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Thrombin activatable fibrinolysis inhibitor (TAFI) in cord blood

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Abstract: Thrombin activatable fibrinolysis inhibitor (TAFI) is a plasma zymogene (procarboxypeptidase B) which can decrease fibrinolysis and thus act as a haemostatic factor. TAFI is now extensively studied in many complications as well as in physiological and complicated pregnancy. The question we posed in the present study was whether TAFI antigen is present in cord blood plasma. The study group consisted of 38 parturient women, 26 primiparous and 12 multiparous with normal course of pregnancy and delivery. The cord blood was sampled from the cord vein, and the mother's blood from the antecubital vein. 3.2% sodium citrate was used as an anticoagulant. TAFIa/ai antigen was measured by ELISA method. TAFIa/ai antigen was identified in all samples of cord blood plasma. Its level was 91.50 ng/ml (range: 71.76 - 160.77 ng/ml) vs. 55.46 ng/ml (range: 39.77 - 68.54 ng/ml) in the mother's blood, which means that the level of TAFIa/ai antigen was significantly higher in fetal blood than in maternal blood ($p<0.00001$). TAFIa/ai antigen is an integral component of cord blood plasma. The concentration of TAFIa/ai antigen is about two times higher in fetal blood than in maternal blood.

Key words: TAFI - cord blood - fibrinolysis

Introduction

Thrombin Activatable Fibrinolysis Inhibitor - TAFI, also called procarboxypeptidase B or carboxypeptidase U and carboxypeptidase R, is formed in the liver as a unichain glycoprotein of molecular weight of 60 000 Da and circulates in the blood in the form of zymogene (pro-TAFI). It is activated by the thrombin/thrombomodulin complex, functioning on the walls of vessels (thrombomodulin exposed on the endothelium is a receptor of thrombin and forms complexes with it) [Fig. 1]. Activated pro-TAFI, that is TAFIa (m.w. 35 000 Da), is a proteolytic enzyme which changes the fibrin structure in such a way that it becomes less susceptible to fibrinolysis. This phenomenon consists in splitting off of C-final lysine and arginine which decreases adsorption of tPA (tissue plasminogen activator) and plasminogen on fibrin, leading in consequence to a decrease of plasmin generation and thus to prolongation of fibrinolysis [4,17]. TAFI is treated as a link connecting coagulation with fibrinolysis [5]. Anti-inflammatory effect is another property of TAFI [6]. TAFI has beneficial influence on hemostasis, which is supposed to be an effect of antifibrinolytic activity; this has been observed during therapeutic application of recombined factor VIIa in hemophilia [2,11]. Increased level of TAFI antigen was described for many diseases. The increase of the level of TAFI and of fibrinolysis inhibitor of type PAI-1 in the coronary disease create together a high risk of cardiac infarction [9,12]. In hepatocirrhosis the levels of TAFI, antithrombin and alpha-2-antiplasmin go down, which is explained by weaker synthesis [10,15]. In disseminated intravascular coagulation (DIC), TAFI level is lower, which can be a consequence of consumption [18]. A lower level of TAFI antigen was observed in women in severe preeclampsia and in intrauterine growth retardation syndrome (IUGR) [1]. But nothing is known about TAFI in the fetal blood. The aim of the present study was to find out whether and at what level TAFI antigen appears in cord blood.

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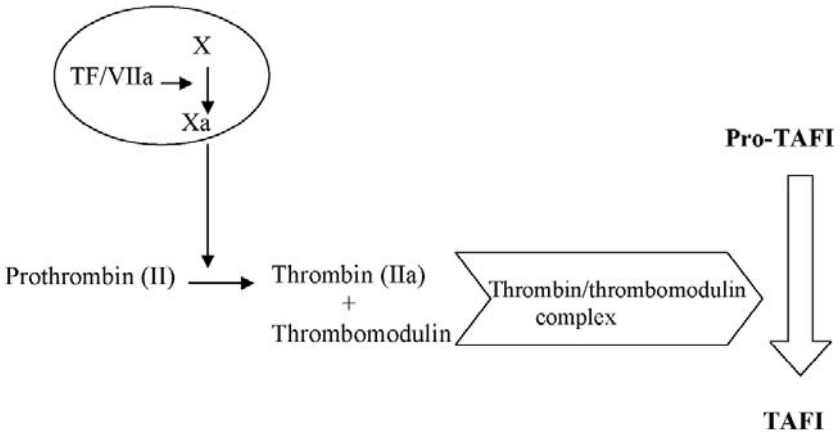

