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Coagulation profile in normal full-term neonate in the first week of life in Lagos-Nigeria

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ABSTRACT

Normal reference values of haemostatic profile are frequently needed to assist in diagnosis and management of bleeding disorders. Because of the anatomical and physiological differences between neonates and adults, it is essential to know the reference range of coagulation profile in neonates in the first week of life. The aim of this study is to establish a normal reference range for coagulation profile in normal full-term neonates in the first week of life in Lagos-Nigeria. This is a cross-sectional study carried out among normal full-term neonates born in Lagos Island Maternity Hospital and Lagos University Teaching Hospital. Neonates' demographic data were documented. Citrated plasma was collected and tested for Prothrombin Time (PT), Partial Thromboplastin Time with Kaolin (PTTK) and Thrombin Time (TT) using Coatron M2 Coagulation Analyzer (TECO GmbH in Germany). Reference values were established for neonates in the 1st week of life by using the formula of Mean ± 2 Standard Deviation (SD) that is at 95% confidence level. Comparative analysis was carried out between the mean values of neonates and adults established mean values. The Mean ± Standard Deviation of PT, PTTK and TT at the first week of life were 13.41 ± 1.33 seconds, 43.38 ± 6.75 seconds and 24.01 ± 3.03 seconds respectively. Using the formula of Mean ± 2SD, the reference ranges of PT, PTTK and TT were 10.7-16.07seconds, 29.88-56.88 seconds and 17.95-30.07 seconds respectively. Statistically significant difference was observed when mean values of PT, PTTK and TT of neonates at the 1st week of life were compared with adults established values done in the country and elsewhere in the world (p-value < 0.05). This research provides a reference range of PT, PTTK and TT for the management of neonates in the 1st week of life in Lagos, Nigeria. It indicates that reference values of PT, PTTK and TT in neonates during the 1st week of life are different from that of adults as described in literature. Therefore we cannot use adult reference values of PT, PTTK and TT to manage neonates during the 1st week of life.

Key words: Reference values, full-term neonates, prothrombin time, partial thromboplastin time with kaolin, thrombin time.

INTRODUCTION

Normal haemostasis is the process of forming clots in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system [1]. In the event of tissue or blood vessels damage, three mechanisms operate locally to control the bleeding; vessel contraction, formation of platelet plug and formation of fibrin. Normal haemostatic mechanism consists of four components; Blood vessel wall, Platelets, Coagulation and procoagulants factors and the Fibrinolytic system [2].

The development of haemostasis in the newborn differs from that of adult and coagulation factors do not cross the placenta barrier. The coagulation factors are synthesized independently in the fetus and are dependent on two factors: the gestational age and the level of maturity of the liver [3].

At birth the level of the contact factors (XII, XI, high molecular weight kininogen) and Vitamin K dependent factors are decreased to about 50% of adult values [4,5]. Thrombin generation is also reduced by 30-50% of adult levels [6].

This implies that screening test like Prothrombin Time (PT), Partial Thromboplastin Time with Kaolin (PTTK) and Thrombin Time (TT) may be longer in neonates. But the fact that the neonates do not bleed is suggestive of a possible compensatory balance. For instance the fibrinolytic system is also down regulated with a decrease in the levels of plasminogen to 50% of adult level and an increase in plasminogen activator inhibitors [7].

The most useful screening tests in neonates are: the prothrombin time, partial thromboplastin time with kaolin, thrombin time, fibrinogen assay and platelet count, however age specific reference ranges are required for appropriate interpretation [8,9].

In Nigeria, little information is available about coagulation profiles in neonates. Adult normal reference values are available in most hospitals but those of neonate are very scarce. The aim of this study was therefore to determine the reference value of coagulation profile in apparently healthy neonates in the first week of life and compare with established adult values in the country and other parts of the world.

MATERIALS AND METHODS

Study Population

Two hundred normal full-term neonates (200) born in Lagos Island Maternity Hospital and Lagos University Teaching Hospital were enrolled into a study for a period of seven months; August, 2013 through February, 2014. The neonates were randomly selected upon acceptance by their parents, but there were parents who refused to participate in this study, we did not actually recorded number of parents who refused since some of them did not explain the reasons for their refusal while some expressed sympathy to their neonates. Demographic data were taken in delivery register and by interviews with the parents. The data were documented in the data collection sheet.

Ethical Consideration

Approval for the study was obtained from the Lagos State Health Service Commission and the Health Research and Ethics Committee of Lagos Island Maternity Hospital and Lagos University Teaching Hospital respectively, permission from the respective Head of Departments and informed consent of the parents were also obtained.

Sample Size

The sample size was calculated using single proportion formula ($n = (z^2pq)/d^2$). The minimum sample size was found to be 180. However, 200 samples were studied as to increase the precision of the study.

