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Regular Article

Activation contact system (ACS) and tissue factor (TF) in human amniotic fluid: Measurements of ACS components and TF, and some implications on the pathophysiology of amniotic fluid embolism

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ABSTRACT

Background/Aim: It is believed that the amniotic fluid-derived TF, in the case of amniotic fluid embolism(AFE), contributes to acute disseminated intravascular coagulation (DIC) and obstetric shock in the mother. However, the role of amniotic fluid-derived contact phase coagulation factors that irrupt into the bloodstream simultaneously with TF is still unknown. Our study objective was to identify and measure the concentrations of CAS components and TF in amniotic fluid.

Material and methods: The study group consisted of 30 healthy parturients with uneventful pregnancy and birth. Amniotic fluid (AF) and maternal blood were sampled at the end of the first stage of labor. The components of ACS, i.e. factors XII and XI (FXII, FXI), prekallikrein (PK), high molecular weight kininogen (HMWK), and tissue factor (TF) were measured by immunoenzymatic method (ELISA).

Results: All ACS components were detected in AF; their levels were higher in AF than in the maternal plasma: FXII - 29.17 ng/mg protein vs. 0.94 ng/mg protein (medians); FXI - 27.28 ng/mg protein vs. 0.92 ng/mg protein (medians); PK - 88442.04 ng/mg protein vs. 113.44 ng/mg protein (medians); HMWK - 4253.82 ng/mg protein vs. 2857.96 ng/mg protein (medians). The concentration of TF in amniotic fluid was 39.46 pg/mg protein (median) vs. 0.41 pg/mg protein (median) in blood plasma.

Conclusions: 1.The ACS components, i.e. FXII, FXI, PK and HMWK, are the constituents of amniotic fluid. 2.The concentrations of the amniotic fluid-derived factors having a coagulation initiation potential, i.e. TF and contact phase coagulation factors, are higher in amniotic fluid than in mother's blood plasma.

Introduction

The activation contact system (ACS) is made up of four proteins that have been long known as the coagulation system components. They are called contact factors and include factor XII (FXII), factor XI (FXI) and prekallikrein (PK) which are serine proenzymes, and high molecular weight kininogen (HMWK), being a cofactor protein [1–7]). It is now clear that ACS starts not only procoagulant reactions, but also the proinflammatory ones, via the intrinsic pathway of coagulation or the kallikrein-kinin system, respectively. Additionally, the crosstalk between ACS and renin-angiotensin system [1], fibrinolytic system and some others contributes to the multilayered character of the system [1,8].

According to the waterfall cascade hypothesis, there are two pathways of blood coagulation: extrinsic (TF-dependent) and intrinsic pathways that are triggered by TF/VIIa complex and CAS, respectively; the two pathways converge to form thrombin, a serine coagulation enzyme. TF-dependent coagulation is deemed to be the major coagulation route, ensuring body hemostasis in the cooperation of plasmatic coagulation factors with blood platelets and vascular wall components.

CAS activation starts with FXII activation, which *in vivo* occurs in two circumstances: (i) in endothelial injury and when subendothelial matrix proteins, mainly negatively charged collagen and laminin, are exposed to FXII; (ii) or when FXII gets in contact with surface active blood-foreign materials. Then, FXII autoactivates upon exposure to negatively charged surfaces and is converted to the enzyme FXIIa. The enzyme activates a cascade of a few proteolytic reactions [2], including FXI conversion to FXIa, which in turn change inactive FIX to the active form FIXa, becoming part of the intrinsic tenase complex (factors VIIIa + IXa + X) to form Xa, a serine enzyme that transforms prothrombin into thrombin. Thus, FIXa as a link between the intrinsic and

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extrinsic pathways of coagulation is a product of procoagulological activity of CAS [7].

TF (formerly called tissue thromboplastin) has been the most frequently investigated coagulation factor of the amniotic fluid with regard to its role in amniotic fluid embolism (AFE). However, the involvement of ACS in AFE has not been considered. The current study objective was (i) to identify and measure the concentrations of all the four CAS components in amniotic fluid,(ii) to measure TF and (iii) to compare the results with those for mother's blood plasma.

Material and Methods

Patients

The study group consisted of 30 healthy parturient women at term (20 primiparas and 10 multiparas) with singletons (29 cases) or twins (1 case), aged 26 ± 5.3, with physiological course of pregnancy, who gave natural birth (26 births) or who had cesarean section due to obstetric emergency (4 births). Cases with diabetes, preeclampsia, pregnancy induced hypertension (PIH), infection during pregnancy or patients taking any medications except for folic acid, vitamins and minerals, were excluded.

The control group consisted of 10 non-pregnant healthy women, with no medical history, not on medication, in luteal phase, matched in age with the study group.

