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Prediction of Threats to Multiple Pregnancy Interruption Depending On the Cause of Its Occurrence.

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ABSTRACT

The article shows the main ultrasound signs of abortion threat, depending on its causes. The peculiarities of distributing the gene polymorphisms of IL-8 (-781 C/T), MTHFR C677T, FII G20210A, FV (Leiden) G1691A and PAI-1675 5G/4G were studied and their role at women with a multiple pregnancy miscarriage. The prognostic role of identified genetic predictors in the development of miscarriage is evaluated. In analyzing the frequency of alleles and genotypes indicated risk of reproductive losses, the most significant genetic markers are discovered and the interaction on polymorphisms of studied genes is determined. Careful monitoring the patients with multiple pregnancy in the antenatal period, prevention of the complications of multiple pregnancy, monitoring from the early terms of gestation make it possible to carry out a differentiated approach to women- aging pregnancy and delivery, thus helping decrease perinatal morbidity and mortality.

Keywords: multiple pregnancies, miscarriage, threat of premature birth, genetic polymorphism, transvaginal ultrasonography.

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INTRODUCTION

Among the major problems of obstetrics practice one of the first place takes miscarriage, which is a major cause of perinatal morbidity and mortality. Despite some progress made in the diagnosis, treatment, prevention of complications, the frequency of miscarriage in I trimester remained stable over the years, accounting for 15-20% of all desired pregnancies [1-3, 24, 36]. Equally alarming is the problem of miscarriage in the second trimester. Particularly acute this applies to multiple pregnancies. The incidence of multiple miscarriages is at 54.3% compared to 9.7% in singleton pregnancies [4, 25, 33, 34]. Lost pregnancy account for almost 17% of all desired pregnancies, while 75 - 80% of miscarriages occur in early (before 12 weeks of pregnancy), and unfortunately, there is no downward trend in these indicators. [7] About half of pregnancies are twins and 80% of triplets are interrupted for 36 weeks. According to J. Lumley (1993), in Europe, 17% of multiple pregnancies were suspended at 20 - 27 weeks, 21% - between 28 and 31 weeks and 17% of births take place between 32 and 36 weeks. In the second trimester 18 - 20% of interrupted multiple pregnancies occur, in the third - 7 - 30% [5]. Among births that occur before 32 weeks, 25% were multiple births. In view of the above, the maximum prolongation of multiple pregnancies is essential for reducing perinatal morbidity and mortality in this group of patients at high risk.

Analysis of recent research and publications: analyzing data publications, it should be noted a number of miscarriage causes, including separate social and medical factors - genetic (80% of all causes), immune (27 - 44%), endocrine (30 - 78%), infections (50%), uterine (11 - 14%), leading to spontaneous abortions, missed pregnancy and preterm delivery [5, 21]. In the first trimester are 70 - 80% of all terminations of pregnancy. Most of these losses occur in early pregnancy in the form of missed - from 45 to 88.6% [9, 19, 26]. The main reasons for abortion up to 5-6 weeks are genetic and immunological factors [24]. By genetic causes of miscarriages include chromosomal abnormalities, gene mutations, and the presence of polymorphic alleles of genes of susceptibility [5]. It was founded that 60 - 80% of embryos during miscarriage and abortion frustrated with chromosomal abnormalities, most of which occur in gametes for fertilization or in the early stages of division of the zygote. At carrying out the ultrasound, availability of trisomy can be suspected at anembryonya, non-fertilized egg the size of the pregnancy, as well as the presence of multiple fetal malformations. X monosomya causes aplasia of embryo, at ultrasound can be observed only visible umbilical cord, sometimes stored embryos with severe hydrocephalus. The structural abnormalities include inversions, translocations, deletions, their presence in couples lead to miscarriages, congenital malformations of the embryo / fetus, early forms of growth retardation [22, 23]. Ultrasound signs of pregnancy that is not developing embryo absence (anembryonya - "empty" fertilized egg the size of 2-3 cm with fuzzy, thick walls); lack of heartbeat in the embryo / fetus (embryo without a heartbeat may be unchanged, and can be as high echogenicity undifferentiated mass or individual fragments of the lack of blood flow in the area chorionic); size mismatch ovum and embryo gestational age [4, 6, 23, 28].

