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SHEI “Ternopil State Medical University by I. Ya. Horbachevsky”

The corellation of immunodependent mechanisms and Glutathione-S-Transferase Producing Gene Deletions in endomitriosis

Л. М. МАЛАНЧУК, В. М. МАРТИНЮК

ДВНЗ “Тернопільський державний медичний університет імені І. Я. Горбачевського”

ВЗАЄМОЗВ’ЯЗОК ІМУНОЗАЛЕЖНИХ МЕХАНІЗМІВ ІЗ ДЕЛЕЦІЄЮ ГЕНІВ ГЛУТАТІОН-S-ТРАНСФЕРАЗИ У РОЗВИТКУ ГЕНІТАЛЬНОГО ЕНДОМЕТРІОЗУ

The article is devoted to the actual problem today - the study of the relationship immunodependent mechanisms of gene deletion glutathione-S-transferase in a brilliant development of endometriosis. Gene network of this disease is rather complicated and diverse. It includes various genes metabolism (detoxification), genes that are responsible for immune status of genes and cell-cell interactions. Polymorphism of genes of detoxification often influences the functional activity of the molecular structure of DNA. In recent years in world literature there is more data to the pathological processes in the human body is due to immune regulation and characterized by a certain imbalance of pro- or anti-inflammatory cytokines, which contribute to the occurrence of endometriosis and can influence the effectiveness of treatment.

Стаття присвячена актуальній проблемі сьогодення – вивченню взаємозв’язку імунозалежних механізмів із делецією генів глутатіон-S-трансферази у розвитку генітального ендометріозу. Генна мережа цього захворювання є досить складною та різноманітною. Вона включає різні гени метаболізму (детоксикації), гени, що відповідальні за імунний статус, та гени міжклітинних взаємодій. Поліморфізм генів детоксикації дуже часто суттєво впливає на функціональну активність молекулярної структури ДНК. Протягом останніх років у світовій літературі з’являється все більше даних, що перебіг патологічних процесів в організмі людини відбувається за рахунок імунологічної регуляції і характеризується певним дисбалансом про- чи протизапальних цитокінів, які сприяють виникненню ендометріозу та можуть впливати на ефективність його лікування.

Staging of problem and analyses of recent researches and publication. During the last several years genital endometriosis had the leading role in the structure of gynecological diseases, with occurrence ranging between 12 % and 50 % in women of reproductive age. While there are numerous theories explaining the etiology of the disease, none of the fully explain the nature of the pathology [1, 2]. Current opinion examines this disorder as pathological process with a chronic, recurring pattern. Endometriosis develops in the woman's body due to a disbalance between the immune, endocrine and molecular-genetic processes [3] .

It is known that the genesis of genital endometriosis (GE) is related to genes responsible for metabolism, characterized by a highly variable DNA structure. Enzymes of the Guthionine S-Transferase (GST) family play a key role in providing cell resistance to lipid peroxydation by free radicals, alkilizing of proteins and forming resistance to medications preventing DNA damage [4, 5]. The course of all pathological processes in the body happen due to the immune system's regulation and are characterized by certain interactions of anti-inflammatory cytokines. From our literature analysis of the genetic component of endometriosis development, one may assume that a breakdown of the humeral response's intercellular reactions is a possible cause of the pathology in a single layer of the uterus. Currently, the role of the humeral response in regulating immune reactions is well studied [6, 7, 8]. The key endogenous immunoregulators that play an active role in the proliferating processes of the uterus and especially active during endometriosis are interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNFα). Therefore, the goal of our inquiry was to determine the role of polymorphosis of the GSTM1, GSTT1 genes and immune-dependent mechanisms in the development of GE.

Materials and methods. The clinical portion was carried out at the Ternopil Perinatal Center “Mother and Child.” Laboratory diagnostics in the interdepartmental scientific-clinical laboratory of SHEI “Terno-

pil State Medical University by I. Ya. Horbachevsky of MPH of Ukraine". 40 female patients were admitted with GE, aged from 27 to 45. The control group consisted of 20 women determined to be without GE after inspection, as well as without any signs of ovary or menstrual dysfunction. All patients granted informed consent for the collection of venous blood for research purposes. For genetic testing 3–5 ml of venous blood from the v. cubitalis was extracted under standard conditions in the morning, before food intake and stored in a special vaccum system – 3 % EDTA.

Frequencies of GSTM1 and GSTT1 polymorph varieties were obtained with the help of a polymerase chain reaction. Extraction of DNA from peripheral blood was done with the the "АмпліСенс" (Amplisense) testsystem according to the instructions provided with the product. Identifying insertion – deletion polymorphism in the GSTM1 and GSTT1 genes (Glutathione - S-transferase class T1 and M1) was carried out with the polymerase – chain reaction, "NEOGEN", Kyiv. Homozygous and heterozygous positive GSTM1 and GSTT1 gene alleles were determined on an electropherogram by the presence of a genetic material on the 218 and 460 mark respectively. Absence of those markers indicated homozygous deletion of the individual's genes. (Results of genotype distribution are represented in Table 1).

