

Research Article

The Role of Endothelial Dysfunction Markers in Pregnant Women with Chorion Detachment, Included in the Program of Auxiliary Reproductive Technologies

Natalya Lytvyn

Abstract

An urgent medical and social problem is the restoration of reproductive function of women who suffer from infertility, which became possible due to auxiliary reproductive technologies. Women with induced pregnancy make the group of a high-risk on miscarriage, due to interrelated processes – immunological disorders and endothelial dysfunction that occur in the body of pregnant women after the use of extracorporal fertilization programs, and can lead to the chorion detachment and the formation of subchorionic hematomas.

The purpose of the study is to determine the role of endothelial dysfunction as one of the leading factors that determine the development of a local non-progressive chorion detachment in infertile patients included in the program of auxiliary reproductive technologies.

Materials and methods. We have examined 130 pregnant women, who were divided into groups: the control group included 30 women, whose pregnancy occurred in the natural cycle and with uncomplicated gestational course; the main group – 50 patients with induced pregnancy and risk factors of the occurrence of chorion detachment, who were performed the proposed pre-gravidapreparation; the comparative group – 50 pregnant women who received a standard scheme of pregnancy management before and after in-vitro fertilization. A general clinical examination, ultrasound examination, homocysteine level determination, endothelin-1 and nitrogen oxide metabolites were performed.

Results. In women included into the program of auxiliary reproductive technologies with local chorion detachment were recorded changes of vascular endothelial function with a possible increase in endothelin-1 production and a decrease of the nitric oxidesynthesis. During the induced pregnancy with the presence of subchorionic hematoma, an increase of the level of endothelium-damaging factor of homocysteine was noted. **Conclusions.** This study identifies the parameters that reflect the main links of endothelial dysfunction and can be used as markers of local chorion detachment.

Keywords

infertility; miscarriage; subchorionic hematoma; auxiliary reproductive technologies; endothelial dysfunction

Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

Corresponding author: fairorchid85@gmail.com

Problem statement and analysis of the recent research

An extremely urgent medical and social problem is the restoration of reproductive function of the women who suffer from infertility, since the frequency of infertility in married couples in Ukraine is according to various data from 15.0% to 20.0% and there is no tendency to decrease, which creates socio-demographic problems in the national scale. For the past decades, the use of auxiliary reproductive technologies (ART) in our country has allowed pregnancy to be obtained in 33.5% of infertile married couples [1, 8]. Despite the high efficiency of ART, most of the induced pregnancies have a variety of complications, and each second one ends with its interruption. According to the World Report on Assisted Reproductive Methods, only 70-80% of clinical pregnancies after

extracorporal fertilization (IVF) end with live birth [3].

Pregnant women after IVF programs are included into the group of risk factor of miscarriages. It is associated with the burden of obstetric-gynecological anamnesis, various disorders of the reproductive system, concomitant extragenital diseases. The stimulation of super-ovulation in the program of IVF and hormonal therapy with high doses of progesterone to support the early terms of pregnancy is the trigger mechanism for activation of pathological processes, which lead to interruption of pregnancy. According to the research data of a number of authors the complicated course of pregnancy is associated with the immunologic disorders in the organism of the mother, impairment of endometrium receptiveness, antiphospholipid syndrome, hyperhomocysteinemia, defects of coagulation system, presence of hypersusceptibility to chori-

onicgonadotropin of a man, imbalance of different growth factors, endothelial dysfunction etc. [4, 7].

During the development of different obstetric pathologies, considerable attention is paid to interrelated processes – immunological disorders and endothelial dysfunctions that occur in the body of a pregnant woman after the use of IVF and are of a particular importance in ensuring blood circulation in the uterus-placenta-fetus system [2].