Ethical Issues

All the patients were informed about the research and they gave informed consent for sampling of the material. The study was approved by the Bioethics Committee.

Collection of Amniotic Fluid and Blood

Plastic syringes containing 0.5 ml of 3.2% sodium citrate and 20-gauge needles with a vacutainer system were used. A sample of amniotic fluid (4.5 ml) and a sample of venous blood (4.5 ml) were obtained from each patient. The antecubital vein was punctured without any tourniquet. Amniotic fluid was obtained in two ways: a/during cesarean section procedure (4 cases) - after cutting the uterine muscle (lower segment, transverse incision), visualizing the amniotic sac and rinsing its surface with 0.9% NaCl, amniotic fluid was obtained by puncturing fetal membranes under optical control; b/ during natural labor (26 cases) – in advanced first stage (endocervical canal dilation of approximately 8 cm) the lower pole of the amniotic sac was visualized in speculum, rinsed with 0.9% NaCl; amniotic fluid was obtained by puncturing fetal membranes under optical control. The samples of amniotic fluid or blood were transferred to plastic test-tubes and then taken for centrifugation (+4 °C, 1,500 g, 15 min.). The supernatants were decanted and placed in 0.2 ml portions, which were then stored at -70 °C for 3-4 weeks. The samples were defrosted at a room temperature directly before the analysis (thawed specimens were never refrozen).

Statistical analysis confirmed that the results were not correlated with the mode of amniotic fluid collection.

Assays and Reagents

- The coagulation factors XII, XI, prekalikrein (PK) and high molecular weight kininigen (HMWK) as well as tissue factor (TF) were measured by ELISA method (Immunosorbent Assay Kits for coagulation factors XI,XII and PK and HMWK by USCNK Life Science Inc. and Zymutest TF kit by Hyphen BioMed, Enzyme-linked, respectively).
- BCA (bicinchoninic acid assay) method was used to measure the level of total protein.

Statistical Analysis

The statistical analysis was conducted using STATISTICA 8.0 (StatSoft). The distribution of the parameters examined differed from normal (Shapiro-Wilk's test). Their variability was expressed as the median and quartiles (lower, Q 25 and upper, Q 75). The Wilcoxon test was applied to analyze the differences in the parameters between cord blood and maternal blood, whereas the U Mann-Whitney test to investigate the differences between the study groups. Correlation analysis was performed using Spearman correlation coefficient. The p value of 0.05 was considered statistically significant.

Results

Total Protein Concentration

Total protein concentration was 5.21 mg/ml (median) in amniotic fluid, ranging from 4.16 to 5.99 mg/ml, and 78.53 mg/ml (median) in blood plasma, ranging from 73.22 to 83.74 mg/ ml.

Factor XII

The concentration of FXII in amniotic fluid was 29.17 ng/mg protein (median), ranging between 19.56 and 39.14 ng/mg protein, while in blood plasma of parturient women 0.94 ng/mg protein (median), ranging from 0.66 to 1.86 ng/mg protein. The difference between the levels in amniotic fluid and blood plasma was statistically significant (comparison of medians, $p \leq 0.001$) (Fig. 1a).

Factor XI

The concentration of FXI in amniotic fluid was 27.28 ng/mg protein (median), ranging between 0.41 and 156.58 ng/mg protein. In blood plasma of parturient women it was 0.92 ng/mg protein (median), ranging from 0.03 to 10.44 ng/mg protein. The difference between the levels in amniotic fluid and blood plasma was statistically significant (comparison of medians, $p \leq 0.01$) (Fig. 1a).

Prekallikrein (PK)

The concentration of PK in amniotic fluid was 88442.04 ng/mg protein (median), ranging between 72668.11 and 104935.95 ng/mg protein, while that in blood plasma 113.44 ng/mg protein (median), ranging from 79.94 to 146.70 ng/mg protein. The difference between the levels in amniotic fluid and blood plasma was statistically significant (comparison of medians, $p \leq 0.001$) (Fig. 1b).

High Molecular Weight Kininogen (HMWK)

The concentration of *HMWK* in amniotic fluid was 4253.82 ng/mg protein (median), ranging between 1936.40 and 9907.80 ng/mg protein, whereas in blood plasma 2857.96 ng/mg protein (median), ranging between 2541.57 and 3161.04 ng/mg protein. The difference between the levels in amniotic fluid and blood plasma was statistically insignificant (comparison of medians, $p > 0.05$) (Fig. 1b).

Tissue Factor (TF)

The concentration of TF in amniotic fluid was 39.46 pg/mg protein (median), ranging between 21.67 and 66.26 pg/mg protein, while that in blood plasma 0.41 pg/mg protein (median), ranging from 0.29 to 0.50 pg/mg protein. The difference between the levels in amniotic fluid and blood plasma was statistically significant (comparison of medians, $p \leq 0.001$) (Fig. 2).