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Polymorphic variants of detoxification genes and possible gestational complications in pregnant women with retrochorial and retroplacental hematomas

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Fetal health depends on both the genetic program of its development and the structural and functional adequacy of placenta.
***Materials and methods*. Our research envisaged the use of the molecular genetic method to identify deletion and allelic polymorphism of the GSTT1, GSTM1 genes in the homozygous state and the designation of the identified genotypes as the deletion variant (GSTT1 deletion, GSTM1 deletion) or the allelic variant (GSTT1 allele, GSTM1 allele). The research involved 105 women; among them, there were 50 pregnant women with local non-progressive placental abruption. Group I included 33 women with placental dysfunction, who gave birth to babies with normal body weights; Group II comprised 17 women with placental dysfunction and development delay in their newborn babies.**
***Results*. The study of deletion polymorphism of the GSTT1, GSTM1 genes and A313G polymorphism of the GSTP1 gene in all the women (n=105) (50 patients of the main study groups and 55 women of the control group) revealed the following distribution of polymorphic variants of the studied genes: the GSTT1 allele genotype has made up 81.90%; the GSTT1 deletion genotype was found in 18.10% of cases; the GSTM1 allele genotype was observed in 55.24% of cases; the GSTM1 deletion genotype was detected in 44.76% of women. The frequency of the GSTP1 polymorphic variants was as follows: the AA genotype – 54.29%, the AG genotype – 38.10%, the GG genotype – 9.52%.**
***Conclusions*. The genotype combinations of the GSTT1 allele, GSTM1 allele, 313AA of the GSTT1, GSTM1, GSTP1 genes create no conditions for and clearly reduce the risk of placental dysfunction and intrauterine growth restriction with local non-progressive placental abruption.**
Key words: *abruption of placenta, genes of detoxification, obstetric complications, polymorphism of genes.*

According to current scientific knowledge, the development of placental dysfunction is determined by various factors including biological, chemical and radiation ones, as well as maternal metabolites, i.e. the occurrence of pathological conditions in the antenatal and neonatal periods should be attributed to multifactorial nature of different endogenous and exogenous damaging factors [1, 2]. Fetal health depends on both the genetic program of its development and the structural and functional placental adequacy. The role of the placenta is known to consist in the implementation of a number of functional mechanisms, namely trophism, protein synthesis, hormonal function and hormone modulation, synthesis of biologically active substances, anti-toxic function, utilization and excretion of metabolites, regulation of lipid peroxidation (LPO) and antioxidant defense [3, 4].

A series of recent studies have reported the effect of certain polymorphic variants of the glutathione S-transferase (GST) GSTT1, GSTM1 genes that impair detoxification processes and increase the risk of developing multifactorial pathology including placental dysfunction [5, 6, 7, 8]. According to literature, LPO intensification is associated with polymorphism of phase II detoxification genes which exert an anti-oxidant effect at the cellular level. Thus, it can be concluded, that in the presence of certain polymorphic variants of GST genes, namely the GSTT1, GSTM1, GSTP1 genes, the exhaustion of the glutathione-dependent system of antioxidant defense and inhibition of the detoxification function of the placenta occur that leads to the progression of placental dysfunction [3, 7, 9]. Most studies of deletion and allelic polymorphism of the GSTT1, GSTM1 genes were directed to determine the homozygous state without analyzing the heterozygous state [8, 10, 11].

When analyzing literature, we have found only several reports dealing with the study of genetic polymorphism of GST genes in placental dysfunction on the background of miscarriage [5, 10]. However, the number of researches dealing with the study of the intensity of free-radical processes and antioxidant state in women with placental dysfunction increased annually [12, 13]. The role of hereditary factors, namely certain GST polymorphic variants in the development of placental dysfunction and obstetric complications accompanying it remains insufficiently studied.

The objective: was to study the frequency of allelic polymorphism of the GSTT1, GSTM1 genes in women with retrochorial and retroplacental hematomas in conjunction with the development of gestational complications and perinatal pathology in the newborns.

MATERIALS AND METHODS

Our research envisaged the use of the molecular genetic method to identify deletion and allelic polymorphism of the GSTT1, GSTM1 genes in the homozygous state and the designation of the identified genotypes as the deletion variant (GSTT1 deletion, GSTM1 deletion) or the allelic variant (GSTT1 allele, GSTM1 allele). According to the results of the experimental studies, A313G polymorphism of the GSTP1 gene determined the activity of the corresponding isomeric enzyme. The 313AA genotype was associated with normal enzyme activity; in case of the 313AG genotype, enzyme activity decreased by 30%; in case of the 313GG genotype, enzyme activity decreased by 70%.

