnancy were excluded from studied population. Women contacting with exogenous risk factors, such as alcohol, electromagnetic radiation, industrial noise, vibration, chemical pollutants were also excluded. All smokers also were excluded from studied population because there are data, that tobacco smoking is a factor that increases the risk of spontaneous abortion (Ness *et al.*, 1999). Although it is stated that there are no difference in genotype frequency of polymorphism in *CYP1A1* and *CYP2A6* genes as far as in *GSTT1* deletion frequency between smokers and non-smokers with early pregnancy loss (Aagaard-Tillery *et al.*, 2010). The study was approved by the Southern Federal University Bioethics Committee. The participants willingly signed the informed consent. After approval by institutional review board, 71 women (mean age 29) with spontaneous abortion in 6-11 week of gestation and 101 women (mean age 30) with normally progressing pregnancies and without any history of spontaneous or missed abortion were studied.

Genomic DNA was isolated from the EDTA-anticoagulated peripheral blood using the commercial kit "DNA Express" (termo-coagulation method, Lytech, Russia).

Polymorphism *Ile462Val* of cytochrome P-450 gene *CYP1A1* (MIM *108330), *Arg47His* (rs122) alcohol dehydrogenase gene *ADH1B* (MIM +103720), *Glu487Lys* alcohol dehydrogenase gene *ALDH2* (MIM +100650), *I105V GSTP1* gene (MIM *134660) was detected by allele-specific polymerase chain reaction method using SNP-express reaction kits (Lytech, Russia). The PCR products were analyzed by horizontal 3% agarose gel electrophoresis. Gel images were captured using GelDoc XR system (Bio-Rad, USA). Densitometry was performed using ImageJ (NIH, USA). The background was subtracted with the rolling ball radius of 50 pixels.

Hardy-Weinberg equilibrium analyses were performed using Hardy-Weinberg equilibrium calculator in www.oege.org/software/Hardy-Weinberg (Rodriguez *et al.*, 2009). Differences in distribution of allele variants between studied groups were assessed by χ^2-analyses. To evaluate pregnancy loss risk we calculated Odd Ratios (OR).

Results

The frequencies of genotypes of studied groups are shown in Table 1. The distributions of all genotypes in each group were in Hardy-Weinberg equilibrium. The distributions of genotype and allele frequency between women with early pregnancy loss and the controls were equal for *CYP1A1, ADH1B* and *GSTP1* gene polymorphism.

Table 1. Distribution of *CYP1A1*, *ALDH2*, *ADH1B* and *GSTP1* genotypes among 71 cases with Early Pregnancy Loss (EPL) and 101 controls

Genotype, allele	Controls (n = 101)	EPL (n = 71)	OR (95% CI)	χ^2 (p)
CYP1A1 Ile462Val				
Ile/Ile	85 (84.2%)	63 (88.7%)	1.48 (0.6-3.68)	0.73
Ile/Val	16 (15.8%)	8 (11.3%)	0.67 (0.27-1.67)	-0.7
462Val allele	0.056	0.079	0.69 (0.29-1.67)	0.67 (0.41)
ALDH2 Glu487Lys				
Glu/Glu	99 (98.0%)	62 (87.3%)	0.14 (0.03-0.67)	7.97
Glu/Lys	2 (2.0%)	9 (12.7%)	7.19 (1.5-34.36)	-0.02
487Lys allele	0.01	0.063	6.77 (1.44-31.8)	7.7 (0.006)
ADH1B Arg47His				
Arg/Arg	92 (91.1%)	58 (81.7%)	0.44 (0.18-1.09)	3.38
Arg/His	8 (7.9%)	11 (15.5%)	2.13 (0.81-5.6)	-0.18
His/His	1 (1.0%)	2 (2.8%)	2.9 (0.26-32.6)	
47His allele	0.05	0.106	2.27 (0.99-5.21)	3.9 (0.05)
GSTP Ile105Val				
Ile/Ile	42 (41.6%)	25 (35.2%)	0.76 (0.41-1.43)	1.15
Ile/Val	48 (47.5%)	35 (49.3%)	1.07 (0.58-1.97)	-0.56
Val/Val	11 (10.9%)	11 (15.5%)	1.5 (0.61-3.68)	
105Val allele	0.347	0.401	1.26 (0.81-1.97)	1.08 (0.3)

Table 2. A combined analysis of distribution of *ALDH2*, *GSTP*, *ADH1B* genotypes among 71 cases with early pregnan-cy loss and 101 controls

Genotype	Controls, n (%)	EPL, n (%)	OR (95% CI)	p
ALDH2 Glu487Lys or *487LysLys/GSTP Ile105Val* or *105ValVal*	2 (1.9)	7 (9.9)	5.4 (1.1-26.9)	0.034
ALDH2 Glu487Lys or *487LysLys/ADH1B Arg47His* or *47HisHi/ GSTP Ile105Val* or *105ValVal*	1 (0.9)	6 (8.4)	9.2 (1.1-78.5)	0.020
ADH1B Arg47His or *47HisHis/GSTP Ile105Val* or *105ValVal*	2 (1.9)	9 (12.7)	7.2 (1.5-34.4)	0.008

For *ALDH2* gene polymorphism genotype and allele frequency differed significantly between controls and EPL group (Table 1). Occurrence rate of the *Glu487Lys* heterozygous genotype were 6 times higher among women with early pregnancy loss. Women with heterozygous *ALDH2 Glu487Lys* genotype have 7 fold early pregnancy loss risk increase. The *487Lys* allele frequency was 6 times higher among women with early pregnancy loss compared to controls.

The combination of polymorphic variants of genes of first and second detoxification phase has additive effect and results in multifactorial pathology risk increase. Table 2 represents the results of analysis of gene polymorphism combinations.