At present, more than three hundred identified gene mutations are common in the population and determine predisposition to various diseases. Gene's susceptibility to miscarriage merged into seven groups: genes metabolism of folic acid and vitamin B12 genes clotting factors, genes of the immune system, hormonal metabolism genes, growth factor genes, genes of detoxification genes and epithelial dysfunction [5, 35]. The role of non-pregnancy allelic polymorphism of the gene methyl entetrahidrofolatreduktaza was investigated. (MTHFR) S677T. Reduced activity of the enzyme MTHFR leads to the accumulation of homo cysteine, which promotes excessive proliferation of myositis vascular wall, reduces the synthesis of nitric oxide, leading to vascular spasm at the microcirculation, endothelial damage of the vascular wall, improve the activity of platelets, V and II coagulation factors, reduces the activity of ant thrombin III, leading to widespread thrombosis, especially in the "mother-placenta-fetus" [27]. Thrombosis chorionic areas is the cause of it detachment that at ultrasound investigation has a linear ehonehatyvnoe gloss. At progressing the retinal detachment can be observed the distribution of retrohorial hematoma [28]. Local thrombosis in the chorionic area with it further detachment occur in the presence of mutations in the genes of different coagulation factors, so gene mutation V clotting factor (mutation Leiden, G1691A, FVL), in the gene II coagulation factor - pro thrombin (G20210A, PTM), in Genius and coagulation factor - fibrinogen (G455A) [5, 27].

About 40% pregnancy losses are associated with immune disorders that lead to the rejection of the embryo / fetus. They are based on autoimmune and hello immune processes. At autoimmune processes aggression of immune system is against its own tissues. These include the anti-phospholipid syndrome, antibodies to β -human chorionic gonadotropin (β -hCG), progesterone, estradiol, thyroid hormones. At the

hello immune process, immune response is directed against antigens of the fetus, which he received from his father and is a foreign body for pregnant women. Hello immune violations include incompatibility parents NLA-system, the predominance of Th2-lymphocytes (T-helper type 2) in the blood of pregnant Th1-lymphocytes (T-helper type 1) [32]. There are 5 categories of immune disorders that lead to abortion failures when attempting in vitro fertilization and infertility, incompatibility parents NLA-system; anti phospholipid syndrome (APS) associated with circulating antibodies to phospholipids and phospholipid-binding proteins, the morphological study in pregnant chorionic revealed violations of maturation and focal necrosis of the chorionic, presence of blood clots, excessive in amount of fibrin in the space between the villous; and the presence of antinuclear and antibodies; the presence of anti-sperm antibodies; abnormal activation of CD 56 + CD 16 - natural killers and CD 19, CD + 5 + B lymphocytes [11, 21]. At pathological levels of CD 19, CD + 5 + B lymphocytes in the blood of pregnant women - over 10% - is the formation of antibodies to the hormones that are important for normal development of the pregnancy - progesterone, estradiol, β -human chorionic gonadotropin. Progesterone enhances blood flow to the uterus, blocking the synthesis of oxytocin receptors, lowering the tone of the uterus. At a low level of progesterone in the blood of pregnant as a result of the activation of CD 19 + CD 5 + B lymphocytes, and at the lack of corpus luteum function on the background of various endocrine pathology [19, 24, 29], chronic adnexitis, endometriosis, as well as normal progesterone in the blood of pregnant women, but the violation of its effects on the endometrium due to mutations in the gene progesterone receptor - 1031S, 1978T, 2310T RGR [5] are not sufficient changes of endometrium in the second-phase of the menstrual cycle, disrupted the process of implantation, leading to spontaneous abortion or formation of defective chorine, placental insufficiency. Chorionic gonadotropin is synthesized by trophoblastic cells, supports the corpus luteum. In the presence of antibodies to human chorionic gonadotropin bag is damaged, there retrohorialni hematoma due to local thrombosis in the chorionic area [18, 23, 26].