Serum interleukin levels were determined through Enzymed linked immunosorbant assay (ELISA) test using an ELISA test system. (Immune system indicators are represented in Table 2).

Table 1. Distribution of GSTM1 and GSTT1 genotypes in the observed and control group

Gene	Genotype	Observed group	Control group
		n=40	n=20
GSTM1	0/0	22 (55 %)	9 (45 %)
	+/+	18 (45 %)	11 (55 %)
GSTT1	0/0	19 (47.5 %)	5 (25 %)
	+/+	21 (52.5 %)	15 (75 %)

Table 2. Immune system indicators in the observed, control group and clinical norm

Indicator	Observed group, n=40	Control group, n=20	Normal Levels
IL=6 (pg/ml)	14.5	7.5	<10 (pg/ml)
IL=8 (pg/ml)	148, 0	21.5	<30 (pg/ml)
TNFα (pg/ml)	5.5, 0	3.5	<5 (pg/ml)

Researsh results and their discussions. The analysis of polimorfism variants of the GSTM1 gene did not yield a statistically significant difference between the mutation frequencies among the group diagnosed with endometriosis as compared to the control group (55 % and 45 % respectively).

The occurrence of a homozygous GSTT1 zero allele turned out to be significantly higher in women diagnosed with endometriosis as opposed to the control group. We analyzed the genotype combinations of two guluthionine S-transferase genes in the observed and control groups. Worth mentioning, deletions both in the GSTM1 and GSTT1 genes occurred considerably more often in the observed group compared to the control group.

From our analysis of the immunological indicators in both patient groups, we observed that IL-6 levels were approximately twice as high in patients with GE than in asymptomatic patients. This data supports the localization of IL-6 function; the concentrations observed in the treatment group indicate an inflammatory and immune response. IL-8 levels were observed to be almost seven times higher in patients with GE as opposed to the healthy group. These results may suggest the importance of the role of the non-specific immune system cells during GE inflammation.

The group of patients with adenomyosis had TNFα levels aprox. 1.5 higher than the control group, which is a positive sign of inflammation. Since tumor necrosis factors fulfill multiple important functions in the body and have a pleiotropic effect on other genes, it follows that TNFα has a substantial effect on the differentiation, proliferation and activation of cells involved in the inflammation process.

Conclusions. 1. The clinical inquiry establishes a positive correlation between endometriosis and the null genotypes of the two 2nd stage detoxification genes (GSTM1, GSTT1) and this will hopefully have a significant impact on the development of new methods to treat and prevent the disease given the individual properties of the genome.

2. Women with adenomiosis had an observed elevation of IL-6 and TNFα blood serum levels that

suggests an adequate cytokinetic regulation of the immune response during inflammation. Since the primary function of IL-8 is to encourage neutrophil migration to the site of inflammation, a high concentration suggests an activated innate immune response.

Perspectives of further studies. Therefore, further research into the role and function of factors regulating the immune response and deletions of gluthionine S-transferase is a potential route for determining possible markers for genital endometriosis without the use of invasive procedures.

REFERENCES

1. A possible mechanism of autoimmune-mediated infertility in women with endometriosis / J. Inagaki, L. Hao, M. Nakatsuka [et al.] // Am. J. Reprod. Immunol. – 2011 – Vol. 66 (2). – P. 90-99.
2. Adenomyosis: epidemiological factors. / P. Vercellini, P. Viganò, E. Somigliana [et al.] // Best Pract. Res. Clin. Obstet. Gynaecol. – 2006 – Vol. 20 (4). – P. 465–477.
3. Glutathine S-transferase Ml gene polymorphisms and susceptibility to endometriosis in a French population / H. Baranova, R. Bothorishvilli, M. Canis [et al.] // Mol. Hum. Reprod. – 1997. – Vol. 3. – № 9. – P. 775–780.
4. Glutathione-S-transferase PI gene polymorphism and susceptibility to endometriosis / D. Ertunc, M. Aban, E. C. Tок [et al.] // Hum. Reprod. – 2005.
5. Guo S. W. Epigenetics of endometriosis / S. W. Guo // Mol. Hum. Reprod. – 2009. – Vol. 15 (10). – P. 587–607.
6. Haider S. Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium / S. Haider, M. Knofler // Placenta. – 2009. – Vol. 30. – P. 111–123.
7. Berkkanoglu M. Immunology and endometriosis / M. Berkkanoglu, A. Arici // Am. J. Reprod. Immunol. – 2003. – Vol. 50. –P. 48–59.
8. Immunological factors and their role in the genesis and development of endometriosis / C. Siristatidis, C. Nissotakis, C. Chrelias [et al.] // J. Obstet. Gynaecol. Res. – 2006. – Vol. 32 (2). – P. 162–170.

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