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RESEARCH STUDY

Preterm birth prediction in pregnant women over than 35 years. Observational analytical cohort study

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ABSTRACT

Introduction. Premature birth can occur at any age; however, it is important to note that the risk of preterm birth can vary based on several factors, including the mother's medical history, general health, and lifestyle. There is thought to be a relationship between maternal age and the risk of preterm birth, although the exact nature of this relationship may vary. At the same time, it is considered for ages over 35, an increased risk factor for the evolution of pregnancies with complications. Pregnant women over 35 face a higher risk of premature birth. This increased risk may be associated with age-related factors such as underlying health conditions, higher rates of multiple pregnancies (due to fertility treatments), and potential placental dysfunction.

Material and methods. In the given study, the biomarkers IL-6, IL-8, IL-10, IL-12, SDF-1α and VEGF in amniotic fluid (AF) and maternal blood were investigated, considering the above as predictive of premature birth outcome. At the same time, the oxidative stress status of maternal blood and amniotic fluid collected in the second trimester of pregnancy was identified.

Results. In the research, we obtained statistically significant increases in the biomarkers AAT-isopropyl, G-GTP, HPL-isopropyl from the amniotic fluid taken from pregnant women over 35 years of age in the second trimester of pregnancy in those pregnant women who had a preterm birth. In the serum of pregnant women with premature birth, an increase in the concentration of carnosine-histidine peptides, G-GTP, GR and SH (thiol) groups was identified, and the decrease in the values of SDF 1α, HPL – hexane and IL-12 were statistically significant in the serum pregnant women compared to that of the amniotic fluid.

Identifying the values of biochemical mediators during pregnancy can be a method of predictive diagnosis

Conclusions. Our study shows the relationship between some concentrations of oxidative stress biomarkers (AAT-isopropyl, HPL-isopropyl and G-GTP, IL-12) in amniotic fluid, and values of (Carnosine Histidine Peptide, GR and SH and SDF-1α) in the serum of pregnant women, in the second trimester of pregnancy.

Keywords: premature birth, preterm delivery, risk factors, pregnancy over the age of 35 years, cytokines, inflammation, biomarkers, IL-6, IL-8, IL-10, IL-12, SDF-1α, VEGF, oxidative stress, amniotic fluid.

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Key messages

What is not yet known about the issue addressed in the submitted manuscript

Premature birth can be associated with a number of complications and risks for both mother and baby. Babies born prematurely may have breathing difficulties due to the immaturity of their lungs, which could diminish their quality of life.

Immune biomarkers, determined from the blood of pregnant women over 35 years of age and from the amniotic fluid, are informative with predictive value for pregnancies ending in premature birth.

The research hypothesis

Observational analytical cohort study in preterm birth prediction in women over than 35 years from the second trimester of pregnancy by investigating the correlation between the level of oxidative stress and cytokines in fetal amniotic fluid (AF) and maternal blood, taken in the second trimester of pregnancy in pregnant women aged over 35 years at which the premature birth was subsequently certified.

The novelty added by manuscript the already published scientific literature

Following the results received in the study, the identified immune mediators were found to be involved in the prediction of pregnancy complications, due to premature birth. Importance multifunctional of cytokine, involved in the immune response mechanisms, in the case of the given study it is associated with the complication of pregnancy through preterm birth in women over than 35 years.

Introduction

Premature birth is one of the main causes of perinatal morbidity and mortality [1-3]. For women with pregnancy over 35 years, the risk of pregnancy complications is worrying.

It is considered one of the main causes of maternal morbidity, and it refers to the presence or occurrence of diseases conditions, or health complications [4, 5].

In recent years, in the Republic of Moldova, there is a tendency to postpone pregnancy after the age of 35 [6, 7].

With age, the woman's body is more susceptible to chronic pathological manifestations, medical complications during pregnancy in pregnant women over 35 years of age are determined by the aging of the reproductive system, which includes the increased risk of complications during pregnancy.

One of the complications during pregnancy and birth is hemorrhage, which is often due to premature births. Anticipating the pathologies of the pregnancy evolution, as well as an early diagnosis of the pathologies that complicate the pregnancy evolution would significantly influence the prognosis of maternal risk in pregnancy [6].

Premature birth is one of the main causes of perinatal morbidity and mortality. For women with pregnancy obtained through the in vitro fertilization method, over 35 years old, the risk of pregnancy complications is worrying.

It is considered one of the main causes of maternal morbidity, and it refers to the presence or occurrence of diseases, conditions or health complications

Numerous studies have identified the role of oxidative stress and cytokines in the pathogenesis of preterm birth [7, 8].

The purpose of the study: to identify the correlation between preterm birth and the level of oxidative stress in fetal amniotic fluid and maternal serum collected in the first trimester of pregnancy in pregnant women over 35 years old.

