New Approaches to Molecular Diagnostics of Prenatal Pathology

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Analysis of the spectrum of amniotic fluid proteins in physiological and abnormal pregnancy using proteomic analysis allowed detection of a number of difference proteins, that are absent or, alternatively, appear in gestosis. Among absent proteins, there were NADPH-dependent carbonyl reductase, epidermal fatty acid-binding protein, haptoglobin, calgranulins A and B. In contrast to proteomic spectrum of amniotic fluid in physiological pregnancy, 7 new proteins appear during gestosis, 3 of them were identified: C area of immunoglobulin K-chain, breast cancer metastasis suppressor-1, and protein-1 containing AIG2-like domain. Possible effects of revealed differences in proteomic spectrum on development of main disturbances during gestosis are discussed. Difference proteins detected in amniotic fluid may serve as gestosis markers.

Key Words: *proteomic analysis; difference proteins; amniotic fluid; gestosis*

Recent decade was characterized by intensive development of modern biomedical technologies providing new insight into causes of gestation complications. These technologies include proteomic studies providing information on protein repertoire of the studied object and helping to elucidate previously unknown mechanisms of pathology development [1]. Gestosis is an important obstetric condition leading to prenatal pathology. Genesis of gestosis remains poorly studied [2,3]. At the same time, disturbances in the expression of proteins playing the important role in all processes in the cell at the molecular level can serve as a trigger for complicated train of disturbances resulting in this pregnancy complication. However, the data concerning proteomic profile in gestosis are scanty and controversial [12,15].

Amniotic fluid (AF) is an informative object for proteomic investigations in pregnancy; AF proteins have both maternal and fetoplacental origin, which allows comprehensive assessment of the imbalance in the mother–placenta–fetus system and detection of protein markers of prenatal pathology.

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The objective of this work was to investigate proteomic spectra of AF in physiological pregnancy and in pregnancy complicated by gestosis.

MATERIALS AND METHODS

We examined 32 women at the age of 23-33 years, 20 of them had uncomplicated pregnancy and parturition and in 12 women pregnancy was complicated by late gestosis. AF obtained from examined patients during the parturition (weeks 39-40) served as material for analysis.

Total protein content in AF was measured after Bradford. The proteins were fractionated using two-dimensional polyacrylamide gel electrophoresis (PAAG) [9]. Protein load 200 μg/gel was used to obtain analytical gel. Isoelectric focusing was carried out on IPG-strips (pH 3-10) using Protein IEF Cell device (Bio-Rad). Separation at the second direction was carried out in vertically 8-16% PAAG gradient on Protean II xi Multi-Cell device (Bio-Rad).

Upon completion of two-dimensional electrophoresis, to visualize protein spots, phoregramms were stained with silver nitrate, scanned, and analyzed using PDQuest software package (Bio-Rad). The spots were

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cut out from the gel, treated with trypsin [13], and protein were identified using time-of-flight mass spectrometry (MALDI-TOF-MS) on Autoflex II (Bruker) mass spectrometer, Mascot MS Search software (Matrix Science), and NCBI and Swiss-Prot data bases.

Comparative analysis of proteomic maps was carried out using virtual integrated "master gels" of two-dimensional electrophoregramms (PDQuest software) of AF during normal pregnancy and pregnancy complicated by gestosis.

RESULTS

Proteomic profile of AF is characterized by substantial heterogeneity (~100 electrophoretic fractions in molecular weight range of 12-80 kDa), moreover, most proteins are detected in both physiological and complicated pregnancies (Fig. 1). At the same time, a number of difference proteins was detected; the presence or absence of these proteins depended on pregnancy course (Table 1).

Amniotic fluid from women with gestosis, in contrast to that in physiological pregnancy, lacks 10 proteins with isoelectric points in the range 5.2 to 6.4 and molecular weight 13-33 kDa, 5 of which were identified. They included NADPH-dependent carbonyl reductase involved in regulation of oxidation-reduction reactions [11], fatty acid-binding epidermal protein participating in regulation of cell differentiation and transmembrane processes [6], non-enzyme antioxidant haptoglobin, and calgranulins A (protein S100-A8) and B (protein S100-A9). Two latter proteins control cell cycle phase shift. In addition, calgranulin effects are associated with providing normal intensity of arachidonic acid cascade and, consequently, of prostaglandin synthesis [10].

Lack of proteins with various regulating functions in AF can be explained by disturbances in their production primarily in the placenta, and may be of importance in the development of main functional manifestations of gestosis.

Proteins not expressed in normal pregnancy also play an important role. Our investigations showed that AF in gestosis contains 7 additional proteins with isoelectric points of 5.8 to 6.6 and molecular weights 14-32 kDa, among which 4 proteins were not identified and 3 proteins were identified: C-area of immunoglobulin K-chain, breast cancer metastasis suppressor 1 (BRMS1), and protein 1 containing AIG2-like domain. BRMS1 is known to suppress gene of nuclear factor NF-κB [7], which plays an important role in cell proliferation, apoptosis, generation of cytokines and their receptors, NO and other free radical molecules, which is associated with regulation of gene expression involved in these processes [5].