Inclusion Criteria

Only apparently healthy full-term neonates delivered vaginally who were between 38-40 weeks gestational age, weighted between 2.5-4.0kg and APGAR (Appearance, Pulse, Grimace, Activity, and Respiration) score of $\geq$ 7 at 1^{st} and 5^{th} minutes of life were considered eligible and enrolled into this study.

Exclusion Criteria

All neonates with any of the following were excluded: birth weight less than 2.5kg or >4.0kg, asphyxia, any congenital abnormality on physical examination, jaundice, obvious bleeding disorders, a family history of hereditary clotting disorder and APGAR score of $\leq$ 7 at 5^{th} minute of life. Neonates whose mothers had hypertension and/or diabetes or were on anticoagulants like warfarin or aspirin were also excluded.

Sample Collection

Both written and oral consent were obtained from the parents before sample collection. One milliliter (ml) of blood was collected from a peripheral vein under aseptic conditions using a 23 gauge needle, 0.9ml of blood was put into a bottle containing 0.1ml of 3.2% tri-sodium citrate (i.e. in 1:9 ratio). The sample collected was gently inverted 5-6 times to prevent clotting. The samples were taken to the laboratory in cooler with ice packs and spun in centrifuge at 2000 *g* for 15 min to obtain platelet-poor plasma within an hour of sample collection. All blood samples were collected within the first week of life. The platelet-poor plasma was separated and stored at -80^0C until they were ready for analysis. This is due the fact that the stability of plasma is as follows: 4hours at $18\text{-}26^0$C, 8hours at $2\text{-}8^0$C, 30days at -20^0C and 6months at -70^0C. The entire research occurs within 7 months but the storage of plasma at -80^0C was within 3 months. After obtaining the required sample size, the frozen plasma was allowed to thaw and then tested for the prothrombin time, partial thromboplastin time with kaolin and thrombin time using Coatron M2 Coagulation Analyzer made by TECO GmbH in Germany. Measurement of PT, PTTK and TT for each sample was carried out in duplicates and the mean values recorded.

Data Analysis

Data processing was done using the Graph pad *Instat* (2007) computer statistical software package (Version 5.00) [10]. Mean ± Standard Deviation on PT, PTTK and TT were calculated and reference range was established from mean and standard deviation. One-way Analysis of Variance (ANOVA) was used for comparison of neonatal mean and known adult mean values in Nigeria (Source: Alao *et al.,* 2009) [11] and Canada (Source: Andrew *et al.,* 1987) [4]. All statistical analyses were at 5% level of significance, $p \leq 0.05$ (i.e. 95% confidence level).

RESULTS

Two hundred (200) normal full-term neonates aged between 1 to 7 days were recruited for this study. 110 (55%) were males and 90 (45%) were females. The mean age of males was 3.05±2.00 days and that of females was 2.83±1.64 days. The overall mean age was 2.95 ± 1.85 days. Seventy eight (39%) of neonates were within the 1st day of life while the remaining 122 (61%) were between 2-7 days old.

Table1: Reference ranges of the coagulation profile are illustrated in Table 1. The Mean Prothrombin time, Partial thromboplastin time with kaolin and Thrombin time were 13.41±1.33 seconds, 43.38±6.75 seconds and 24.01±3.03 seconds respectively.

Table2: Comparison of the neonatal coagulation profile with established adult values shows that the mean neonatal PT, PTTK and TT were significantly higher than adult values (Tables 2).

Table 1: Coagulation profile values in healthy full-term neonates during the first week of life

Parameters	Mean ± SD	Reference Range
Prothrombin Time (Seconds)	13.41±1.33	10.75-16.07
Partial Thromboplastin Time with Kaolin (Seconds)	43.38±6.75	29.88-56.88
ThrombinTime (Seconds)	24.01±3.03	17.95-30.07

Key: SD-Standard Deviation, Reference ranges had been calculated by using the formula of mean±2Sd (i.e. 95% confidence level).

Table 2: Comparison of coagulation profile of neonates in the 1st week of life, Nigerian adults (Source: Alao *et al.*, 2009)[11] and Canadian adults (Source: Andrew *et al.*, 1987)[4]

Parameters	1st week of life Mean±SD	Nigerian Adults (Alao *et al.*, 2009)[11] Mean±SD	Canadian adults (Andrew *et al.*, 1987)[4] Mean±SD	p-value
PT (seconds)	13.41±1.33(200)	14.9±2.3(50)	12.4±0.78(29)	<0.0001
PTTK (seconds)	43.38±6.75(200)	25.3±3.9(50)	33.5±3.44(29)	<0.0001
TT (seconds)	24.01±3.03(200)	17.1±2.9(50)	25.0±2.66(29)	<0.0001

PT = Prothrombin Time, PTTK=Partial Thromboplastin Time with Kaolin, TT=Thrombin Time, SD= Standard Deviation

DISCUSSION

In this study, reference range of coagulation profile at 1st week of life was determined.