At physiological pregnancy in peripheral blood increases lymphocytes that have receptors for progesterone. During the interaction of progesterone with lymphocytes the last synthesize and secrete "progesterone-induced blocking factor", which activates the CD 56 + CD 16 + natural killer. When they activate the immune response of the mother to embryo that has NLA antigens father carried the T-helper type 2 (Th2) through anti-inflammatory cytokines (interleukins 3, 4, 10, 13). Anti-inflammatory cytokines, in turn, increase the production of asymmetric antibodies is unable to activate complement fixation, phagocytosis, and cytotoxicity. At a low level of progesterone a small number of "progesterone-induced blocking factor" is synthesized, CD 56 + CD 16 + natural killer is activated, the immune response of the mother carry the T-helper type 1 (Th1) through pro inflammatory cytokines (interleukins 1, 2, 6, tumor necrosis factor α). Inflammatory cytokines cause necrosis of decidua tissue, leading to the surface of trophoblastic invasion, hypoplasia chorionic, impaired formation of vascular network chorionic, local ischemia Chorionic the development of local DIC and thrombosis of between villous space, damage of bag, the death of the embryo [14, 18, 19, 23, 29, 32].

Endothelial dysfunction is manifested by reduced synthesis of prostacyclin, nitric oxide, exceeding the physiological synthesis rate increase of endothelin-1, thromboxane, which leads to vascular spasm and reduced blood flow in the chorionic area or placental local DIC, thrombosis of between villous space, detachment of chorionic / placenta [14, 27]. The cause of increased tone and impaired blood flow in the chorionic / placenta is gene polymorphism of endothelial nitro oksydsyntetazy - 4a / 4b eNOS, which reduces the synthesis of nitric oxide and angiotensinogen gene polymorphism - AGT M235T and T174M, enhancing synthesis of angiotensinogen II [5].

Gene's group of II phase of detoxification is represented by glutathione-S-transferase, which neutralize the products of lipid peroxidation, causing a toxic effect on cell membranes, including cell embryo. The presence of genes weak alleles in phase II detoxification couple - GSTM1, GSTT1, GSTP1 – causes the missed miscarriages and pregnancy.

Infection is one of the most significant causes of miscarriage, which is 13 times increased incidence of spontaneous abortions, 6 times - preterm birth [6, 20]. At the termination of pregnancy plays a role both viral and bacterial infection. Viruses and bacteria that directly affect the embryo, leading to missed abortion, the phenomenon of "missing twin" with twins, violate the differentiation of major anatomical structures of the fetus [4] and cause inflammatory changes in the chorionic, disrupting its function, increase the level of pro-inflammatory cytokines, prostaglandins causing uterine contractions [13, 30]. Result of infection is defined by gestation in which it occurred. Since the emergence of infection during the first two weeks the following

fertilization results in blastopatiyi and terminations of pregnancy. Infection in pregnancy from 2 to 10-11 weeks can lead to missed abortion, spontaneous abortion, birth defects result in the formation of fetal inflammatory, chorionic changes [8, 20]. Histologically scrape from the uterus after spontaneous abortion against the backdrop of infection are morphological changes, vacuities, endometritis, common leukocyte infiltration of tissues, thrombosis of between villous space, micro thrombosis of microvasculature, a significant number of fibrynoyidu that bond nap in conglomerates necrosis in decidua tissue, forming without violating their when viral lesions - polymorphism cell nuclei with increasing [17]. For timely formation of an increased risk of preterm birth among women with multiple pregnancies is searched by many scientists' prognostic criteria as the threat of premature birth in singleton and multiple pregnancies with. Ultrasonic monitoring at multiple pregnancies carries valuable information about the fetus, cervix (length, internal pharynx, cervical canal) and contributes to the timely formation of an increased risk of miscarriage.

The aim of our study: reducing the frequency of miscarriage by studying and predicting the impact of genetic predictors at women with multiple gestation and the dynamic ultrasound fetal monitoring starting at early pregnancy.