NF-κB-signal pathway is of great importance in embryonic period of ontogeny [14]. First of all, it is important in the placenta development, trophoblast invasion into endometrium, and gestational remodeling of spiral arteries. Trophoblast (particularly villous), similarly to tumor cells possess high migration, proliferation, and invasion capacities, but unlike mentioned

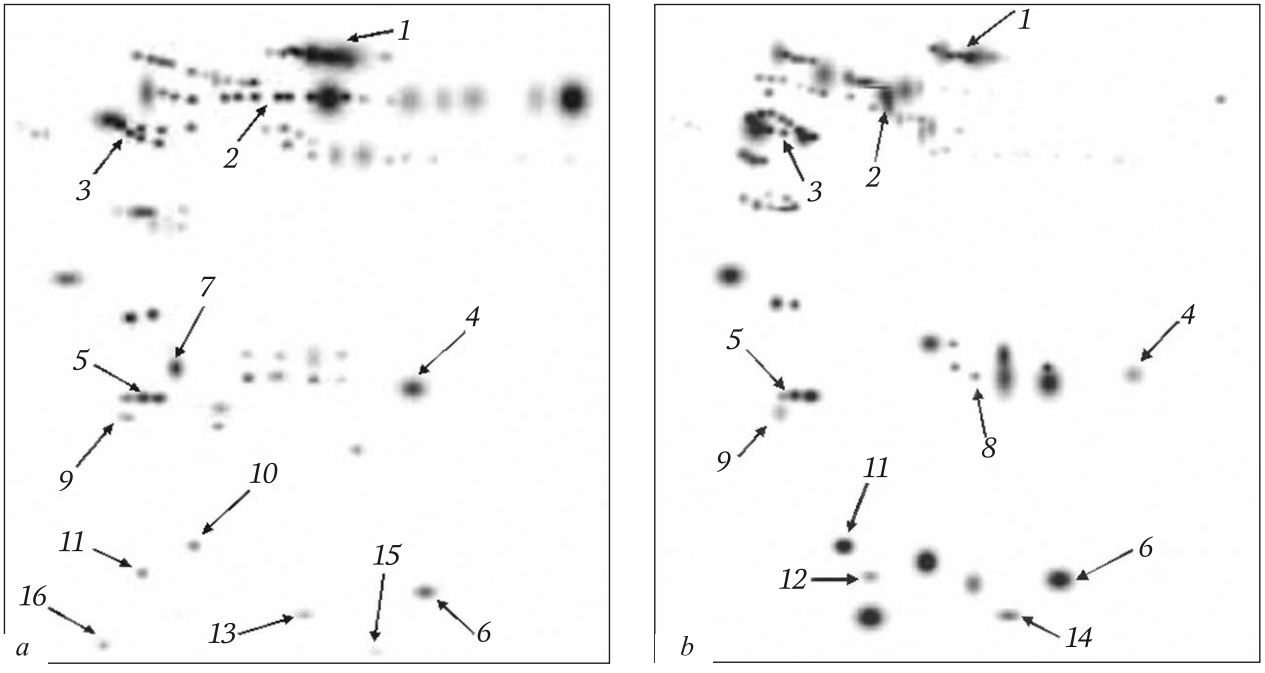

Fig. 1. Proteomic maps ("master-gels") of amniotic fluid in physiological pregnancy (*a*) and gestosis (*б*). Protein numeration corresponds to that in Table 1.

TABLE 1. Amniotic Fluid Proteins Identified in Physiological Pregnancy and in Gestosis

No.	Protein name	No. in Swiss-Prot database	Molecular weight, kDa	Isoelectric point	Physiological pregnancy	Gestosis
1	Serotransferrin	P02787	77	6.24	+	+
2	Serum albumin	P02768	69.3	5.9	+	+
3	Alpha-1-antitrypsin	P01009	46.7	5.3	+	+
4	HLA class II histocompatibility antigen gamma chain	P04441	32.3	6.83	+	+
5	Apolipoprotein A-I	P02647	30.8	5.5	+	+
6	Hemoglobin subunit beta	P68871	15.9	6.9	+	+
7	Carbonyl reductase [NADPH] 3	O75828	32.3	5.57	+	-
8	Breast cancer metastasis suppressor 1	Q9HCU9	31.75	6.13	-	+
9	Peroxiredoxin-2	P32119	28.9	5.3	+	+
10	Haptoglobin	P00738	18.6	5.84	+	-
11	Transthyretin	P02766	17	5.38	+	+
12	AIG2-like domain-containing protein 1	Q9BVM4	16.1	5.83	-	+
13	Fatty acid-binding protein, epidermal	Q01469	14.7	6.18	+	-
14	Ig kappa chain C region	P01834	14	6.62	-	+
15	Protein S100-A9	P06702	13.2	5.2	+	-
16	Protein S100-A8	P05109	12.9	6.61	+	-

Note. "+" – presence of the protein, "-" – absence of the protein.

cells trophoblast invasion under physiological conditions is a strictly controlled process. Disturbances in controlling mechanisms, particularly observed modification in BRMS1 expression, apparently, will promote intensification of apoptosis, accumulation of active oxygen forms, resulting eventually in oxidative stress, imbalance of pro- and antiinflamatory cytokines, with subsequent development of inflammatory response.

Crucial importance in gestosis development is now assigned to the above-mentioned disturbances [4]. Prolongation of the mentioned processes against the background of oxidative stress may result in apone-crosis, intermediate type of cell death with signs of both apoptosis and necrosis [8] associated with damage to trophoblast biomembranes and release of cell components, including toxic and proinflamatory ones. Detection of membrane-bound protein with AIG2-like domain in gestosis AF supports the fact of cellular membrane damage. The absence of epidermal fatty acid-binding protein in gestosis apparently plays a role in disturbances of transmembrane processes. Possible release of proinflammatory cytokines and free-radical compounds may result in damage to endothelium and its dysfunction typical for this pathology [2,3].

Thus, our findings suggest that gestosis develops against the background of changed production of some regulatory proteins. Since these proteins realize cell informational program, the data we obtained using proteomic analysis extent our knowledge concerning the molecular mechanisms of gestosis, which still occupies one of the first places in the structure of maternal and perinatal morbidity. Identified difference proteins in amniotic fluid may be used as informative gestosis markers.

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