The mean PT in the first week of life obtained in this study was 13.41±1.33 seconds which is slightly higher than the 12.4±1.46 seconds reported by Andrew *et al.,* 1987 at 5th day of life [4]. Federico *et al*., 1994 reported a mean PT of 13.1± 0.9 seconds among healthy neonates in Trieste, Italy [12] which is similar to our findings. A study done in Ibadan, Nigeria by Okunade *et al.,* 1998 also reported a mean PT value of 15.2 ± 0.9 (14-17) seconds among neonate at first day of life [13]. This mean value is much higher than our values. This may be related to sample size (30 versus 200) or the age differences in the study unit. The study by Okunade *et al.,* 1998 involved only 30 neonates in the first day of life whereas this study involved 200 neonates in the first week of life.

Federico *et al*., 1994 also reported a mean PT of 11.9±0.6 seconds among adults in Trieste, Italy [12]. The Mean±SD of PT was found to 14.04±1.93 seconds by Adama *et al.,* 2013 among adults [14]. The mean PT among adults was also reported to be 15.7±1.6 seconds by Isaac *et al.,* 2014 which is different from our neonatal mean PT [15]. Abdulrahaman *et al.,* 2012 reported 14.920±1.209 seconds as Mean±SD of PT among adults [16].

The reference range obtained for PT in this study was 10.75-16.07 seconds. This is similar to that reported by Andrew *et al.,* 1987 which had a reference range of 10.1-15.9 seconds for healthy full term infants in the first day of life [4]. In a study by Lippi *et al.,* 2007 normal value of prothrombin time in healthy full term infants was also found to be 11-15 seconds [17]. Chakrapani *et al.,* 2010 reported 12–17 seconds as reference value of prothrombin time in term babies [18]. Reverdiau-Moalic *et al.,* 1996 reported 1 1.4-14.0 seconds as reference range in adults [19]. There is hence slight variation in reference ranges from the various studies. This may reflect genetic or racial differences,

or the different reagents used. This emphasizes the need to generate local neonatal reference values for proper interpretation of result.

The PT mean value we obtained was about 1 minute greater than that of adults and this difference was statistically significant (p-value < 0.0001). This reflects the peculiarity of coagulation profile in neonates.

The mean PTTK in the 1st week of life was 43.38±6.75 seconds in this study. This value is closer to that obtained by Andrew *et al.,* 1987 (mean PTTK at 5th day of life of 42.6±8.62 seconds) [4]; but much higher than the 35.0±4.5 seconds reported by Federico *et al.,* 1994 in healthy full-term newborns [12]. The mean value reported by Okunade *et al.,* 1998 among neonates at first day of life is 47 ± 2.0 (41.50) seconds [13].

Federico *et al*., 1994 also reported 28.8±2.7 seconds among adults in Trieste, Italy [12]. The Mean±SD of PTTK among adults was found to 34.20± 7.91 seconds by Adama *et al.,* 2013. The mean PTTK among adults was reported to be 36.3±3.5 seconds by Isaac *et al.,* 2014 which is different from our neonatal mean PTTK [15]. Abdulrahaman *et al.,* 2012 reported 41.380±4.295 seconds as Mean±SD of PTTK among adults [16].

The reference range obtained for PTTK was 29.88-56.88 seconds. Andrew *et al.,* 1987 reported a reference range of PTTK in healthy full term infants to be 31.3– 54.5 seconds in the first day of life which is closer to our result [4]. Lippi *et al.,* 2007 reported lower upper limit (30-40seconds in healthy full term infants) compared to our findings in this study [17]. Chakrapani *et al.,* 2010 reported a PTTK reference value of 25–45 seconds which is lower than our findings [18]. Reverdiau-Moalic *et a.,* 1996 reported 25-39 seconds as reference range in adults [19].

The mean PTTK in the 1st week of life in this study was significantly higher than values reported in adult studies [4, 11]. This indicates some differences between mean values of coagulation profile among neonates and adults and this may be due to the fact that most coagulation factors in the intrinsic pathway are lower in neonates compared to the adults [20].

The mean TT at 1st week of life was 24.01±3.03 seconds in this study which is similar to that obtained by Andrew *et al.,* 1987 where the mean TT at 5th day of life was 23.1±3.07 seconds [4]. Andrew et al., 1987 also reported 25.0±2.66 seconds as Mean±SD of TT among adults [4]. Our reference range of TT was however higher than that reported by Lippi *et al.,* 2007 [17] in healthy full term infants (17.95-30.07 seconds vs 15-20 seconds). Reverdiau-Moalic *et a.,* 1996 reported 12-16 seconds as reference range in adults [19].

The difference between the neonatal mean values and those of adults might be due to the anatomical and physiological differences between neonates and adults.

CONCLUSION

Reference ranges in coagulation profiles are unique in neonates and adult reference ranges of PT, PTTK and TT may not be useful in managing neonates in the first week of life. Therefore, it is advisable to establish Neonatal reference range of coagulation profiles in our communities.

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