Detection of PAMG-1 oncoantigen using nanogold conjugates with monoclonal antibodies in samples of biological fluids.

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Abstract

Invested the diagnostic value of the determination of free IGF protein PAMG-1 in the diagnosis of rupture of membranes in pregnant women. For this purpose was developed by a rapid test, based on conjugates of nanogolds withmonoclonal antibodies to specific epitopes of the IGFBP-1. It is shown that the specificity of the test was 100%, sensitivity 98.2%, positive predictive value of 100%, positive predictive value, negative 99.2%.

Keywords: PAMG-1 oncoantigen using nanogold conjugates with monoclonal antibodies

Rupture of the fetal membrane of the human fetus occurring before the birth process is called premature rupture of the amniotic membrane (PRAO). This complication of pregnancy is observed in about 10% of pregnancies. In the absence of appropriate treatment, PRRAO poses a serious risk to the life of the fetus and the mother. In regions where Prao is not diagnosed, IT causes 10% of perinatal mortality[1]. With small tears of the amniotic membrane, the leakage of the fetal waters is poorly determined not only by patients, but also by medical personnel during examination in mirrors, especially in the case of a high location of the amniotic membrane tear. In such cases, infection of the fetus occurs in almost 100% of cases[2].

Laboratory methods for the diagnosis of PRA [3] are quite numerous, but largely ineffective because of the large number of false-positive and false-negative results. Such tests are nitrogenous test, whose sensitivity 70%, accuracy 90% and a specificity of 97%., a fern test whose sensitivity is 70% and accuracy is 93%[4]. Therefore, immunochemical tests have been proposed, both immunoenzyme[5] and immunochromatographic [6, 16, 17], on the basis of markers of amniotic fluid, such as alfafetoprotein, prolactin, chorionic gonadotropin and some others.

The most promising tests for such diagnostics are tests based on the IGFBP-1 protein determination. This protein binding the growth factor of IGF and performing the function of its temporary deposition was discovered by D. Petrunin [7] as a protein of amniotic fluid. In connection with its function, IGFBP-1 plays a significant role in regulating the growth processes of the fetus and, due to this, accumulates in significant amounts, which are 2-4 orders of magnitude higher than its concentration in the blood of pregnant women of the corresponding gestation period. The test for the leaking of fruit water, called PROM, firm OYMEDIXBIOCHEMICA, Finland, was proposed by the group under the leadership of E. M. Rutanen [8]. However, in cases of bleeding, the result of this test may not be reliable, since the blood obtained directly from the placental site has a higher IGPBP-1 content than the blood of the cervical blood vessels. If used to detect IGFBP-1 enzyme immunoassay test is not applicable at home, as well as in small medical institutions and in the offices of private doctors in the process of admission.

We have proposed testnaya the basis of monoclonal antibodies free of IGFбелкуIGFBP-1, kongugirovannah with nanocolloidal gold, with a diameter of 30 nm, as the concentration of free PAMG-1 in the blood of pregnant women second and third trimester is significantly lower than the General PAMG and blood impurities in the sample will not significantly affect the appearance of false positive results.

Materials and methods:

Monoclonal antibodies against the free PAMG-1 [9], these conjugates of antibody with nanogold and the mouse IgG standards free PAMG-1 was obtained from OOO “Nano-lab”, Russia. Nitrocellulose membranes, fiberglass matrices, lavsan self-adhesive films are obtained from MDI, India. Samples of vaginal contents of pregnant women with rupture and without rupture of the fetal bladder were obtained from firms “Virol” (Ukraine) And the network of clinics “MSCH 03” (Russia). The application of the chromatograms was performed using the setup EasyPrintermodelLPM-02. (MDI, India). Immunochromatography quantification was performed using a complex Expert-lab (Russia). Statistical processing was carried out using the Exel program, $p<0.05$ was considered as the criterion of reliability.

To determine the free PAMG-1, immunochromatographic strips were collected. (Figure 1)

Fig 1. Scheme of Assembly of immunochromatographic test based on nanocolloid gold to detect free PAMG-1. A-side view, B — front projection. 10,12,14,16, 18,22,24,26,30.

