Hemostatic Profile in Healthy Premature Neonates; Does Birth Weight Affect the Coagulation Profile?

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ABSTRACT

Context: There are limited studies assessing the neonatal hemostatic factors in relation to birth weight. **Aims:** This study aims to compare the coagulation factors between three groups of neonates with different birth weight for gestational age (GA). **Settings and Design:** In a cross-sectional study, 74 healthy premature neonates were involved. **Subjects and Methods:** Serum prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), thrombin clotting time (TT), and levels of fibrinogen, anti-thrombin III, protein S and protein C were measured. Neonates were classified into three groups according to birth weight, including small, appropriate and large for gestational age (SGA, AGA, and LGA). **Statistical Analysis Used:** Statistical analysis was performed using SPSS software. **Results:** There was a significant difference in the levels of protein S ($P < 0.001$), protein C ($P = 0.004$), and values of APTT ($P = 0.01$) between three groups. Other coagulation factors however, did not represent a significant pattern ($P > 0.05$). Protein S concentration, directly ($B = 0.78$, $P < 0.001$), and APTT, inversely ($B = -0.29$, $P = 0.03$), associated with birth weight after adjustment for GA and sex. **Conclusions:** Despite the decrease in APTT from SGA to AGA, and LGA neonates, levels of protein S increases directly with birth weight. However, no other coagulation factors revealed an explainable pattern in relation to the state of SGA, AGA, or LGA.

Key words:
Birth weight, coagulation, neonate, prematurity

INTRODUCTION

Hemostasis in human is a dynamic process, under enormous influence of age. Serum levels of anti-coagulation factors, pro-coagulants and fibrinolytic proteins vary with gestational age (GA) and consequently the birth weight.[1-8] Although developing of hemostatic system rarely accompanies by clinical symptoms in healthy term neonates, premature small for GA (SGA) neonates have higher morbidity and mortality rate due to their hemostatic disturbances predisposing them to hemorrhages or thrombotic disorders.[1-3,7,9,10]

Owing to the scant data on hemostatic factors of neonatal population, this study assess the hemostasis parameters and coagulation inhibitors in preterm SGA, appropriate for GA (AGA) and large for GA (LGA) neonates and found whether hemostasis parameters correlated with birth weight independent of GA and sex.

SUBJECTS AND METHODS

Subjects

This study was performed on 74 healthy preterm neonates born in Imam Hospital, Tehran University of Medical Sciences (TUMS), between January 2011 and December 2012. All neonates were the result of normal pregnancies with Apgar score of more than 7 in 1st min of birth and without any prenatal complications. GA was assessed by the last menstrual period and first trimester ultra sonography.[11] The study was consistent by the principles of Helsinki Declaration. Institutional Review Board of Imam Hospital and the Ethic Committee of TUMS approved the study protocol on human subject.

After obtaining parental written informed consent, demographic data such as sex, delivery mode, GA, birth weight, and Apgar score were collected. Subjects were classified into three groups including SGAs (birth weight below the 10th percentile for GA), AGAs (weight between 10th and 90th percentile for GA), and LGA (birth weight over the 90th percentile for GA), according to the

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birth weight curve by Ray *et al.*[12] Neonates of mothers with a history of pre-eclampsia, coagulation disorder, autoimmune disease, medication intake such as aspirin, anticonvulsant, warfarin or anti-tuberculosis drugs, congenital coagulation disorders and neonates with conditions predisposing to sepsis, birth trauma, prenatal asphyxia, systemic infection, cardiovascular or respiratory failure,[2,4] and premature rupture of membrane were excluded from the study.

Laboratory study

Blood samples were obtained from the umbilical cord, before administration of intravenous vitamin K, and collected in ethylene diamine tetra acetic acid tubes. Platelet count was measured by Sysmex XT1800 I cell counter (Japan). Prothrombin time (PT), activated partial thromboplastin time (APTT), and international normalized ratio (INR) were measured by Sysmex CA500 coagulometer (Japan) using 3.2% buffered sodium citrate anticoagulant tubes. Thrombin clotting time (TT), antithrombin III (AT-III), protein C and protein S levels were assessed by Sysmex CA1500 automated coagulometer (Japan). Serum fibrinogen concentration was measured by manual method using Clauss assay.