The endothelium carries out a barrier function to support homeostasis by preserving the dynamic equilibrium of a number of diverse processes: 1) vascular tone; 2) anatomical structure and remodeling of blood vessels; 3) local inflammation; 4) processes of hemostasis and thrombolysis. As it is well-known, endothelial dysfunction is a universal pathogenetic mechanism of most diseases, and also plays an important role in reproductive disorders, in particular infertility, the usual miscarriage of pregnancy. Endothelium produces vasodilators and anti-aggregants – nitric oxide (NO), bradykinin, prostacyclin, prostaglandin E2, endothelial hyperpolarization factor, vasoconstrictors and pro-aggregants – endothelin-1 (ET-1), angiotensin II, serotonin, prostaglandin F2a, leukotrienes, thromboxane A2, heparin, plasminogen activators, growth factors. Among the numerous endothelial factors, the commonly recognized marker of endothelial dysfunction is NO. Nitric oxide is formed of L-arginine under the action of the enzyme NO synthase (NOS). It is the system of L-arginine – NO modern obstetrics gives the leading vasoregulatory role during the period of gestation. Some of the most vasoactive substances are endothelial peptides – endothelins. The most common among endothelins is endothelin-1, which is a large bicyclic polypeptide. The synthesis of ET-1 is stimulated by thrombin, adrenaline, angiotensin, insulin, interleukins, cell growth factors, etc. The ET-1 synthesis inhibitors include natriuretic peptides. ET-1 is involved in the remodeling of blood vessels, increases blood pressure and production of cytokines, reduces uterus-placental blood flow, and causes haemoconcentration and proteinuria [5].

Another important factor in reproductive and obstetric disorders is hyperhomocysteinemia, which leads to damage and activation of endothelial cells, thereby breaking the balance-endothelial complex. On the other hand, hyperhomocysteinemia may be accompanied by the development of secondary autoimmune reactions, and may also reduce the biological activity of nitric oxide, which leads to vasospasm and endothelial dysfunction. Homocysteine induces apoptosis of trophoblast cells and significantly reduces the production of chorionic gonadotropin, which may be the cause of complications associated with violation of implantation and, as a result of these disorders there is a detachment of the chorion and the formation of subchorionic hematomas [6].

It should be noted that the biological effect of NO and endothelin-1 in the development of pregnancy requires further detailed study and opens up new perspectives in the development of modern methods of diagnosis and therapy of miscarriage, aimed at improvement of the perinatal and maternal

morbidity rates [9]. Considering the above-mentioned facts, it is important to study the state of the endothelial complex in order to determine its pathogenetic role in the loss of pregnancy. Taking into account contemporary ideas about endothelial dysfunction as a universal trigger mechanism of reproductive disorders and the role of vascular changes in the violation of processes by placentation, in such women there were performed studies on the metabolites NO, endothelium-damaging factor – homocysteine and vasoconstrictor peptide – endothelin-1.

The objective of the study is to determine the role of endothelial dysfunction as one of the leading factors that determine the development of a local, non-progressive chorionic detachment of patients with infertility treated with auxiliary reproductive technologies programs.

1. Materials and methods

To realize the objective to be achieved, a comprehensive study of 130 pregnant women, who depending on the characteristics of onset and the course of pregnancy, were divided into groups.

The control group (CG) included 30 women, whose pregnancy occurred in the natural cycle and with uncomplicated gestational period; the main group (MG) – 50 patients with induced pregnancy and risk factors of chorionic detachment, who were performed pre-gravida preparation, which included receiving of the drug with a high content of folic acid and omega-3 – fatty acids for three months according to the program of ART, in a month before the program – the amino acid L-arginine aspartate and a hepato-cardioprotective medicine, the hormonal background was corrected by retroprogesterone in the second phase for 3 months. The comparison group included 50 pregnant women who received a standard scheme of pregnancy management before and after IVF. General clinical examination, ultrasound examination and determination of homocysteine level by immunoassay (ELISA) using standard production kits produced by Ltd. Company “BioKhimMak” (Russia) were performed. The research of endothelin-1 level in blood plasma was studied using the ELISA method using “Biomedica” reagents. The content of stable NO metabolites was determined in blood serum by ELISA method using test-systems manufactured by the company “R&D system” supplied by BioKhimMak Ltd., based on the principle of converting the nitrate into nitrite during the reaction, which is catalyzed by the enzyme nitrate-reductase. Subsequently, total nitrite is determined by absorption of azo-stain in the Griss reaction at a wavelength of 540 nm. Blood was taken twice: at 5-7 weeks and at 11-12 weeks of pregnancy.

Statistical processing of the results of the study was carried out using the standard program “STATISTICA 6.0” with the main variation indicators. The reliability of the obtained results was determined using the Student’s t-criterion.

