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BIOCHEMICAL MARKERS OF PREMATURE BIRTH

Kayumova G.M.
Bukhara State Medical Institute named after
Abu Ali ibn Sina, Bukhara, Republic of Uzbekistan

Premature birth remains one of the pressing problems of modern obstetrics, as it determines the level of perinatal mortality and morbidity [1]. Of no small importance are the psycho -social , economic, and demographic aspects of the problem of preterm pregnancy, as well as the frequency of this pathology, which over the past 20 years has not shown a tendency to decrease. All this indicates the need for a comprehensive study of the problem of premature birth and the search for new approaches to prediction. Unfortunately, anamnestic data and clinical manifestations do not always sufficiently allow timely prediction of premature birth. In this regard, researchers, based on pathogenetic mechanisms, propose using hormonal parameters— estriol in saliva [6], immunological parameters—cytokines in amniotic fluid and cervical mucus [7–9], and others— as markers of preterm birth . As is known, maternal, placental and fetal factors take part in the development of premature birth. Today, the role of the fetus in the initiation of labor, including premature labor, is beyond doubt. In this regard, it is of interest to study the diagnostic significance of determining markers of fetal origin in predicting preterm birth. For this purpose, we examined 132 pregnant women with a history of recurrent miscarriage (risk group for preterm birth) over the dynamics of the gestational period. Of these, 92 women experienced early termination of pregnancy at 22–37 weeks of gestation (main group). The comparison group included 40 women whose pregnancy ended in term birth. The control group consisted of 48 women with a physiological pregnancy. Of these, 30 women were examined in the first and second trimesters of pregnancy (termination of pregnancy for social reasons) and 18 women with a normal pregnancy at 37–40 weeks. Pregnant women underwent general clinical and special research methods, laboratory methods for determining myoglobin in peripheral blood and amniotic fluid, and determining fetal fibronectin in cervical contents. We selected the above biochemical parameters due to the fact that, according to the literature [2, 3], the role of a triggering factor in the onset of labor can be played by fetal myoglobin, which appears as a result of centralization of the fetal blood circulation, accompanied by a reduction in blood flow in its skeletal muscles. Fetal myoglobin, by stimulating the synthesis of prostaglandins by the fetal membranes, can contribute to the onset of labor. Fetal fibronectin is a protein of the extracellular matrix of fetal membranes, which is present in amniotic fluid and placental extracts; it is considered one of the informative biochemical markers determined in cervicovaginal contents. The material for laboratory testing of myoglobin was the mother's venous

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blood, amniotic fluid obtained by amniocentesis during a planned cesarean section and termination of pregnancy for social reasons (control group), directly during childbirth (main group). Determination of myoglobin in plasma and amniotic fluid of pregnant women was carried out by enzyme immunoassay using Cortez test systems Diagnostics MYOGLOBIN. For the quantitative determination of fetal fibronectin in the contents of the cervix, a test system was used (Adeza Biomedical Fetal Fibronectin Enzyme Immunoassay). Tests for fibronectin were taken from pregnant women with intact amniotic fluid at 22–35 weeks of pregnancy, since at these gestational periods during physiological pregnancy it is practically not detected in the cervical -vaginal contents (less than 50 μg/ml). The age of the women examined ranged from 21 to 39 years. All women examined lived in the same climatic and geographical conditions. The study of menstrual function showed that the average age at menarche in the main and comparative groups was significantly higher compared to the control ($p < 0.05$). In pregnant women with recurrent miscarriage, menstrual dysfunction was significantly more common compared to pregnant women in the control group (26.0% and 27.5% versus 6.3%). Menstrual dysfunction manifested itself in the form of irregular menstruation, algomenorrhea and hypomenstrual syndrome. The average duration of the menstrual cycle in the examined pregnant women did not differ significantly and averaged 27.0 ± 0.5 days in the main group; in the comparative group – 27.2 ± 0.5 days; in the control group – 26.8 ± 0.7 days. An analysis of the reproductive function of the examined pregnant women showed that in the comparative and main groups there were more first-time pregnant women among repeatedly pregnant women compared to the control group (38.0% and 37.5% versus 16.7%). The obstetric history of women with recurrent miscarriage is aggravated by the presence of medical abortions (30.4% and 27.5%), spontaneous miscarriages (53.3% and 42.5%), premature births (38.3% and 30.0%) . A study of the gynecological history showed that in pregnant women with recurrent miscarriage , inflammatory processes were more common, including from the appendages and body of the uterus, cervical erosion, and colpitis . Benign tumors (uterine fibroids and cervical canal polyps) occurred in women of the main and comparative groups. There was a history of infertility in 5.4% of women in the main group and 5.0% in the comparison group. In 1 case (1.1%) there was a saddle-shaped uterus (main group). Analysis of the somatic anamnesis revealed an unfavorable premorbid background in women with recurrent miscarriage . They were more likely to have extragenital diseases such as anemia, varicose veins, liver disease, endemic goiter, urinary tract diseases and various infections. Laboratory studies have shown that during physiological pregnancy, the level of myoglobin in the peripheral blood of pregnant women in the first trimester is 18.5±1.7 ng /ml; in the second trimester – 25.0 ± 2.5 ng /ml and in the third trimester – 34.8 ± 2.7 ng /ml. In the comparative group, the myoglobin content was, respectively, 32.4 ± 2.9 ng /ml;