Materials and methods

A clinical trial has been performed in the National Center of Reproductive Health and Genetics in the Republic of Moldova and has included the pregnant women after 35 years, who have been subjected to an invasive prenatal diagnosis (amniocentesis). According to the pregnancy outcome, from a cohort of 65 women, which were divided into 2 groups: the first group of pregnant women over 35 years (37.8±0.48), the study group, was made up of 11 patients who have been included in the group with premature birth, and second group control from 35 to 37 years old (36.4±0.83) who had no complications in pregnancy.

The study has been approved in 20 June 2011, by the "Research ethics committee of State University of Medicine and Pharmacy *Nicolae Testemitanu"*, created by Senate Decision no. 6/1 of August 30, 2010 in accordance with the provisions of the National Legislation. An informed consent has been obtained from each subject at the beginning of the study.

Amniocentesis and blood samples: The intervention has been performed aseptically, transabdominal ultrasound-guided, 20 ml of amniotic fluid has been extracted first for the further diagnostic tests according to the cytogenetically screening, subsequently 10 ml of amniotic fluid has been removed and divided into 2 ml aliquots and stored at -48°C until being analyzed.

The venous blood has been collected after the amniocentesis procedure and drawn into the specimen tubes for serum extractions and tubes containing EDTA as anticoagulant for plasma extractions. The plasma and serum have been prepared by centrifugation, aliquoted and stored at -48°C until being analyzed.

Assay of serum and amniotic fluid cytokines IL-6, IL-8, IL-10, IL-12, SDF-1a, VEGF, concentrations have been measured by specific quantitatively affordable enzyme linked immunosorbent assay (ELISA) kits (PeproTech Inc., Minneapolis, USA.) according to the manufacturer's instructions. The assays have been carried out in flat-bottomed 96-well immunoplates (MaxiSorp, Nunc, Wiesbaden, Germany). The amniotic fluid and serum have been measured using BioTek's PowerWave HT microplate spectrophotometer. A standard curve has been made in parallel to each assay and the results have been converted into pg/mL.

Oxidative stress markers were also analyzed: AAT%-hexane, AAT-isopropyl, HPL-hexane-early, HPL-hexane-late, HPL-hexane-intermediate, HPL-isopr-early HPL, HPL-isopr-intermediate, DAM, NO, Histidine peptides Carnosine,

Catalase, ROS, G-GTP, Total proteins, Albumin, PPOA,Thiol groups of prot, Thyoredoxin, Glutaredoxin, AIM, GR, K, Ca, G-S-T, AGE, S-nitrosothiols, GPO, SH groups, He had dammed.

The analysis of the obtained data was performed on a personal computer using standard programs such as Microsoft Word 2010, Microsoft Excel 2010, Statistica 10. The qualitative indicators were analyzed using the non-parametric method - the calculation of the Chi-square test, which allows to evaluate the statistical significance of the differences between two or more relative measures and the Chi-square corrected by Yates for continuity.

Results

In a cohort of 65 pregnant women over 35 years old examined in the present study, 11 developed premature births.

Examining the biomarkers of both groups, up to 21 weeks of gestation, proved informative. Of all the cases investigated, the take-off premature birth were registered in the term after 35 weeks of gestation and resulted in premature birth.

No specific difference was found between the groups included in the study the stage of the second trimester of pregnancy related to the obstetric and anamnestic antecedents of pregnant women over 35 years.

Although many studies have shown that elevated levels of cytokines and oxidative stress may be associated with an increased risk of preterm birth in certain situations, such as intrauterine infections, cervical and amniotic sac inflammation, or complications such as preeclampsia.

In the blood serum taken from the pregnant women in the given study, who later developed premature birth, an insignificant increase of the following markers was attested: The level of cytokine biomarkers IL-6, from the blood of the pregnant woman (score 0.108, p = 0.742), IL-6, from the amniotic fluid (score 1.3930, p = 0.238), the level of IL-8, from the blood of the pregnant woman (score 0.018 , p = 0.892), IL-8, from the amniotic fluid (score 1.837, p= 0.175), IL-10 level, from the blood of the pregnant woman (score 0.594, p = 0.441). Nevertheless, there has been no difference between IL-10 levels in the AF in both groups, and the average value has been approximately equal. IL-10, from the amniotic fluid (score 3.418, p = 0.065), IL-12 level, from the blood of the pregnant woman (score 0.015, p = 0.903), IL-12, from the amniotic fluid, however, proved to be statistically informal (the score 4.229, p = 0.04) in the research group its level was found to be increasing.

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS - reactive molecules that include hydrogen peroxide, free oxygen radicals, and other similar molecules) and the ability of the body's antioxidant system to neutralize and repair these ROS.