The GSTM1 and GSTP1 genes are expressed directly in the placenta; when studying the GSTT1 gene, a significant effect of the cellular level on the state of the antioxidant defense system was found indicating the relevance of our research [8, 14]. The determination of allelic polymorphism of the GSTT1, GSTM1 genes was carried out using a multiplex polymerase chain reaction (PCR) in an automatic mode in GeneAmp 2400, GeneAmp 2700 (Applied Biosystems) thermal cyclers with primers by amplifying multiple sequences proposed by Arand M. et al. (1996).

To calculate and compare the average values of digital data, as well as to assess statistical significance of the results obtained, there were used the methods of evaluating the difference between the mean trends (Student's t-test), correlation analysis. The presence (or absence) of a certain allele, genotype or their comparison in several genes was considered as an indicator. If there was a significant difference between the control group (or population

sample) and the study group, the odds ratio (OR) was calculated. The 95% confidence interval (CI) was used to estimate the precision of the OR. A p-value of <0.05 was considered statistically significant. To predict placental dysfunction, the need to study three genes of the GST gene family was considered, as a three-locus model had better accuracy.

The intergenic and gene-factor interactions were studied by the method of binary logistic regression using MDR_2.0 software. To assess the potential risk of developing placental dysfunction at the individual level, there was conducted the discriminant analysis using the ROC curves. The effectiveness of the models studied was evaluated by the area under the curve (AUC) considering their sensitivity and specificity. The closer to 1 the AUC of the gene studied was, the higher its effectiveness was. If the AUC was 0.5 and less, that model indicated the absence of discriminating properties of the gene; in our case, it was the GSTT1 gene (AUC=0.556).

The research involved 105 women; among them, there were 50 pregnant women with local non-progressive placental abruption. Group I included 33 women with placental dysfunction, who gave birth to babies with normal body weights; Group II comprised 17 women with placental dysfunction and development delay in their newborn babies. All the women were observed in the Ivano-Frankivsk Regional Perinatal Center. Among women with placental dysfunction, there were 30 (60%) females with clinical signs of undifferentiated connective tissue dysplasia (UCTD): 20 (66.67%) women of Group I (60.61%) and 10 (33.33%) females of Group II (58.82%). The control group included 55 women with the preserved reproductive status without UCTD who gave birth to healthy full-term babies.

RESULTS

The study of deletion polymorphism of the GSTT1, GSTM1 genes and A313G polymorphism of the GSTP1 gene in all the women (n=105) (50 patients of the main study groups and 55 women of the control group) revealed the following distribution of polymorphic variants of the studied genes: the GSTT1 allele genotype has made up 81.90%; the GSTT1 deletion genotype was found in 18.10% of cases; the GSTM1 allele genotype was observed in 55.24% of cases; the GSTM1 deletion genotype was detected in 44.76% of women. The frequency of the GSTP1 polymorphic variants was as follows: the AA genotype – 54.29%, the AG genotype – 38.10%, the GG genotype – 9.52%.

According to the indicators of the OR obtained, the risk of developing placental dysfunction and fetal distress during pregnancy on its background in the GSTM1 deletion genotype increased from 2.59 times to almost 4 times. The risk of intrauterine growth restriction (IUGR) on the background of placental dysfunction increased by more than 11 times (as compared to the control group).

Thus, statistical calculations allowed us to establish that the presence of the GSTM1 deletion genotype in mothers is a risk factor for prenatal damage to the fetus and the probability of IUGR development if there are clinical signs of placental dysfunction.

In the GSTM1 allele genotype, there was observed a decrease in the risk of IUGR development on the background of placental dysfunction (OR = 0.23 95% CI (0.05-0.94), however, its presence did not exclude prenatal fetal hypoxia.

The patterns presented are quite understandable, since maternal microenvironment affects the state of embryonic and fetal metabolic processes, and the GSTM1 isomeric enzymes are expressed in the placenta, i.e. are directly involved in the antioxidant and detoxification placental functions.