Statistical analysis

Data analyses were performed using statistical package for windows (SPSS version 16, Inc., Chicago, USA). Data were presented by mean ± standard deviation. One-way ANOVA test was used to compare differences of hemostatic parameters between SGA, AGA, and LGA groups. Linear regression was used to evaluate the association between neonatal coagulation profiles and birth weight after adjustment for GA and sex. Statistically significant level was considered to be $P < 0.05$.

RESULTS

Hemostasis parameters were evaluated in 74 healthy preterm neonates. The mean GA was 35.46 ± 1.34 weeks (ranged between 30 and 37 weeks). Subjects were divided into three groups based on their birth weight including 22 (29.73%) SGA neonates, 28 (37.84%) AGA neonates, and 24 (32.43%) LGA neonates. No significant differences observed in the sex ratio or delivery mode between these groups. Table 1 presents demographic and hemostatic parameters of the study population. Mean APTT was significantly higher in SGA neonates compared with AGA, and LGA groups. SGA neonates were found to have significantly lower levels of protein S, and platelet; along with significant higher levels of protein C compared with AGA and LGA neonates ($P < 0.001$). Mean level of protein S was also lower in AGA group than in LGA group ($P = 0.03$). There were no statistical differences in levels of PT, INR, TT, fibrinogen, and AT-III between the three groups.

Table 2 shows the GA-, and sex-adjusted β coefficients of coagulation profile and neonatal birth weight. There is a significant direct association between the level of protein S and birth weight ($B = 0.78$, $P < 0.001$), while APTT was inversely associated with birth weight ($B = -0.29$, $P = 0.03$) considering all neonates.

DISCUSSION

This study showed that protein S level is increased with increased birth weight while value of APTT decrease as birth weight increases. Other coagulation factors did not correlate significantly with birth weight.

A similar study by Mitsiakos *et al.* assessed PT, INR, APTT, fibrinogen, coagulation factors, Von Willebrand factor, protein C and free protein S, AT and some other fibrinolytic

Table 1: Demographic and hemostatic characteristics of the SGA, AGA, and LGA neonates

	SGA (n=22)	AGA (n=28)	LGA (n=24)	P value for trend	Adult reference range
Gestational age (weeks)	35.04±1.81	35.60±1.34	35.67±0.56	0.22	
Male (%)	13 (59.1)	14 (50)	13 (54.2)	0.81	
Weight (g)	2049.2±270.34	2977.9±456.9	3501.3±162.7	<0.001	
Apgar 1/Apgar 2 (median, range)	8/9 (7-9)/(8-10)	8/9 (7-9)/(8-10)	8/9 (7-9)/(8-10)	0.28	
PT (s)	17.20±2.21	17.50±2.15	16.78±2.82	0.56	11-14
APTT (s)	45.93±11.57	38.56±9.44	39.56±6.29	0.01	25-40
INR	1.82±0.41	1.84±0.37	1.74±0.52	0.71	1.0-1.3
Thrombin time (s)	29.33±5.76	30.40±5.95	29.55±12.94	0.89	17-27
Protein S (%)	78.59±10.73	104.32±19.77	115.17±13.14	<0.001	10-150
Protein C (%)	30.77±10.30	22.09±7.42	27.12±9.49	0.004	70-130
Fibrinogen (mg/dl)	195.18±54.27	197.53±70.61	197.62±34.15	0.98	200-400
AT-III	119.05±7.91	121.50±2.78	118.71±10.78	0.43	80-120
Platelet	169.86±72.22	194.75±29.06	217.17±53.10	0.01	150-450

Data represent as mean±SD and the range. Apgar score calculated in the 1st and 5th min of life. SD – Standard deviation; SGA – Small gestational age; AGA – Appropriate gestational age; LGA – Large gestational age; PT – Prothrombin time; APTT – Activated partial thromboplastin time; INR – International normalized ratio; AT-III – Antithrombin III