Oxidative stress can damage fetal membranes, which are responsible for maintaining the integrity of the amniotic sac. If the membranes break prematurely, premature rupture of the membranes (water breaks) can occur and premature birth can occur. At the same time, oxidative stress can stimulate the release of inflammatory cytokines, such as interleukins, which can trigger inflammatory processes in the uterus. This inflammation can lead to premature uterine contractions and trigger premature labor.

The value of SDF-1α in the serum of the pregnant woman is statistically significant (value of 6.838 p =0.009) but SDF-1α in the amniotic fluid (value 3.001 p = 0.083), with the statistical value for the given study insignificant. Biomarker VEGF - in pregnant serum was not significantly different between the given groups (value 1.030 p = 0.310) and in the amniotic fluid it was not statistically informative either VEGF (value 1.762 p = 0.184).

This is why we also studied markers of oxidative stress to identify the possibility of predicting premature birth in the second trimester of pregnancy (Table 1).

Table 1. Statistical variable in the amniotic fluid and blood of pregnant women taken in the second trimester of pregnancy

	Value	Statistical significance	Value	Statistical significance
Researched marker	Serum of pregnant women		Amniotic fluid	
AAT%- hexane mM/s.l	2,116	p =0,146	0,466	p =0,495
AAT-isopropyl mM/s.l	0,640	p =0,424	4,093	p =0,043*
HPL-hexane-time uc/ml	0,046	p =0,830	8,564	p =0,003**
HPL-hexane-intermediate uc/ml	0,336	p =0,562	3,015	p =0,082
HPL-hexane-late uc/ml	0,001	p =0,975	0,942	p =0,332
HPL-isopr-time uc/ml	3,117	p =0,077	3,351	p =0,067
HPL-isopr-interm uc/ml	3,562	p =0,059	3,983	p =0,046*
HPL-isopr-late uc/ml	2,269	p =0,132	2,693	p =0,101
DAM μM/l	1,449	p =0,229	3,785	p =0,052
NO μM/l	2,892	p =0,089	0,599	p =0,439
Histidine peptides Carnosine μM/l	6,898	p =0,009**	0,005	p =0,944
Catalase μM/l	0,172	p =0,678	1,567	p =0,211
SOD u/c	1,402	p =0,236	2,647	p =0,104
G-GTP U/L	4,535	p =0,033*	3,829	p =0,050*
Prot all g/L	0,418	p =0,518	0,876	p =0,349
Albumin g/L	1,265	p =0,261	1,486	p =0,223
PPOA	1,306	p =0,253	0,063	p =0,802
Thiol groups of protein	0,777	p =0,378	2,375	p =0,123
Thyoredoxin	0,036	p =0,850	0,429	p =0,512
Glutaredoxin	2,360	p =0,124	1,597	p =0,206
GRM/s.L	0,523	p =0,469	4,829	p =0,028*
K mM/L	0,054	p =0,817	0,075	p =0,784
G-S-TμM/min. IT	0,024	p =0,876	0,163	p =0,686
AGE μg/L	1,117	p =0,291	0,747	p =0,387*
SH groups, mkM/g protein	9,019	p =0,003**	-	-

Note: AAT - total antioxidant activity; HPL - lipid hydroperoxides; DAM - malonic dialdehyde; NO - nitric oxide; SOD - superoxide dismutase; G-GTP - γ Glutamyltransferase; Prot – protein; PPOA - protein products of advanced oxidation; GR - glutathione reductase; K – Potassium; G-S-T - glutathione-S-transferase enzyme; AGE - advanced glycated end-products; SH groups - thiol group * - p <0,05, ** p <0,001**

A tendency to decrease IL-12 concentration (4.229, p=0.04) was found in the group with the risk of premature births. At the same time, SDF-1α biomarkers were also de-

creased in the serum of the pregnant woman, statistically significant (value of 6.838 p =0.009). Early hexane HPL in the amniotic fluid collected in the second trimester of pregnancy (8.564, p =0.003**), unlike AAT-isopropyl in the amniotic fluid whose value increased in the studied group (4.093 p =0.043*), HPL-isopropyl- intermediate also had a tendency to increase in amniotic fluid in the second trimester of pregnancy in pregnant women over 35 who were going to develop premature labor (3.983 p =0.046*). As well as the values of carnosine histidine peptides, from the single pregnant woman, G-GTP and from the pregnant woman's serum, and from the amniotic fluid sampled, GR and SH thiol groups from the sampled serum, with statistically significant values.

Discussion

The increased risk of preterm birth among older mothers is largely explained by early induction of labor for medical conditions. Our study contributes to the identification of variables, the testing of hypotheses and the attempt to predict certain particularities in the evolution of pregnancy, which may occur in pregnant women over 35 years old.