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35.6 ± 2.7 ng /ml and 57.2 ± 3.4 ng /ml. In the main group, there was a significant increase in the level of myoglobin in the peripheral blood of pregnant women at all stages of gestation compared to the control and comparison groups ($p < 0.001$). The myoglobin content in amniotic fluid during premature birth was 125.0 ± 16.2 ng /ml in the second trimester and 111.8 ± 13.6 ng /ml in the third trimester of pregnancy. During physiological pregnancy, these indicators were respectively 20.6 ± 4.8 ng /ml and 39.5 ± 8.2 ng /ml. It is known that myoglobin (heme-containing protein with a molecular weight of 17100 D) is one of the key compounds that determine the high intensity of oxidative metabolism in skeletal muscle and especially in the myocardium [4]. It has the ability to bind to oxygen. The main function of myoglobin is the transport of oxygen from hemoglobin to the oxidase system of muscle cells and the maintenance of an optimal oxygen gradient near mitochondria [5]. Due to the lack of strong bonds with intracellular structures and its small molecular weight, myoglobin can quickly leave the muscle cell when it is damaged into the blood and be excreted by the kidneys in the urine. According to the literature [2,3], myoglobin in amniotic fluid is of fruit origin. Therefore, the increased content of myoglobin in the amniotic fluid that we established

in case of early termination of pregnancy, it may indicate intrauterine fetal hypoxia. Analysis of correlations between the content of myoglobin in plasma and amniotic fluid in pregnant women revealed a positive correlation ($r = 0.614$). Consequently, the myoglobin content in peripheral blood can indirectly judge the intrauterine state of the fetus. It should be noted that during the subsequent analysis of perinatal outcomes in preterm birth, the following pattern was established. Thus, when comparing myoglobin levels in the peripheral blood of pregnant women and the condition of the fetus at birth, it was found that severe asphyxia in newborns was accompanied by an increase in the level of myoglobin in the mother's blood by 1.3–1.5 times compared with similar parameters for mild asphyxia in newborns . In order to clarify the diagnostic significance of the determination of myoglobin, a transgression analysis of the level of this biochemical marker in the peripheral blood of pregnant women of the main and comparative groups was carried out. The analysis showed that the transgression of the distribution series of myoglobin indicators in the first trimester of pregnancy is high (Tr = 85.9%), while in the second trimester it is relatively low (Tr = 49.15%). Therefore, determining the level of myoglobin in the peripheral blood of pregnant women in the second trimester is more significant compared to the first trimester of pregnancy for predicting preterm birth. It was found that when myoglobin levels are more than 69.75 ng /ml (2.0 times higher than in the comparative group and 2.8 times higher than in the control group), it is possible to judge the development of premature birth with an accuracy of 91.3%. To establish the diagnostic value of determining fetal fibronectin , an analysis was taken from the contents of the cervix in 92 out of 132 pregnant women with recurrent miscarriage . 52 women subsequently

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experienced premature birth, and therefore were included in the main group. In 40 women, pregnancy ended in term birth; they were assigned to the comparison group. In 25 women at risk for preterm birth, a study for the presence of fibronectin was carried out without clinical symptoms of threatened preterm birth; in 67 pregnant women, signs of threatened miscarriage were noted.

When collecting the analysis, samples from women with bleeding were not used, as this could lead to false-positive results; collection was also carried out before any manipulations and examinations of the vagina, which could provoke the release of fetal fibronectin from the membranes. We carried out a quantitative analysis for the presence of this biochemical marker. The result was considered negative if the concentration of fetal fibronectin was less than 50 μg/ml. With higher values, this test was considered positive. The analysis found that the content of fetal fibronectin in cases of threatened premature birth is 0.19 ± 0.02 μg/ml; and when starting – 1.27 ± 0.09 μg/ml. It has been established that when fetal fibronectin levels exceed 0.35 μg/ml, the development of premature birth can be judged with an accuracy of 90.6%. It should be noted that there were 5 false-positive tests for the presence of fibronectin in the contents of the cervix and 1 false-negative test. False-positive tests were associated with the presence of bacterial vaginosis in the pregnant women examined. The predictive value for a positive test was 91.1%; and for negative – 97.2%. It should be said that various authors [10] discuss the issue of possible mechanisms for the appearance of fetal fibronectin in the cervicovaginal contents. It is believed that the chorionic trophoblast in the extracellular matrix is an important source of fibronectin in cervico -vaginal secretions. Due to the fact that fetal fibronectin is expressed predominantly in the area of the lower segment; two possible ways of its appearance in the cervico -vaginal secretion are suggested. 1st way - as a result of an increase in the tone and contractility of the uterus, mechanical stress increases, changes occur in the cervix, separation of the choriodecidua , which leads to the loss of fetal fibronectin from its surface and the entry of the protein of the extracellular matrix of the fetal membranes into the cervicovaginal secretion. 2nd way - a bacterial infection ascends into the decidua , an inflammatory reaction develops, bacteria and leukocyte protease destroy the decidua and chorionic extracellular matrix, as a result of which fibronectin appears in the vagina. The same inflammatory process ensures a local release of cytokines and prostaglandins, premature ripening of the cervix occurs, and labor pains begin. Thus, determination of the level of myoglobin in peripheral blood in the second trimester of pregnancy and the concentration of fetal fibronectin in the contents of the cervix from 22 weeks of pregnancy can be used as biochemical markers of preterm birth.

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