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Assessment of coagulation parameters and D dimer levels among neonates in Lagos, Nigeria – An attempt at establishing normal values for clinical decision making

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Abstract: *Background:* The management of newborn haemostatic defects pose challenges due to lack of reference values. This study aimed to assess coagulation parameters among newborns with attempt to define normal values, and any significant biologic or pathologic variables.
Methods: A cross-sectional study involving 96 singleton newborns (regardless of term) from selected hospitals in Lagos. Umbilical cord blood samples were collected and analyzed for coagulation profile parameters: prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and D-dimer. Assay results were described as mean ± SD, median, range and percentiles. Categorical data were described as frequency and proportions.
Results: Mean gestational age was 37.96 ± 2.75 weeks, and mean birth weight was 3.29 ± 0.49 kg. The mean±SD values for coagulation profile parameters were: PT: 22.09±7.58 seconds, aPTT: 60.86±17.99 seconds, TT: 11.21±3.56 seconds, and D-dimer: 266.59±144.25 ng/ml FEU. Determined 2.5 to 97.5 percentiles for umbilical cord blood coagulation profile parameters were: PT: 6.93-37.25 seconds, aPTT: 24.88-96.84 seconds, TT: 4.09-18.33 seconds, and D-dimer: <555.09 ng/ml FEU. No association was found between sex, gestational age, birth weight, APGAR score, and coagulation studies.
Conclusion: This study provides the coagulation parameters among newborns using umbilical cord blood based on identified clinical and biologic characteristics of study participants. The findings highlight the need for more expansive research into age-, sex-, geographical location-specific reference ranges for coagulation parameters in newborns, compared with other populations. The results will aid in the interpretation of coagulation tests in neonates, facilitating the diagnosis and management of coagulation disorders.

Key words: cord blood, neonates, newborns, D-dimer, coagulation profile, neonatal haemostasis, clotting profile, prothrombin time, activated partial thromboplastin time

Résumé: *Contexte :* La prise en charge des anomalies hémostatiquesnéonatales pose des défisen raison du manque de valeurs de référence. Cette étude visait à évaluer les paramètres de coagulation chez les nouveau-nésententant de définir des valeursnormales et toute variable biologiqueoupathologique significative.
Méthodes: Une étude transversaleportant sur 96 nouveau-nésuniques (quel que soit le terme) provenantd'hôpitauxsélectionnés à Lagos. Des échantillons de sang de cordon ombilicalontétécollectés et analysés pour les paramètres du profil de coagulation : temps de prothrombine (PT), temps de céphalineactivée (aPTT), temps de thrombine (TT) et D-dimères. Les résultats des tests ontétédécrits sous forme de moyenne ± écart-type, médiane, plage et centiles. Les donnéescatégoriellesontétédécrites sous forme de fréquence et de proportions.
Résultats: L'âgegestationnelmoyenétait de 37,96 ± 2,75 semaines et le poidsmoyen à la naissance

était de 3,29 ± 0,49 kg. Les valeurs-moyennes ± SD pour les paramètres du profil de coagulation étaientPT: 22,09 ± 7,58 secondes, aPTT : 60,86 ± 17,99 secondes, TT : 11,21 ± 3,56 secondes et D-dimères: 266,59 ± 144,25 ng/ml FEU. Les 2,5 à 97,5 percentiles déterminés pour les paramètres du profil de coagulation du sang du cordon ombilicalétaient : PT : 6,93 à 37,25 secondes, aPTT : 24,88 à 96,84 secondes, TT : 4,09 à 18,33 secondes et D-dimères : <555,09 ng/ml FEU. Aucune association n'aététrouvée entre le sexe, l'âgegestationnel, le poids à la naissance, le score APGAR et les études de coagulation.

Conclusion: Cette étude fournit les paramètres de coagulation chez les nouveau-nésutilisant du sang de cordon ombilical sur la base des caractéristiquescliniques et biologiquesidentifiées des participants à l'étude. Les résultatssoulignent la nécessité de recherches plus approfondies sur les plages de reference spécifiques à l'âge, au sexe et à l'emplacement-géographique pour les paramètres de coagulation chez les nouveau-nés, par rapport à d'autres populations. Les resultants faciliteront l'interprétation des tests de coagulation chez les nouveau-nés, facilitantainsi le diagnostic et la prise en charge des troubles de la coagulation.