As the second important genetic factor for both placental dysfunction and IUGR on the background of local non-progressive placental abruption, there was analyzed A313G polymorphism of the GSTP1 gene. According to literature, the GSTP1 gene is expressed in the placenta, and its expression increases during pregnancy. The state of the GSTM1 isomeric enzyme activity plays an important role in the detoxification and antioxidant processes in the placenta. The comparison of Group II and the control group showed significant differences in the frequencies of the GSTP1 (A313G) gene genotypes as compared to Group I and the control group. In the 313GG genotype of the GSTP1 gene, the risk of developing placental dysfunction and IUGR increased by 9 times GSTP1 (χ^2 = 4.12, p = 0.036, OR = 9.64 95% CI (1.07-56.50), while in the 313AA genotype, this risk decreased significantly (χ^2 = 21.56, p = 0.001, OR = 0.07 95% CI (0.02-0.24)). In the comparison groups, there were no significant differences in the frequencies of the 313AG genotype of the GSTP1 gene. In case of dominant inheritance pattern (313AG + 313GG as compared to 313AA), the risk of developing placental dysfunction increased by almost 3 times and the risk of developing placental dysfunction and IUGR increased by 6 times.

Thus, statistical analysis conducted in the comparison groups allowed us to establish the prognostic value of studying the GSTM1, GSTP1 genes in women and the need to determine their combined effects considering the intergenic interactions as well as their interactions with other unfavorable risk factors.

For a more detailed assessment of the effect of the GSTT1, GSTM1, GSTP1 gene polymorphism on the development of placental dysfunction and the manifestations of IUGR on its background, the results obtained were analyzed in conjunction with other exogenous risk factors, anamnestic data and clinical and laboratory findings being determined during pregnancy. Unfortunately, there were no gene-factor interactions, that was obviously due to the fact that women received prophylactic agents and treatment during pregnancy which resulted in a pronounced effect of gene-factor interaction. The only risk factor which showed the cumulative effect together with genetic polymorphism was gestosis of pregnancy. In Group I, gestosis was diagnosed in 15 out of 33 (45.45%) women; in Group II, it was diagnosed in 10 out of 17 (58.82%) women. In women of Group II with gestosis, the frequency of the GSTM1 deletion genotype increased as compared to women of Group I (χ^2=5.24, p=0.022, OR=8.00 95% CI (1.21–52.7)). Considering the fact that pregnant women of Group II gave birth to children with the signs of IUGR, we can conclude that the clinical course of pregnancy in women with placental dysfunction, clinical manifestations of gestosis and the GSTM1 deletion genotype is associated with the increased risk of IUGR (by more than 5 times).

This result indicates the need to search for the ways of implementation of unfavorable effect of the genetic factor and the possibilities of prevention which consist in developing specific approaches to monitoring women with the GSTM1 deletion genotype and mandatory prevention of placental dysfunction and gestosis.

The comparative analysis of the studied women with placental dysfunction and diagnosed UCTD showed significant differences in the GSTM1, GSTT1 genes as compared to the control group. The GSTM1 deletion genotype (χ^2=4.86, p=0.028, OR=2.79 95% CI (1.11–7.02) was more often observed in women with UCTD manifestations, while the GSTM1 allele genotype (χ^2=4.86, p=0.028, OR=0.36 95% CI (0.14–0.90) prevailed in women of the control group. To identify the combined effect of A313G polymorphism of the GSTP1 gene and UCTD on the development of placental dysfunction, we studied the information value and reliability of various inheritance models. The analysis conducted identified the associations of a dominant model with the development of placental dysfunction in pregnant women with UCTD (p=0.03, OR=2.84, 95% CI (1.15–7.28).

CONCLUSIONS

Thus, the analysis of genetic polymorphism in the studied women and its comparison with the results obtained when analyzing the patients of the control group contributed to the establishment of important features. There were identified

the associations of the GSTM1 deletion genotype with the increased risk of placental dysfunction in pregnant women and the associations of the GSTM1 allele genotype with reduced risk of developing this gestational complication. The data on the differences in this polymorphic variant between Group I and Group II indicated that the GSTM1 deletion genotype in mothers with impaired placentation and local non-progressive placental abruption is a prognostic marker for risk of IUGR, while the GSTM1 allele genotype reduces the risk of fetal hypotrophy.

The increase or decrease in the risk of progressive premature placental separation is associated with polymorphic variants of the GSTM1 gene, and impaired placentation with the presence of retrochorial or retroplacental hematoma is associated with polymorphic variants of the GSTT1, GSTP1 genes.

Поліморфні варіанти генів детоксикації і можливі гестаційні ускладнення у вагітних з ретрохоріальними та ретроплацентарними гематомами

М.І. Римарчук

Здоров'я плода залежить як від генетичної програми його розвитку, так і від структурної та функціональної повноцінності плаценти.