Presentation of basic material: research was conducted at the Sumy Regional Clinical perinatal center and laboratories molecular genetic studies of SSU. During 2012 - 2015 years under our supervision there were 160 patients with twins, at 84 (52.5%) of whom pregnancy was against the background of the threat of spontaneous abortion or premature birth, they were the main group. The control group was 47 pregnant women with physiological course of gestation. All patients got through molecular genetic testing gene mutation IL-8 (-781 C / T), MTHFR S677T, FII G20210A, FV (Leiden) G1691A 1675 and PAI- + 5G / 4G. To determine the relation of polymorphism of miscarriage was conducted DNA isolation from peripheral blood of pregnant women, it recruited a venous blood monovet of 2.7 ml with anticoagulant («Sarstedt», Germany). DNA was extracted from whole blood leukocytes using sets DIAtomDNAPrep 100 («Isogene», Russia). Definition of allele polymorphism was performed using allele-specific polymerase chain reaction (PCR) followed by hydrolysis of the corresponding amplicon restryktasis endonuclease. Genomic DNA was isolated from blood samples by the standard method using proteinase K, phenol-chloroform extraction and ethanol precipitation. Identification of allele variants was carried by the presence of recognition for that endonuclease restryktasis by electrophoresis. The presence of mutations showed the formation of two low bands appearing under the action of the enzyme. Full splitting the PCR product indicates the presence of the homozygous form of the analyzed DNA mutations and partial heterozygous. The analysis studied the distribution of genotypes was carried out using test χ^2 . The distribution of genotypes for the studied polymorphic loci was tested against the Hardy-Weinberg equilibrium with the help of Fisher's test. To compare allele frequencies between groups Pearson criterion was used. Differences were considered statistically significant at $p < 0.05$. To analyze the association of alleles and genotypes were studied genes with the risk of obstetric complications calculated odds ratios (OR) with 95% confidence intervals. In conventional clinical examination and laboratory studies was conducted ultrasound monitoring of early pregnancy. In first trimester ultrasound diagnosis was carried out using two standard methods: transvaginal sonography and transabdominal scanning techniques filled bladder. All studies were conducted on modern ultrasound devices using transvaginal probe 6.5 MHz convex sensors 3, 5 and 5 MHz in the two-dimensional echo mode. In the second and third trimesters was carried out transvaginal tservikomometriya, ultrasound fetoplatsentometriya, doppler blood flow. Statistical analysis of the data was performed with parametric methods using statistical computer programs [16].

The results of the molecular genetic testing showed that the MTHFR mutation S677T found at 32 women (38.1%) of the main group polymorphism gene PAI-1 675 5G / 4G in 16 (19,0%), FII G20210A at 1 (1, 2%), FV (Leiden) G1691A at 7 (8.3%) and various combinations of mutations at 28 (33.3%). To detect the possible association of polymorphisms of genes miscarriage was used a comparative analysis of the frequency of alleles and genotypes between patients with miscarriage and controls. According to the results of the MTHFR polymorphism S677T women met in the main group of heterozygous variant (51.2%) in the control group (13.5%), homozygous MTHFR gene mutation S677T was (15.4%), which is three times higher than in the control group (5.4%). Analysis of gene mutation PAI-1 675 5G / 4G in the intervention group showed a decrease in the normal genotype 5G / 4G (54,8%) compared with the control group (75.7%, $p < 0.05$), while the proportion of hetero- and homozygous carriers of genotypes was higher compared with the control group (25.0% and 20.2%, against 18.9% and 5.4%). The high frequency of homo- and heterozygous carriers of the mutation FV (Leiden) G1691A (12, 8%, 2, 6%) compared with the control group (2.7%, 0%). At studying gene polymorphism distribution of IL-8 locus - 781S / T was received results as allelic gene fragments. For gene

polymorphism 781S / T IL-8 homozygote for the dominant type was marked as - SS heterozygote - PT homozygotes for the recessive type - TT. At women with miscarriage the following results: CC - (19, 1%) cases, centuries - (57.1%) cases, CT - (23.8%) cases. In the control group the corresponding figures were (42.5%), (40.4%) and (17.1%). Genetic markers of successful pregnancy are the existence of a locus allele C - 781 C / T gene encoding IL-8. Carriers of the T allele have a 1.85 times higher risk of pregnancy complications. TT homozygotes for allele (-781 C / T) have significantly lower chances of successful pregnancy than the total of all dominant gene carriers, namely 1.5 times. All carriers of recessive allele gene have three times higher risk of pregnancy pathology compared with homozygosity of dominant alleles. Holders of genotype C / T and T / T have the relative risks of 2 and 1.5 times higher than the native homozygous for the dominant of the allele gene of miscarriage.