Mots clés: sang de cordon, nouveau-nés, nouveau-nés, D-dimères, profil de coagulation, hémostasenéonatale, profil de coagulation, temps de prothrombine, temps de céphalineactivée.

Introduction

Neonatal haemostatic system differs from that of adults with full maturity being achieved after 6 months to after 1 year.[1] In developmental haemostasis, there is a balance of decreased coagulation factors and anticoagulant proteins which confers the newborn haemostatic protection resulting in a decreased risk of thromboembolic and/or haemorrhagic events compared to adults.[2,3] As a result of the decreased coagulation factors and anticoagulant proteins, screening tests for the haemostatic system are expected to be prolonged in newborns and it should be noted that this increase is physiological and not usually due to underlying pathological conditions.

Due to the gradual maturation of the neonatal haemostatic system, several studies have been conducted to determine the performance of the coagulation parameters at various age groups.[4,5,6] Clearly, the reference intervals are reported to be influenced by age, sample type, ethnicity, sex, and methodology used including analyser and reagent type.[7,8]

There is limited literature available on various components of the haemostatic system in newborns in our environment with adult reference intervals or reference intervals from other populations applied when managing neonatal cases with haemostatic challenges. Laboratory reference intervals are important in clinical decision making and patient care. The Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH) thus strongly recommends that each laboratory defines the age-dependent reference ranges under its own technical conditions.[9]

Index study assessed the coagulation status of newborns using umbilical cord blood. Parameters tested included prothrombin time, Activated partial thromboplastin time, thrombin time and D-dimer. The study attempted to establish normal values for the identified coagulation parametersaccording to guidelines provided by the Clinical and Laboratory Standards Institute.[10]. As well, the determined values were stratified based on biologic and clinical variables such as gestational age, sex, maternal age, APGAR scores and birth weight. Differences in mean for each coagulation parameter for variables such as gestational age were also tested.

Methods

Study design and setting

This is a cross-sectional study conducted between September 2019 and March 2020 with live births at 3selected general/tertiary hospitals in Lagos State, Nigeria. These hospitals were selected based on convenience of access to the labour room data and patient load.

Subjects

Umbilical cord blood: Umbilical cord blood samples were obtained using convenience, non-random sampling technique from 96 singleton newborns of normal pregnancy (irrespective of term), delivered vaginally in the labour wards of selected hospitals in Lagos State, Nigeria. Blood from the umbilical vein of freshly delivered placenta which had the cord clamped at both ends was collected using the syringe technique. All samples were manually checked for clot and excluded if detected. Informed consent was obtained from the mothers of the newborns studied.

Plasma preparation

Appropriate volumes (4.5ml) of cord blood was collected into anticoagulant sample bottles containing 0.5 ml of 3.1% trisodium citrate followed by gentle mixing. Citrated platelet-poor plasma was then obtained by centrifugation within 1 hour of sample collection which was then stored at -80°C till measurement within 2 months of sample collection.

Materials

Prothrombin Time (PT); activated Partial Thromboplastin Time (aPTT) and Thrombin Time (TT) were

estimated using the Coatron M2 coagulation analyser made in Milano, Italy and the TECO GmbH coagulation reagent from MEGALAB, China. The D-dimer assay was carried out using a commercial D-dimer ELISA kit by Elabscience Biotechnology Corporation which uses Sandwich-ELISA method. The BiotekELx 800 absorbance microplate reader (serial no- 205808) was used to read the absorbance.

Parameters or data collected: Clinical history of study participants were obtained from the case notes after umbilical cord samples had been collected. Information collected included: birth weight, sex, APGAR score, gestational age at birth and maternal age.

Data analysis

Statistical analysis was carried out using IBM SPSS Version 23.0 (SPSS Inc., Chicago, Illinois, USA). Categorical data are presented in frequencies and proportions. Numeric data are presented in means, median and measures of dispersion. As a rule, values are considered normal if they fall within the range of mean SD (mean ± 2 SD).[11] Arithmetic mean ± 2SD was considered as normal range for coagulations parameters (prothrombin time, partial thromboplastin time and thrombin time). Normal D-dimer range is presented as less than mean+2SD. Comparison of coagulations parameters for statistical significance among various categories such as gestational age, APGAR scores, sex and birth weight was tested with Analysis of variance. All tests of significance were two-tailed and considered statistically significant at $P \leq 0.05$.