Conjugate of nanocolloidal gold (Fig.1) with monoclonal antibodies to one epitope, PAMG-1 was applied to zone 10, located on a fiberglass Pad that does not Sorb the protein. Antibodies were products of mouse hybridoma. On the nitrocellulose membrane (22) nanodimensional antibodies to drugambien-1 (14)closer to the Pad (test area) and, in parallel, at a distance of 2-3mm affine monospecific antibodies to mouse immunoglobulins (control area)(16). The reaction products formed in the Pad and on the nitrocellulose membrane in the process of lateral movement fall into the suction filter (24), which is a special filter paper. The strip surface was covered with protective films (28.30). On the film (28) there are arrows (18), which show the direction and depth of the strip immersion in the sample. All products were mounted on a rigid plastic base (26), which has a special adhesive coating. The study of nanozolote particle size was carried out by emission electron microscopy using electron microscope Libra 200FE by Carl Zesis Group.

To obtain the material studied by immunochromatography, a Dacron probe was inserted into the vagina for one minute on the strips collected as described in the materials and methods. The probe was then immersed in a buffer solution containing 0.05 M PBS pH 7.2 and 0.02% sodium azide. The probe is rotated in the Eppendorf tube containing 0.4 ml buffer for one minute, then the probe was removed and the strip pad was immersed in the tube. The sample begins to migrate under the action of capillary forces in the pad, then in nitrocellulose and suction filter. If the sample enters the zone (10), the liquid part of the sample dissolves the conjugate and binds PAMG-1 to the monoclonal antibody of the conjugate. Further, the liquid migrates to the nitrocellulose membrane, where in the presence of a sufficient amount of PAMG-1 in the sample, a complex of conjugate — PAMG-1 — immobilized antibody is formed. Due to the presence of nanozolote in the conjugate, colored due to the effect of plasmon resonance in red and yellow, a band with a high concentration of nanozolote is formed, which visualizes the presence of PAMG-1 in the sample. In the control zone, the uncoupled conjugate is captured by antibodies against mouse immunoglobulin and forms a control strip, which indicates the serviceability of the test and the completeness of the reaction.

Fig2. Electronic microphotography of nanozolote conjugate with monoclonal antibodies against IGF-free PAMG-1 protein. Increase x10000 times.

The sensitivity of the test was chosen in such a way that 10 minutes after the start of the production, it was visually possible to determine the presence of at least 5 ng of free PAMG-1 in the sample.

In addition, for quantitative research, was chosen a test with a sensitivity of 60 PCG/ml. With the help of this option was studied the amount of free PAMG-1 in vaginal secretion of pregnant women 29-38 weeks of gestation. 16 samples of vaginal contents of non-pregnant women and 17 samples from pregnant women without PRA were investigated. These test sensitivity values were chosen based on the results

of our previously published data obtained in enzyme immunoassay [10].It was shown that during pregnancy the concentration of PAMG-1 in the vagina does not exceed 5 nanograms [11]. The result of the study is shown in Fig.2. The figure shows that significant differences between the concentration of free PAMG-1 in the vagina of non-pregnant and pregnant women without rupture of the fetal bladder are not significant. Statistical analysis showed that such differences are statistically unreliable P< 0.012.Fig2. Electronic microphotography of nanozolote conjugate with monoclonal antibodies against IGF-free PAMG-1 protein. Increase x10000 times.

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Comparison of the concentration of free PAMG-1 in the vagina of pregnant women without rupture of the amniotic bladder and non-pregnant women.

Rice.2 comparison of the concentration of free PAMG-1 in the vagina of pregnant women (2) without rupture of the amniotic bladder and non-pregnant women (1)

With the help of the test, the sensitivity of which was established in 5 ng/ml of free PAMG-1, the vaginal contents of 176 patients were examined, some of whom were diagnosed with PRAO. The number of patients is 176. The test was evaluated visually 10 minutes after the start of the production (table 1).

Table. 1. Comparison of the test to detect IGF-free protein PAMG-1 with clinical confirmation of rupture of the fetal bladder.

The test parameters were calculated using the following formulas:

Sensitivity = a / a+c=176/(176+0)=100%

Specificity = d/(b+d)=206/2+206=99%

a is the number of observed true positive cases

b-number of false negative cases observed

c — number of false positive cases observed

d is the number of truly negative cases observed

Thus, a sensitive specific test for the diagnosis of PRA is proposed. The test parameters are superior to the described in the literature nitrogenous[12], fern [13], PROM [14] and Amnisure [15] tests.

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