Fig. 1. The mechanism of procarboxypeptidase B activation by thrombin/antithrombin complex. * on the left side - thrombin formation, and on the right side - TAFI formation, * TF - tissue factor, ** TAFI - thrombin activatable fibrinolysis inhibitor

Materials and method

Patients. The study groups consisted of 38 parturient women, at the age of 21.3±3.2 years, including 26 primiparous and 12 multiparous with a single fetus and a physiological course of pregnancy (cases with hypertension and restricted growth of the fetus were excluded). The control group consisted of 20 healthy women of 21.1±0.9 of age, in the 18-25 day of menstrual cycle. All the patients were informed about the aim of the study and gave their permission for blood sampling. The permission of the Regional Bioethics Committee was also obtained.

Sampling of material. Cord blood was sampled immediately after delivery of the fetus, before omphalotomy, by puncturing the cord vein and aspirating blood into a syringe with 3.2% sodium citrate (anticoagulant/blood proportion: 1:9). The blood was next transferred to a test-tube for centrifugation, put into water with ice and taken to the laboratory where centrifugation was done (2000 × g for 20 min, temp. +4°C). The blood plasma was put in test-tubes in 200 µl portions, tightly closed and kept until next examination (4-6 weeks) at -70°C. The maternal blood and the blood of women from the control group was sampled from the antecubital vein and then processed in the same way as described above for cord blood.

Measurement of the level of TAFI antigen. The TAFI antigen level was measured using the immunoenzymatic (ELISA) method with the laboratory kit IMUBIND TAFIa/ai Antigen ELISA (American Diagnostica Inc.). That test measures TAFIa antigen (active enzyme) and TAFIai antigen (inactivated enzyme).

According to the manufacturer, the sensitivity of that method is 10 ng/ml. In our laboratory the interassay and intraassay coefficients of variability were lower than 10%.

Statistical analysis. The program STATISTICA for Windows by StatSoft was used for statistical analysis.

A characteristic feature of the AgTAFI variable in cord blood and in the maternal blood was decomposition, different from normal, and that is why that variability was described by a median (Me) and lower (Q1) and upper (Q3) quartiles. In the control group the distribution of the feature under analysis was close to normal, yet, for the uniformity of analysis it was also described as Me and Q1 and Q3. The statistical analysis of dependent variables was done with Friedman test. The value $p<0.05$ was adopted as a statistically significant level.

Results

The level of TAFIa/ai antigen in cord blood was statistically significantly higher than in maternal blood ($p<0.0001$). Accordingly, in cord blood (n=38) the level was 91.50 ng/ml (median), range: 71.76 - 160.77 ng/ml (Q1-Q3) vs. the level in the maternal blood (n=38): 55.46 ng/ml (median), range: 39.77 - 68.54 ng/ml (Q1-Q3).

The level of TAFIa/ai antigen in the blood of the control group (non pregnant women n=20) was 72.55 ng/ml, range: 67.50-76.69 ng/ml (Q1-Q3). It was statistically significantly higher than in parturient women ($p<0.0004$). Fig. 2 presents those results in a graphic form

Discussion

At the beginning of the discussion it seems appropriate to comment on the terminology concerning TAFI. Not so long ago, American and European authors used various terms. Now, the following unified terms are commonly used: pro - TAFI, i.e. procarboxypeptidase B, thus TAFI proenzyme; TAFIa is carboxypeptidase B in the form of an active enzyme, while TAFIai is the inactivated form of carboxypeptidase B. In the present study we measured the total level of TAFI antigen, both as an active enzyme and as an inactivated enzyme (TAFIa + TAFIai).

TAFI antigen was present in all samples of the cord blood. We take that result to be evidence that TAFI is an integral component of the blood. This is a new observation. The total level of TAFI (TAFIa + TAFIai) in the fetal blood is almost two times higher than in the maternal blood (91.50 ng/ml vs. 55.46 ng/ml). But, as we demonstrated in our report at the XXIII Congress of the Polish Gynecological Society, the activity of TAFI in cord blood is about 50% lower than in the maternal blood [16]. Most probably, the inactivated TAFI (TAFIai) is a dominant component of the total level.