The study was based on a cohort of 65 pregnant women over 35 years of age who had indications for amniocentesis at 16-21 weeks of gestation.

The aim of the research was to confirm the hypothesis of the existence of a correlation between the risks of pregnancy evolution through premature birth and immune biochemical markers in pregnant women over 35 years old. Biochemical markers were collected from maternal serum and amniotic fluid during the second trimester of pregnancy.

In a series of researches, many authors have developed statistical predictive models of premature birth [9-11]. Other studies focus on clinical characteristics, which could be predictors in preterm birth [12-15].

Besides predicting the risk of premature birth, its prevention is of major importance, because it is very complex, it remains open for further research.

The variety of methods used to measure, for example, oxidative stress is great. Many authors such as Ferguson K., Gunko V. O., Abiaka C., Machado L. in their studies, determine biomarkers by different methods [16-18].

There are also reports of some studies on the importance of immune (interleukin), vascular (VEGF) markers in predicting the evolution of pregnancy with risk of premature birth [19, 20].

At the same time, all these methods are considered to be quite difficult, since oxidative stress biomarkers are very reactive and have very short half-lives. The reason it may appear both in our study and in other hypothesis-based studies of preterm birth risk prediction is that the results of the studies depend on the method used and how quickly these data are measured.

Validation of results identified in adjacent studies is difficult to determine due to the lack of a typical standard for establishing research methods [21].

The study analyzed the panel of markers related to maternal immune response in the second trimester of pregnancy, cytokines, angiogenesis and oxidative stress, in association with the evolution of preterm birth.

For this, samples were determined from the amniotic fluid collected from pregnant women over 35 years old in the second trimester of pregnancy and from the maternal serum, during the same period of time.

In amniotic fluid we obtained statistically significant increases in AAT-isopropyl, G-GTP, HPL-isopropyl.

In the serum of the pregnant woman, the increase in the concentration of carnosine-histidine peptides, G-GTP, GR and SH (thiol) groups was identified, and the decrease in the values of SDF-1α, HPL – hexane and IL-12 were statistically significant in the serum of the pregnant woman compared to that of amniotic fluid.

Identifying the values of biochemical mediators during pregnancy can be a method of predictive diagnosis.

Immunological and inflammatory factors evaluated in pregnant women over 35 years suggest their importance in predicting the occurrence of early miscarriages at the molecular level.

Carrying out research on the behavior of the values of immune mediators in human amniotic fluid and maternal plasma, in pregnancies that ended with premature births compared to physiological births, the aim was to understand the metabolism that takes place during the period of high-risk pregnancy. This allows to select markers, which will be able to be used as rapid tests to identify patients at risk of premature birth.

Currently in the Republic of Moldova, the diagnostic algorithm for patients with imminent or premature labor includes the test: detection of fetal fibronectin (fFN) - a glycoprotein present in the amniotic fluid, placental tissue, which has a predictive value in pregnant women who are at risk of imminent birth premature [22]. The positive prediction, however, is modest (< 20%), because most women have a good outcome because they are treated.

Precisely for these reasons, the World Health Organization (WHO) declares in research priorities during pregnancy, with the aim of reducing the rate of premature births, including epidemiological studies related to the identification of the causes of premature birth, understanding the mechanisms that lead to the initiation of labor, the development of simple screening tests, based on biological and genetic findings with the aim of identifying pregnant women at high risk of labour. Thus, the conducted study falls within the level of research, which presents the scientific value in reducing premature births [23].

Conclusions

This study demonstrates the involvement of immune biomarkers for pregnancies that ended through preterm birth by increasing the concentration of AAT-isopropyl, HPL-isopropyl and G-GTP in amniotic fluid, while decreasing the concentration of IL-12 in amniotic fluid. In the blood, the increased values of Carnosine Histidine Peptide, GR and SH in the serum of pregnant women, as well as the biomarker SDF-1α, in the second trimester of pregnancy.

The predictive value of these markers for pregnancies with risk of premature birth in pregnant women over 35 years of age is of interest for further research.

Abbreviations

AAT – total antioxidant activity; HPL – lipid hydroperoxides; DAM – malonic dialdehyde; NO – nitric oxide; SOD – superoxide dismutase; G-GTP – γ Glutamyltransferase; Prot – protein; PPOA - protein products of advanced oxidation; GR - glutathione reductase; K – Potassium; G-S-T – glutathione-S-transferase enzyme; AGE – advanced glycated end-products; SH groups - thiol group, AF – amniotic fluid; IL – interleukin; SDF-1α – Stromal cell-derived factor 1α.

Competing interests

None declared.

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