Table 2: Age and sex adjusted beta coefficient of neonatal coagulation factors on weight after adjustment for sex and GA

	Standardized beta	*P* value
PT	−0.05	0.74
PTT	−0.29	0.03
TT	−0.03	0.83
Protein S	0.78	<0.001
Protein C	−0.18	0.17
Fibrinogen	0.08	0.52
AT-III	0.04	0.76
Platelet	0.14	0.25

GA – Gestational age; PT – Prothrombin time; PTT – Partial thromboplastin time; TT – Thrombin clotting time; AT-III – Antithrombin III

parameters between SGA and AGA premature neonates. Although there was a significant difference in fibrinogen, factor VIIIc, similar to our findings, PT, INR, protein C, AT were not significantly differ between SGA and AGA group.[13] This study showed a direct relationship between protein S levels and birth weight independent of sex and GA. There are very limited studies regarding the coagulation factors in relation to the birth weight in neonatal population. Existing data are heterogeneous and also insignificantly supports each other. Fuse observed that plasminogen and plasmin are decreased in SGA group.[7] However, their population comprised of neonates with uncertain health status and small sample size. On the other hands, Peters *et al.*[14] demonstrated decreased levels of AT-III and a_2 antiplasmin in SGA neonates that is not in the same line with our results with no definite association of AT-III with birth weight. Their population includes premature neonates with asphyxia in some cases. It has been shown that prematurity, asphyxia, and even the mode of delivery could affect most of the coagulant factors.[6,15,16] Moreover, before interpretation of the results, attention should be paid to the administration of IV vitamin K in neonates. In our population, IV Vitamin K was administered after obtaining blood samples. Furthermore, no difference was presented between three groups of neonates that could adversely affect our results. Salonvaara *et al.* observed decreased levels of factor V and VII with no remarkable difference in PT, APTT, and INR between SGA and AGA neonates.[8] Varying inclusion eligibility among different studies makes it difficult to comment on the existing inconsistency between literature for relationship between coagulation factors and birth weight in SGA, AGA, and LGA neonates.

The evolution of neonatal coagulation system has been stated to be independent of maternal side due to placental barrier for large coagulation proteins between the two blood circulations.[3] Most of hemostatic indices gradually evolve during early life and reach their adult levels by 6 month. Available studies concern about coagulation system in association with GA and neonatal maturity at the time of birth.[3,5,7,14,17]

Studies by Wasiluk *et al.* demonstrated that platelet indices are altered by prematurity and GA.[1,18] Although, platelet changes in premature neonates have been already contributed to GA and birth weight,[19] these authors concluded that decreased platelet count and increased distribution width occurs in relation with low GA and dysfunction of placenta. Further study also confirmed these findings with more emphasis on the effect of intrauterine growth retardation on thrombopoiesis and subsequent platelet impairments.[1] In this study, we found that protein S, directly, and APTT, inversely, associated with birth weight after adjusting for GA and sex, in order to minimize the effect of prematurity.

There is no study pointing to the difference in hemostatic factors in relation to weight, except for a single study that indicate a significant difference in the level of fibrinogen and VIIIc factor in premature SGA and AGA neonates.[13] There is no discussion about LGA neonates nor there is any role found for birth weight. Our study found that coagulation factors are different between different birth weight groups. Although statistically significant for protein S, protein C, and APTT only, other parameters did not reveal a rational justifiable pattern in relation to the birth weight. This could occur as a result of small sample size. However, neither of these differences in hemostatic parameters accompanied clinically significant symptoms.

CONCLUSION

In contrast to APTT, which decreased from SGA, to LGA, level of protein, S was increased directly with birth weight. However, no other coagulation factors revealed an explainable pattern in relation to state of SGA, AGA, or LGA.

ETHICS

This study was approved by the Ethic Committee of TUMS (number of 88-04-91-9636, vice-chancellor for research).

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