Results

Most (75%) of the newborns were term deliveries between 37 and 42 weeks. APGAR score of 0 – 3 at 1 and 5 minutes was present in 1% of the newborns. Most (96.8 and 99%) of the newborns had Apgar score of 6 – 10 at 1and 5 minutes respectively. Normal birthweight (2.5 – 4kg) was recorded in 91.7% of the newborns, followed by Low body weight in 6.3% (Table 1).

The mean±SD prothrombin time, partial thromboplastin time, thrombin time and D-dimers were 22.09±7.58, 60.86±17.99, 11.21±3.56, 266.59±144.25 seconds respectively (Table 2). The mean±2SD ranges for prothrombin time, partial thromboplastin time and thrombin time were 6.93 – 37.25, 24.88 – 96.84 and 4.09 – 18.33 seconds. The mean±2SD range for D dimer was determined to be <555.09 ng/ml FEU. There was no statistically significant difference in coagulation parameters including D dimer based on gestational age, maternal age, and birth weight (Table 3) Coagulation profile tend to get prolonged with lower APGAR scores though not statistically significant. (Table 3).

Table 1: Biologic and clinical characteristics of the newborns

Variable		Frequency (n)	Percentage (%)
Gestational Age (weeks)			
	<37	23	24
	37 – 42	72	75
	>42	1	1
Mean±SD = 37.96±2.75; Range = 30 - 44			
Sex			
	Female	44	45.8
	Male	52	54.2
APGAR 1			
	8 – 10	61	63.5
	6 – 7	32	33.3
	4 – 5	2	2.1
	0 – 3	1	1.0
APGAR 5			
	8 – 10	93	96.9
	6 – 7	2	2.1
	4 – 5	0	0
	0 – 3	1	1.0
Birth Weight (kg)			
	< 2.5kg	6	6.3
	2.5 – 4.0kg	88	91.7
	>4.0kg	2	2.1
Mean±SD = 3.29±0.49; Range = 1.5 – 4.2			

N = 96 (100%)

Table 2: Coagulation profile and D dimer parameters of study participants and D-dimer results (cord blood)

Measures	Prothrombin time (sec)	Activated Partial Thromboplastin Time (Sec)	Thrombin Time (sec)	D-dimer (ng/ml FEU)
Mean	22.09	60.86	11.21	266.59
SD	7.58	17.99	3.56	144.25
2SD	15.16	35.98	7.12	288.5
Mean±2SD	6.93 -37.25	24.88 – 96.84	4.09 – 18.33	<555.09
SEM	0.77	1.84	0.36	14.72
Median	21.60	61.85	9.80	245.00
Minimum	13.80	26.80	7.20	15.00
Maximum	73.40	100.30	19.30	901.00
2.5 Centile	13.80	29.21	7.20	39.10
97.5 Centile	42.60	88.23	19.30	583.65

N = 96 (100%)

Table 3: Relationship of coagulation profile parameters with independent variables

Variable	PT (sec)	F, p-value	APTT (sec)	F, p-value	TT (sec)	F, p-value	D-dimer (ng/ml FEU)	F, p-value
Gestational age (weeks)		0.549, 0.579		0.210, 0.811		0.298, 0.743		0.292, 0.748
< 37	23.55		62.33		11.48		284.87	
37 – 42	21.64		60.52		11.16		261.49	
>42	21.60		51.90		8.80		214.00	
Maternal age (years)		1.976, 0.163		0.030, 0.863		0.518, 0.474		0.277, 0.600
18 – 35	21.67		60.74		11.11		263.52	
>35	24.83		61.67		11.88		286.23	
Apgar1		2.769, 0.046		0.793, 0.501		0.521, 0.669		1.583, 0.199
8 – 10	22.28		60.39		11.09		253.56	
6 – 7	21.13		60.84		11.55		275.78	
4 – 5	21.75		62.10		8.80		428.00	
0 – 3	42.60		88.40		13.20		445.00	
Apgar5		3.922, 0.023		1.195, 0.307		0.614, 0.544		2.131, 0.124
8 – 10	21.88		60.54		11.25		261.20	
6 – 7	21.75		62.10		8.80		428.00	
4 – 5	0		0		0		0	
0 – 3	42.60		88.40		13.20		445.00	
Sex		4.318, 0.040		5.993, 0.016		0.245, 0.622		0.012, 0.914
Female	20.38		56.10		11.02		268.34	
Male	23.55		64.89		11.38		265.12	
Birth Weight (kg)		0.023, 0.977		0.155, 0.856		1.000 0.372		0.247, 0.782
< 2.5	22.12		63.65		10.25		304.17	
2.5 – 4.0	22.12		60.79		11.35		264.68	
>4.0	20.95		55.60		8.20		238.00	