Among women with early pregnancy loss the percentage of those who had the combination of polymorphisms of both first and second detoxification phases in genotype was increased (Table 2). In case of combination of both *ALDH2* and *GSTP1* genes polymorphisms in genotype the early pregnancy loss risk increases 5 times. In case of combination of *ALDH2*, *ADH1B* and *GSTP1* genes polymorphisms in genotype the early pregnancy loss risk increases 9 times.

Discussion

Metabolic activation of xenobiotics leads to the formation of reactive metabolites that occurs the one of the main causes of genotoxicity of many environmental substances. In human organism individual resistance to toxins depends on xenobiotic detoxification gene system functioning. The data about biotransformation system gene polymorphisms influence on pregnancy complication development remain controversial.

Cytochrome CYP1A1 is active towards polycyclic aromatic hydrocarbons, ethanol, acetone, several drags and biologically active compounds, including estrogens (Martucci and Fishman, 1993). It is known that *A2455G (Ile462Val)* mutation leads to "fast" enzyme type formation, that can cause intermediate toxic metabolites concentration increase in tissues and biological fluids. This event may result in activation of biochemical reactions, which can modulate hormonal activity. Some researches stated that polymorphic variants of *CYP1A1* are associated with higher risk of spontaneous abortion (Suryanarayana *et al*., 2004). In the same time there are controversial data (Saijo *et al*., 2004; Aagaard-Tillery *et al*., 2010). In our study we found no differences between genotype and allele frequencies of *CYP1A1* gene in study groups of women.

Alcohol dehydrogenase catalyses the oxidation of primary and secondary alcohols to aldehydes and ketones. Non-typical enzyme form which is coded by polymorphic variant of *ADH1B* gene has higher alcohol oxidation speed (Warren and Li, 2005; Edenberg, 2007).

Aldehydes also are formed during lipid peroxidation. Aldehydes are reactive compounds that inhibit many metabolic reactions and cellular growth. That's why fast aldehydes detoxification is important for cell survival. Enzymatic elimination of aldehydes is a function of NAD-dependent alcohol dehydrogenase. *ALDH2* gene polymorphism studied in our work causes enzyme activity decrease (Yoshida *et al.*, 1984). In combination with low alcohol dehydrogenase activity this results in acetaldehydes tissue concentration increase. Acetaldehyde is known to be a compound with high reactivity, which causes DNA damage. Lack of *ALDH2* enzyme has negative effect on mammal embryo development (Langevin *et al.*, 2011).

Glutathione S-transferase enzymes group catalyze the detoxification of wide spectrum of electrophilic substances. Detoxification can be achieved by binding of xenobiotics to glutathione, which facilitates the neutralization of electrophilic center by SH group. Binded xenobiotics can be eliminated from the body with the urine or during proceeding the next stages of the bitransformation process in which N-acetilase and transpeptidase take part.

Glutathione S-transferases are presented in many tissues including genital tract and placenta. *GSTP1* is a main enzyme type in fetoplacental system (Zusterzeel *et al.*, 1999; 2000b). *GSTP 105Val* variant has low specific activity and affinity to electrophilic substances (Ali-Osman *et al.*, 1997). Presence of this polymorphism in genotype leads to the accumulation of insoluble toxic activated electrophilic metabolites in cells (Hu *et al.*, 1998). It is known that low functional activity of glutathione S-transferases is associated with preeclampsia risk increase. Furthermore, many investigations demonstrated the association between mutant allele and genotype frequencies and severity index, for example with increased platelet aggregation (Zusterzeel *et al.*, 2000a).

Number of researches had shown *105Val GSTP1* homozygous genotype frequency increase among women with Recurrent Pregnancy Loss (RPL) and no significant relationship between RPL and *CYP1A1* gene polymorphism (Zusterzeel *et al.*, 2000b). Suryanarayana *et al.* (2004) demonstrated controversial data: *CYP1A1* gene polymorphism is related to RPL risk while GST gene family polymorphisms are not associated with recurrent miscarriage.

Our study has shown that *GSTP1* gene polymorphism is not associated with first trimester pregnancy loss risk. However, women with polymorphic variants of genes of the first and second xenobiotics detoxification phases (*ADH1B* and/or *ALDH2* in combination with *GSTP1* gene polymorphism) have the increased early pregnancy loss risk. Such genotype affects the equilibrium between the reaction speeds during I and II phase of biotransformation that results in the longer preservation of toxic intermediate compounds, including the products of lipid peroxidation, in cells. Such conditions can activate free radical reactions and pathological processes. Furthermore, it may affect the apoptosis. Other study examined that *GSTP1* is a transcriptional target of p53 suppressor, which affects p53-binding motif in exon 4 of *GSTP1* gene increasing its expression. This mechanism is a part of genome protection system (Lo *et al.*, 2008) which can be disrupted in case of enzyme isoforms presence.

Conclusion

The present research revealed that multifactorial pathology risk increase is the result of combination of multiple polymorphic variants of first and second xenobiotics detoxification phases.

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Author`s Contributions

Elena Vladimirovna Mashkina: Conception and design, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article.

Konstantin Nikolaevich Saraev: Acquisition of data, Analysis and interpretation of data.

Elena Viktorovna Butenko: Analysis and interpretation of data, Drafting or revising the article.

Galina Ivanovna Volosovtsova: Analysis and interpretation of data, Contributed unpublished essential data or reagents.

Tatyana Pavlovna Shkurat: Conception and design, Analysis and interpretation of data, Drafting or revising the article.

Ethics

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. Each author confirms the manuscript represents honest work. All authors have approved the manuscript. Each author agrees with the order in which his name appears on the title page. Study design and methods were approved by Ethics Committee of Southern Federal University.

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