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Haemostatic profile of healthy premature small for gestational age neonates

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ABSTRACT

Background: The pathogenetic profile of premature Small for Gestational Age (SGA) neonates is strongly related to their haemostatic equilibrium, which is inadequately understood.
Objective: To evaluate coagulation and fibrinolysis in premature SGA neonates before intervening with Vitamin K administration.
Study design: We performed a comparison of coagulation, natural inhibitors and fibrinolysis between SGA and Appropriate for Gestational Age (AGA) infants born prematurely [gestational age (G.A.) <37 weeks]. Study population consisted of 139 preterm newborns, 68 of whom were SGA (25 males and 43 females), while 71 were AGA (37 males and 34 females) that consisted the control group. Blood samples were obtained within 30 minutes following birth and before the administration of vitamin K. Investigation included: PT, INR, APTT, fibrinogen, coagulation factors II, V, VII, VIII, IX, X, XI, XII, vWillebrand factor, protein C and free protein S, antithrombin (AT), APCR, tPA and PAI-1. The independent t-test and the Mann-Whitney U test were used to compare the differences between the values of haemostatic parameters.
Results: Premature SGA infants presented significantly lower levels of fibrinogen ($p<0.029$) and higher levels of VIIIc factor, APCR, tPA and PAI-1 ($p<0.041$, 0.017, 0.021 and 0.019 respectively). The two groups had similar demographic characteristics (except from birth weight), without significant differences in the values of other haemostatic parameters.
Conclusions: Despite the statistically significant differentiation in the levels of fibrinogen, VIIIc factor, APCR, tPA and PAI-1, the rest of haemostatic parameters have similar values between SGA and AGA preterms.

Introduction

Small for gestational age (SGA) neonates account for a large proportion of the neonatal population and constitute the 30-50% of the extremely low birth weight (ELBW) infants. Premature SGA infants present higher morbidity and mortality rates compared to appropriate for gestational age (AGA) premature infants, possibly due to the unfavorable in utero environment [1–5]. As a result of high morbidity, requirement for catheterization of central vessels is increased, predisposing to thromboembolic complications that are usually secondary to the use of intra-arterial and intravenous catheters [6,7]. The pathogenetic profile of SGA neonates has been extensively studied; premature SGA present a higher risk for thromboembolic events [8–10] and necrotizing enterocolitis (NEC) compared to premature AGA infants; NEC is triggered by hypoxia that leads to ischemic thrombosis of the enteric capillary bed as a result of blood redistribution to the vital organs. Massive pulmonary hemorrhage has been reported as the cause of sudden death in an SGA infant [8]. SGA neonates present an increased incidence for vascular cerebral stroke [11]. Moreover, premature infants are at high risk for hemorrhagic complications [7,12]. The incidence of complications is inversely proportional to gestational age and is clearly elevated in SGA in comparison to AGA infants [8].

Physiology of neonatal hemostasis is inadequately understood in comparison to the adult model. In healthy preterm neonates the coagulation system is more immature at birth compared to full-terms and gradually evolves toward the mature adult system [13–18]. Besides the fact that pathophysiology of SGA premature infants is strongly related to their hemostatic status, there is insufficient literature data regarding hemostasis in this neonatal population; there is only one published report on hemostasis of full-term SGA infants [19].

Moreover, laboratories that work out on large amounts of neonatal samples should establish their own reference values, since results are strongly related to the specific analyzer device and the reagents that are being utilized; it is well known that there are many pitfalls and dilemmas in the evaluation of neonatal hemostasis [7,20,21].

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Subjects and methods

Study design and setting

The present prospective study was designed in an effort to determine the effect of intrauterine environment on the hemostatic status of premature SGA newborns before intervening on coagulation with the administration of vitamin K. For this purpose, we performed a comparative evaluation of coagulation and fibrinolytic parameters between healthy premature SGA and healthy premature AGA newborns. All infant subjects were born in our hospital between May 2004 and January 2007. Maternal written consent was obligatory in accordance to the requirements of the Ethics committee.

Study population

A gestational age below 37 weeks was settled as the limit of prematurity; SGA newborns were defined as having a birth weight below the 10th percentile for G.A., while AGA infants weighed between the 10th and 90th percentile according to the birth weight curve in relation to G.A. determined by Alexander et al [22]. Gestational age was based on the last menstrual period, on ultrasonographic determinations between 8th and 14th week of gestation and on the Ballard test in cases of unknown G.A.

Exclusion maternal criteria were hypertension (defined as diastolic blood pressure >90 mm Hg during pregnancy), coagulation disorders, endocrine diseases, any collagen vascular disease, abruptio placentae and use of anticoagulants or anticonvulsant drugs. Infants with a family history of inherited haemostatic disorders were also excluded.

Exclusion neonatal criteria were congenital coagulation disorders, clinical evidence of infection, confirmed sepsis, perinatal asphyxia, cardiovascular and/or respiratory failure and major birth defects.