Discussion

Newborns are faced with risk of bleeding and thrombosis. Hence the development of haemostasis begins in utero with an endpoint of attaining characteristic physiological balance in the components of coagulation and anticoagulation systems. The monitoring of clotting profile components of newborns becomes imperative to ensure prevention, diagnosis, and treatment of haemorrhagic and thrombotic events in neonates. However, the most frequent problem with interpreting neonatal or paediatric coagulation profiling is the improper use of reference ranges.[12] This present study evaluated the clotting profile components of newborns with the aim of establishing reference ranges.

The measures of clotting profiles of the neonates which included prothrombin time, activated partial thromboplastin time, and thrombin time observed resulted in calculated reference ranges (mean±2S.D) which vary comparably when compared to several reference ranges reported in several studies notwithstanding geographical population distribution.[13,14,15,16] This variations might explain the reason why it is advisable for different centers or geographical regions to determine their reference ranges as proposed by the International Society on Thrombosis and Haemostasis (ISTH).[9] Evidence also suggests that there is no reliable normal reference ranges for D-dimers concentration in neonates.[17] The differences in reference ranges could be attributed to several factors including gestational age, age of neonate, gender and birth weight. However, sample size might also play a key crucial role in describing the statistical significance of future studies in this scope of research.

Factors including reduced gestational age, older maternal age and low birth weight characteristically, but not significantly, resulted in prolongation of clotting profile components. Low birth weight is reportedly associated with bleeding in neonates.[13] Since there exists linkage between haemorrhage (bleeding) and low birth weight as well as prolonged generation of clotting profile components and haemorrhage, it could be suggested that low birth weight of neonates could be a factor affecting the prolongation of clotting profile components generation.
Based on gestational age, gradual reduction in prothrombin time, activated partial thromboplastin time, and thrombin time observed is corroborated by several studies that documented decrease in reference ranges of coagulation profile with increasing gestational age.[16] This is indicative of prolonged generation time for clotting profile parameters in pre-term babies in comparison to term neonates. This is reportedly responsible for the

high risk of bleeding associated with pre-term infants. The prolongation of the clotting profile components of neonates with gestational age less than 37 weeks compared to those greater than 37weeks found in this study could be explained by the work of Kenet et al. which suggested that it could be due to variations in the levels of haemostatic proteins because of age differences.[18] Mousa (2021) indicated that coagulation proteins are lower in pre-term babies compared to their full-term counterparts. Therefore, pre-term neonates are at disadvantage of prolonged generation time of prothrombin, activated partial thromboplastin and thrombin, and increased levels of D-dimer.[12]

In this study, the severity of birth asphyxia (defined by low APGAR score) and gender significantly accounted for prolonged clotting profile generation time and increased levels of D-dimer in neonates. In agreement with this present study, previous studies have reported the effect of age and gender on reference intervals of the components of neonatal haemostatic profile.[7,8] Finally, prolongation of the generation time of the clotting profile components that characterized pre-term neonates or less than 37 weeks gestational age, low birth weight, and birth asphyxia (defined by low APGAR score), is indirectly proportional to increased levels of D-dimer. In agreement with the findings of this study, there exist an inverse proportion between the concentration of D-dimer and the age of neonates.[19] This indicates an increased level of active fibrinolysis in pre-term neonates and a possible predictor of thrombotic events.[20]

Conclusion

Coagulation profile reference ranges are unique in newborns, neonatal period and childhood. This indicates that adult and childhood reference ranges of prothrombin time, activated partial thromboplastin time, and thrombin time may not be applicable in managing newborns with haemostatic challenges. We recommend that age-dependent reference ranges for coagulation profiles be established by each reference laboratory. Further research including a larger sample size is needed to explore the clinical implications and impact on perinatal care of these reference intervals.