In contrast to research on fetal blood, the level of the antigen and/or TAFI activity in the pregnant women blood has been the subject of research of many authors [1-3,7,8,13,19]. It has been found that

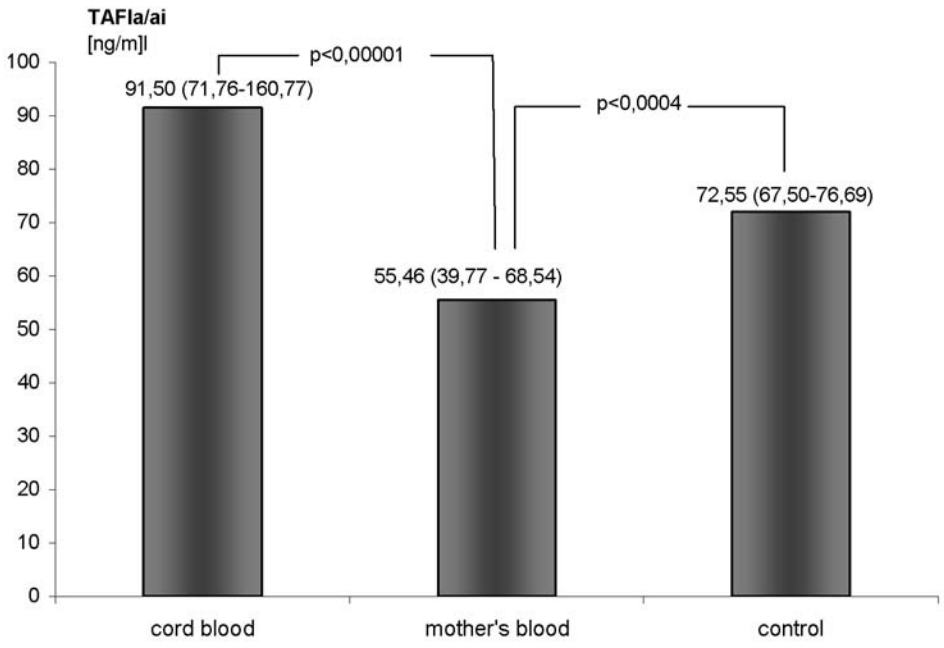

Fig. 2. The level of TAFI antigen (thrombin activatable fibrinolysis inhibitor) in the cord blood plasma. TAFI a/ai - enzymatically active and inactive forms: TAFI(a) and TAFI(ai).

in the course of pregnancy the level of the antigen and TAFI activity increase gradually, though moderately. According to Chabloz *et al.* [7] the level of the antigen in the third trimester of pregnancy constitutes 130% of the level in the first trimester; Mousa *et al.* [13] found the level of the antigen to be 6.6±2 μg/ml at the beginning of pregnancy, and 9.6±2 μg/ml in the third trimester.

An increase of activity in the course of pregnancy was also reported by Watanabe *et al.* [19], with plateau on the 20th week. After delivery, already in the first and second day, the level of the antigen and TAFI activity falls down to the values found in non-pregnant women [13,19].

It is not known what factors influence the increase a relatively high level of TAFI in the fetus. It may be due to an unknown role of that factor in the development of the fetus? Likewise, it is not known when TAFI appears in the fetal blood and when in the extrauterine life the level drops down to that of adults. It is possible that the increase of the level depends on the high level of some of the hormones in the pregnant woman's organism? Perhaps Chettaile *et al.* [8] provided an argument for such an assumption when they reported an increase of the level of TAFI antigen in women using oral contraceptives.

TAFI in maternal blood was not the target of our study; maternal blood was only a reference point of the *gold standard* type thanks to which we could relate our results. It was important because the results of other authors differ among each other (quantitatively, not qualitatively). Most probably, the cause of those differences is the application of different mono- or polyclonal antibodies, as well as insufficient standardization of measurements of TAFI in biological fluids. There are reasons, then, to postulate further studies.

Conclusions

Thrombin activatable fibrinolysis inhibitor (TAFI) is an integral component of cord blood and it can be identified in all samples of the cord blood.

The level of thrombin activatable fibrinolysis inhibitor (TAFI) is statistically significantly higher in cord blood than in maternal blood (about two times higher).

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