Methods

Subjects of our study were hospitalized in the NICU for a period of 6 to 58 days. During this time, they were systematically examined for hemorrhagic or thrombotic events. Clinical evaluation included bleeding from the mucosa, the umbilical stump, the gastrointestinal tract or any other system including the joints, or signs of hemorrhagic predisposition (duration of bleeding at the blood sampling site) [12]. Investigation of intraventicular hemorrhage was performed with brain ultrasound. Clinical evaluation for thrombosis unrelated to catheterization included signs of renal or deep vein thrombosis, cerebral stroke or pulmonary embolism [6,7,23].

To evaluate the influence of intrauterine environment alone on coagulation status of SGA neonates, blood sampling was performed within the first 30 minutes after birth. The same time frame was followed for the AGA group. In all cases blood samples were taken prior to vitamin K administration, in order to evaluate the vitamin-K dependent factors prior to any intervention. Since the same conditions were valid for both groups, the results could be safely compared. After blood sampling, neonates received 0.5 mg iv of vitamin K in order to prevent vitamin K deficiency bleeding [VKDB].

Blood sampling was performed with the G-25 butterfly needle and a total blood volume of 4 ml was obtained from every single neonate.

Freshly drawn blood was mixed with sodium citrate 3.2% in a proportion of 9:1 and centrifuged at 2.500 g for 10 min at 22 °C. Fibrinogen, PT, INR, APTT as well as factors II, V, VII, VIII, IX, X, XI and XII were measured within 4 hours after centrifuging. Evaluation of other proteins [vWillebrand (vWF), protein C, free protein S, antithrombin (AT), activated protein C resistance (APCR), tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1)] was performed after the citrated plasma remained at -20 °C in polypropylene tubes. Storage lasted for a maximum period of 10 days and then the plasma was thawed with water at 37 °C for 15 minutes before serial analysis. No samples were refrozen for later testing. Glass tubes were not used in order to prevent activation of the intrinsic pathway of the coagulation cascade.

Prothrombin time (PT) was measured using the neoplastine – CI plus reagent (Diagnostica Stago, France). According to Langdell RD [24] et al and Larrieu MJ, Weilland C [25], activated partial thromboplastin time was measured using STA®-PTT kit (Diagnostica Stago, France). Fibrinogen measurements were performed with the fibrinogen kit (STA® fibrinogen: lyophilized titrated human thrombin - Diagnostica Stago). Levels of coagulation factors II, V, VII, VIII, IX, X, XI, XII were determined using factor deficient plasma and standard one stage factor assays. Factor deficient plasma samples, calibrator and quality controls used were those commercially available from Diagnostica Stago. Protein C and antithrombin (AT) were measured by synthetic chromogenic substrate using stachrom protein C and stachrom AT III respectively; vWF and free protein S were measured by immunoturbidimetric method using Liatest vWF:Ag (Diagnostica Stago) and Liatest free protein S (Diagnostica Stago) respectively. APCR was evaluated with clot-based method assay using STA- Staclot APC-R kit from Diagnostica Stago; levels of tPA and PAI-1 were measured by Elisa with the use of asseracrom tPA and asseracrom PAI-1 (Diagnostica Stago). All parameters were determined utilising a STA® Compact Analyzer from Diagnostica Stago (Asnières, France).

Statistics

Summary statistics were presented as mean ± standard deviation and 2,5th to 97,5th percentile for continuous variables. Gestational age (GA) and birth weight (BW) were expressed as the mean ± standard deviation. The independent t-test was applied to compare the values of INR, PT, Fibrinogen, IIc, Vc, VIIc, VIIIc, Xc, XIc, XIIc, AT Act and VWF Ag. Comparison of Protein C levels, APTT, IXc, APCR, tPA and PAI-1 was performed with the Mann-Whitney U test. The threshold of significance was $p<0.05$. It was estimated that a sample size of 40 participants per group, could provide significant power to detect sound differences between groups which in most cases was way over 80%.

Results

Approximately 3400 mothers delivered in our hospital during the study period. Two hundred thirty six out of 285 who gave birth to SGA neonates gave informed written consent for their infants to enter the study. One hundred fourteen of them born full-terms (gestational age >37 weeks) were excluded. There were 122 premature SGA newborns, 54 of whom had at least one of the exclusion criteria. Eventually, subject population consisted of 139 premature infants, 68 participants were SGA (25 males and 43 females), while the rest 71 were AGA (37 males and 34 females) and were studied as controls. The SGA preterms had mean G.A. 34.6 ± 2.1 weeks and mean birth weight (B.W.) 1830 ± 549 g, while the AGA group had mean G.A. 33.7 ± 2.8 wks and mean B.W. 2330 ± 695 g. As shown in Table 1, the 2 groups had similar demographic characteristics.

The results of the two groups were comparatively evaluated; the study revealed no differences in PT and APTT. Premature SGA infants presented significantly lower levels of fibrinogen ($p<0.029$). Levels of the vitamin-K dependent coagulation factors (II, VII, IX, X, PS, PC) had no statistical difference between the 2 groups. Of the other coagulation factors (VWF, V, VII, XI, XII), only factor VIII was found increased in the SGA group ($p<0.041$). APCR was also increased in the same group ($p<0.017$) whereas there was no difference in the levels of AT between the two groups.

Moreover, in the study of fibrinolysis we observed an increase in tPA and PAI-1 in the same group ($p<0.021$ and 0.019 respectively)