2. Results of the study and their discussion

An analysis of the age of women, included in auxiliary reproductive technology programs, has shown that more often early reproductive loss was observed in patients aged 26-30 years or more – 48.27% of cases, the mean age of the control group was 24.5 ± 0.8 years, the main group and comparison group – 31.1 ± 0.62 years and 31.5 ± 0.65 years respectively. Infertility of endocrine genesis was observed in 48% of women, tubal-peritoneal – in 24% of women, of combined genesis – in 28%. Evaluation of the effectiveness of ART programs in this category of patients allowed marking in 18% of cases more than three attempts of IVF in anamnesis, in 67% of observations, the IVF program was performed for the first time.

In an ultrasound study, signs of the threat of abortion (local hypertonia of myometrium, the presence of chorion detachment in the form of subchorionic hematoma) were observed in the main group in 8 (16%), in the comparison group – in 21 (42%) of the pregnant women, in the control group the segmental uterine contractions and sites of the chorion detachment are not defined.

During the study of concentration of endothelin-1 in women of the main and comparative groups, a statistically significant increase of this indicator was observed, namely – 0.83 ± 0.03 fmol/ml in the main group and 3.04 ± 0.81 fmol/ml in the comparison group against the patients in the control group, where the concentration of endothelin-1 was 0.52 ± 0.04 fmol/ml. Attention is drawn to the indicators of the main group that received pre-gravida training – they are only 1.5 times higher than those of the control group, while the indicators of the comparison group are 5.8 times higher.

Regarding the content of NO metabolites, this indicator for women of the main group and the comparison group was 38.29 ± 0.97 μ M/l and 26.96 ± 1.17 μ M/l, which is significantly lower than that of the control group – 41.02 ± 1.57 μ M/l. It is noted that the indicator in the comparison group is 1.5 times lower than that in the main group in relation to the control group's indicators.

Thus, the increased production of endothelin-1 and decreased synthesis of nitric oxide were found in women with induced pregnancy and the presence of chorion detachment. These parameters reflect pro-aggregant and pro-inflammatory function of the endothelium. At the same time, a reliable difference in the rates of the women from the comparison group, whose gestational period was complicated by chorion detachment and the formation of subchorionic hematoma, against the women of the main group with signs of the threat of abortion, was revealed.

The imbalance between vasoconstrictor, pro-aggregant endothelin-1 and vasodilator, anti-aggregant nitric oxide leads to violation of morphology and chorionic functioning, which may further contribute to placental insufficiency. The results of the indicators of the main group compared with the results of patients, who did not receive the proposed pre-gravida preparation, indicate the effectiveness of the offered

therapy in the prevention of complications of pregnancy to optimize the gestational period in women after IVF.

Studying the level of homocysteine as the endothelium-damaging factor, it was found that in the main group it corresponded to the one of the control group of 5.57 ± 0.12 mM/l, while in the group of comparison with the presence of non-progressive local chorion detachment it was significantly higher 7.94 ± 0.16 mM/l.

Thus, study has established that pregnancy after ART programs was associated with complications in the patients whose formation and development of chorion and placenta have occurred under conditions of endothelial dysfunction – a decrease of the nitric oxide level and an increase of the endothelin-1 level and the negative effect of homocysteine were at the beginning of pregnancy. These factors may be early prognostic markers of significant obstetric and perinatal disorders in induced pregnancy in women with infertility of different genesis.

3. Prospects of further researches

The promising direction of research is to identify the causes of unsuccessful IVF attempts and early pregnancy loss at the pre-gravida stage with the aim of their further correction.

4. Conclusions

Thus, this study identifies the parameters that reflect the main links of endothelial dysfunction and can be used as markers of local chorion detachment that is a prerequisite for the development of pathogenetically directed preventive complex and treatment of these complications.

1. Women included into the program of auxiliary reproductive technologies, with localized chorionic detachment were recorded changes of vascular-motional function of the endothelium with a significant increase of endothelin-1 production ($p < 0.05$) and a decrease of the synthesis of nitric oxide ($p < 0.05$).
2. During the induced pregnancy with the presence of subchorionic hematoma, an increase of the level of endothelium-damaging factor of homocysteine ($p < 0.05$) was observed.
3. The proposed pre-gravida preparation in women with induced pregnancy due to balancing of endothelial factors improves microcirculatory processes, accelerates the regression of signs of the threat of abortion and helps to successfully prolong the pregnancy in women with subchorionic hematoma.

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