Proof of implementation of identified genetic polymorphisms in miscarriage at the surveyed group was the presence of ultra-sonographer markers in early pregnancy. Identified ultrasonographic signs of corpus luteum function and chorionic inferiority, lack of ovarian corpus luteum; decrease the volume of the corpus luteum to 2 cc or less; chorionic hypoplasia; change parameters of blood flow in the uterine and ovarian arteries on the side of the corpus luteum; chorionic vascularization index decline, reducing blood flow to the chorionic index; local thickening of myometrium, deformation ovum, low location of the fertilized egg in the uterus, the expansion of the internal of the cervix than 2 cm as signs of a miscarriage that started. Ultrasound sign of chorionic hypoplasia is its thickness less than 5 mm; damage of yolk-sac manifests its absence or change in size.

Ultrasound signs of intrauterine infection in MULTIPLE identified in the study were: hypoplasia amnion / amnion, chorionic fragmented / chorionic, chorionic thickening; Resize yolk-sac (less than 2 mm or 5.6 mm) or its absence; early oligohydramnion; size mismatch of ovum gestational age; the presence of amniotic bands; alignment echogenicity of three extra embryonic cavities: chorionic, amniotic, oral, yolk-sac (when infected as a result of greater proliferative-exudative reaction amniotic sac membrane and yolk-sac an increase echogenicity these cavities); local thickening of myometrium, deformation of ovum, low location of the fertilized egg in the uterus, expansion of the internal cervix more than 2 cm; no embryo / embryos; reduction of motor activity of embryos (was determined after 7 weeks of pregnancy); heart rate of the fetus / fetuses less than 90 bpm. / min., no heart beat in one or both fetuses; abnormalities of the fetus / fetuses; lack of blood flow in intermittent or chorionic with blood flow in the Doppler scan; difference systolic-diastolic ratio in the right and left uterine arteries more than 12%.

Ultrasound signs of threatened abortion in the second trimester of the risk of premature birth. The structure of miscarriage in the second trimester cervical incompetence (CIN) is 40%, in the third trimester of CIN is found in every third case of premature birth twins [23]. Cervical immaturity traced to the expansion of the cervical canal. There are organic (secondary, post-traumatic) and functional CIN. The first results from a traumatic injury of the cervix after childbirth, miscarriage, abortion history. In turn, the functional origin of CIN is caused by endocrine disorders. With increased androgen levels, this type of CIN meets every third pregnant. Activation of α -receptors on the background of higher concentrations of estrogen in the blood of women, lack of stimulation of β -progesterone receptors at its reduced level lead to the disclosure of the cervix. In literature there is also the concept of cervical insufficiency (cervical incompetence, insufficiency), which is a risk factor for premature birth, another risk factor is multiple pregnancy, premature birth history, malformations of the uterus, bacterial vaginitis, urinary tract infection, high levels of α -fetoprotein in the blood of pregnant [15]. The mechanism of abortion while CIN due to the fact that the expansion of the isthmus and cervix fertilized egg does not have support, while increasing intrauterine pressure associated with an increase in fetal weight and volume of amniotic fluid, fetal membranes prolapses in the cervical canal, infected and burst. If CIN was diagnosed before pregnancy and its correction was not conducted, in the history there have been spontaneous abortions and premature births, if the state of the cervix changes during pregnancy (she shortened, softened, expanding the cervical canal), this requires suturing the cervix. Surgical correction of CIN was held from the 13th to 27th week of pregnancy. Traditionally, assessment of cervical vaginal performs research, but to more accurately determine the length and position of the cervix applied ultrasound cervikometriya. The average length of the cervix at not pregnant women is 25 - 30 mm. At physiological pregnancy to the 12th week the cervical length reaches 50 - 55 mm due to hypertrophy of the muscular cells isthmus, which is keeping the shape of a cylinder, increasing its length roughly doubled - from 10 - 14 to 28 mm. With increasing gestational age by wedging a fertilized egg into the cavity of the cervical canal, the total length of the cervix reduced. At singleton pregnancy that proceeds without complications, cervix shortens to 0.58 mm per week, in multiple to