Мета дослідження: визначення частоти алельного поліморфізму генів *GSTT1, GSTM1* у вагітних з ретрохоріальними та ретроплацентарними гематомами.

Матеріали та методи. У дослідженні нами було передбачено визначення молекулярно-генетичним методом делеційного та алельного поліморфізму генів *GSTT1, GSTM1* у гомозиготному стані, заплановано позначати визначені генотипи як делеційний варіант (*GSTT1deletion, GSTM1deletion*) або алельний варіант *(GSTT1allele, GSTM1allele)*. Було залучено 105 жінок, з них 50 вагітних з локальним непрогресуючим відшаруванням плаценти. До І основної групи увійшли 33 жінки з плацентарною дисфункцією, які народили дітей з масою тіла у межах гестаційної середньостатичної норми; до ІІ основної групи – 17 жінок з плацентарною дисфункцією та синдромом затримки розвитку народжених ними дітей.

Результати. Під час проведення дослідження делеційного поліморфізму генів *GSTT1, GSTM1* та поліморфізму *A313G* за геном *GSTP1* у всіх жінок (50 пацієнток основних груп та 55 жінок контрольної групи) було виявлено наступне розподілення поліморфних варіантів досліджуваних генів: генотип *GSTT1allele* зафіксовано у 81,90%, *GSTT1deletion* – у 18,10%, *GSTM1allele* – у 55,24%, а генотип *GSTM1deletion* – у 44,76% випадків. Частота поліморфних варіантів за геном *GSTP1* становила: генотип *AA* – 54,29%, *AG* – 38,10%, *GG* – 9,52%.

Заключення. Комбінації генотипів *GSTT1allele, GSTM1allele, 313AA* за генами *GSTT1, GSTM1, GSTP1* не створюють передумов та достовірно знижують ризик виникнення плацентарної дисфункції та затримки внутрішньоутробного розвитку на тлі локального непрогресуючого відшарування плаценти.

Ключові слова: *відшарування плаценти, гени детоксикації, акушерські ускладнення, поліморфізм генів.*

Полиморфные варианты генов детоксикации и возможные гестационные осложнения у беременных с ретрохориальными и ретроплацентарными гематомами

М.И. Рымарчук

Здоровье плода зависит как от генетической программы его развития, так и от структурной и функциональной полноценности плаценты.

Цель исследования: определение частоты аллельного полиморфизма генов *GSTT1, GSTM1* у беременных с ретрохориальными и ретроплацентарными гематомами.

Материалы и методы. В исследовании нами было предусмотрено определение молекулярно-генетическим методом делеционного и аллельного полиморфизма генов *GSTT1, GSTM1* в гомозиготном состоянии, запланировано обозначать определенные генотипы как делеционный вариант *(GSTT1deletion, GSTM1deletion)* или аллельный вариант *(GSTT1allele, GSTM1allele)*. Было привлечено 105 женщин, из них 50 беременных с локальным непрогрессирующим отслоением плаценты. В I основную группу вошли 33 женщины с плацентарной дисфункцией, которые родили детей с массой тела в пределах гестационной среднестатистической нормы; во II основную группу – 17 женщин с плацентарной дисфункцией и синдромом задержки развития рожденных ими детей.

Результаты. Во время проведения исследования делеционного полиморфизма генов *GSTT1, GSTM1* и полиморфизма *A313G* по гену *GSTP1* у всех женщин (50 пациенток основных групп и 55 женщин контрольной группы) было выявлено следующее распределение полиморфных вариантов исследуемых генов: генотип *GSTT1allele* зафиксирован в 81,90%, *GSTT1deletion* – в 18,10%, *GSTM1allele* – в 55,24%, а генотип *GSTM1deletion* – в 44,76% случаев. Частота полиморфных вариантов по гену *GSTP1* составляла: генотип *AA* – 54,29%, *AG* – 38,10%, *GG* – 9,52%.

Заключение. Комбинации генотипов *GSTT1allele*, *GSTM1allele*, *313AA* по генам *GSTT1, GSTM1, GSTP1* не создают предпосылок и достоверно снижают риск возникновения плацентарной дисфункции и задержки внутриутробного развития на фоне локальной непрогрессирующей отслойки плаценты.

Ключевые слова: *отслойка плаценты, гены детоксикации, акушерские осложнения, полиморфизм генов.*

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