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References

1. Reverdiau-Moalic P, Delahousse B, Body G, Bardos P, Leroy J, Gruel Y. Evolution of blood coagulation activators and inhibitors in the healthy human fetus. *Blood 1996:88:900–6.*
2. Lippi G, Franchini M, Montagnana M, Guidi G.C. Coagulation testing in pediatric patients: the young are not just miniature adults. *Semin ThrombHemost 2007; 33:816 -20.*
3. Liu J, Dai Y, Yuan E, Li Y, Wang Q, Wang L, et al. Paediatric reference intervals for common coagulation assays in Chinese children as performed on the STA-R coagulation analyzer. *Int. J. Lab. Hematol. 2019, 41, 697–701.*
4. Monagle P, Barnes C, Ignjatovic V, Furmedge J, Newall F, Chan A, et al. Developmental hemostasis. Impact for clinical hemostasis laboratories. *Thrombosis and Hemostasis 2006:95(2):362–72.*
5. Appel IM, Grimminck B, Geerts J, Stigter R, Cnossen MH, Beishuizen A. Age dependency of coagulation parameters during childhood and puberty. *J ThrombHaemost. 2012 Nov;10(11):2254-63.*
6. Toulon P, Berruyer M, Brionne-François M, Grand F, Lasne D, Telion C, et al. Age dependency for coagulation parameters in paediatric populations. Results of a multicentre study aimed at defining the age-specific reference ranges. *ThrombHaemost. 2016 Jul 4;116(1):9-16.*
7. Ceriotti, F. Establishing Pediatric Reference Intervals: A Challenging Task. *Clin. Chem. 2012, 58(5), 808–10.*
8. Di Felice G, Vidali M, Parisi G, Pezzi S, Di Pede A, Deidda G, et al. Reference Intervals for Coagulation Parameters in Developmental Hemostasis from Infancy to Adolescence. *Diagnostics (Basel). 2022 Oct 20;12(10):2552.*
9. Ignjatovic V, Kenet G, Monagle P. Perinatal and Paediatric Haemostasis Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Developmental haemostasis: Recommendations for laboratories reporting pediatric samples. *J. Thromb. Haemost. 2012, 10, 298–300.*
10. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – 3rd Edition. CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
11. Hathaway WE, Christian MJ, Clarke SL. Study of the various parameters intervening in the pre analytic variables (revue de la literature). *Sang ThrombVaiss1998;10:5 -18.*
12. Mousa, S. Evolution of Hemostasis in Neonates and Children. *Annals of Neonatology J. 2021; 3(2): 9-22.*

13. Neary E, McCallion N, Kevane B, Cotter M, Egan K, Regan I, et al. Coagulation indices in very preterm infants from cord blood and postnatal samples. *J. Thrombosis and Haemostasis. 2015;13 (11):2021-30.*
14. Garba N, Ogbenna AA, Adediran A, Fajolu IB. Coagulation profile in normal full-term neonate in the first week of life in Lagos-Nigeria. *International J. Medical Research & Health Sciences, 2016; 5(3):44-8.*
15. Hochart A, Pierache A, Jeanpierre E, Laffargue A, Susen S, Goudemand J. Coagulation standards in healthy newborns and infants. *Archives de Pédiatrie. 2021;28(2):156-8.*
16. Davenport P, Sola-Visner M. Hemostatic Challenges in Neonates. *Front Pediatr. 2021;9:627715.*
17. Chalmers EA. Neonatal coagulation problems. *Arch Dis Child Fetal Neonatal Ed 2004;89:F475–F478.*
18. Kenet G, Barg AA, Nowak-Göttl U. New insights into neonatal hemostasis. *Thrombosis Update. 2022;7:100103.*
19. Khalilov Zİ, Ünsal A, Altuntaş N. The D-dimer reference intervals in healthy term newborns. *Transfusion and Apheresis Science. 2022;61 (6):103493.*
20. Will A. Neonatal haemostasis and the management of neonatal thrombosis. British Journal of Haematology. 2015;169 (3):324-32.