1, 43 mm. With increasing gestational age increases anterior, posterior and transverse dimensions of the cervix with 24 and 34 mm in 6 weeks pregnant 27 and 40 mm at 38 weeks of pregnancy, respectively, the diameter of the cervical canal 11 mm in 6 weeks to 13 mm at 38 weeks of pregnancy [12, 31]. The threat of abortion based on ultrasound in the second and third trimesters was detected with decreasing cervical length compared with the norm for the duration of pregnancy. Pregnant with twins critical cervical length at 23 weeks of pregnancy is considered equal to 20 mm length, in this case at 40% of pregnant women births occurred before 33 weeks; when you change other settings of the cervix, while expanding internal os 5 mm - 2 cm; sign of threatened abortion is the ratio of the length of the cervix to its diameter $1,16 \pm 0,04$ (rate of $1,53 \pm 0,03$), and change the anatomy of the internal os of the "T" forms a "U" shape in the case of prolapse fetal bladder, by changing the structure of the cervix (the appearance of its thickness ehonehatyv multiple inclusions) in the event of a local tone of the myometrium, the volume of the adrenal glands of the fetus than 442 mm^3 (prematurity developed in the next 5 days). To determine the risk of preterm birth data were used not only vaginal examination and ultrasound, but the results of other methods, such as biochemical. The high risk of preterm birth indicated enhance fetal fibronectin in cervical secretions pregnant at 18 - 34 weeks 50 mg / ml (premature birth before 35 weeks forecast with sensitivity 63%, specificity 81%); if the matrix metalloproteasey - 9 (MMP - 9) in the blood of pregnant women is 67.15 pg / ml (prematurity forecast for 7 days); if the content of insulin-like growth factor, related protein-1 (insulin-like growth-factor binding protein-1 - IGFBP-1) in the cervical mucus in 22 - 32 weeks $\geq 6,4 \text{ mm} / \text{l}$ (predicted preterm delivery before 37 weeks).

In addition, for the rapid diagnosis of preterm labor or willingness of pregnant women to emergency labor with intact membranes used highly sensitive rapid test strips, based on imunohromatohrafiy to determine phosphorylated protein-1 in cervical secretions, which binds insulin-like growth factor - Actim Partus. Phosphorylated protein-1, insulin-binding growth factor synthesizes in the decidua membrane and may appear in the cervical mucus during contractions, with a positive test when its level exceeds 10 mg / l, births occurred within 1-4 days of his conduct (sensitivity, specificity, positive predictive value and negative test results was 89.5%, 94.1%, 94.4% and 88.9% respectively). Ultrasound signs of threatened abortion in the absence of uterine contractions considered an indication for weld overlay on the cervix, the introduction of midwifery obstetric pessary, prophylactic administration of progesterone to 36 weeks of pregnancy (administered at doses from 200 to 500-600 mg per day). With increased excitability of the uterus, uterine contractions our presence meant tocolytic therapy, conducted prevention of respiratory disorders in fetuses to term glucocorticoid 34 weeks of pregnancy.

CONCLUSIONS

1. The study was able to determine the most important predictors associated with the risk of miscarriage in the MTHFR MULTIPLE S677T, FV (Leiden) G1691A and IL-8 (-781 C / T). Combinations of both homo- and heterozygous genotypes of these genes associate with the risk of reproductive losses.
2. Ultrasound method of research is a fundamental for the diagnosis of miscarriage and may indicate its possible causes that point to the sighting diagnosis and adequate treatment of complications of pregnancy.
3. Careful monitoring of patients with multiple pregnancy in the antenatal period, prevention of the most common complications of pregnancy, monitoring of early pregnancy allow differentiated approach to the management of pregnancy and childbirth, helping to reduce perinatal morbidity and mortality.

Prospects for further research are to study the deepening problems antenatal care in multiple pregnancies. Timely detection of genetic predictors will optimize preconception preparation to reduce the risk of reproductive losses and obstetric complications. The presence of genetic markers serve as an indication for intensive control of hemostasis, immune status, homo cysteine levels during pregnancy and the need for treatment. The data suggest that ultrasound placenta in multiple pregnancies may serve as a diagnostic criterion for objective assessment of pregnant women and fetuses, and predict the development of possible complications during pregnancy, childbirth and perinatal complications.

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