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Original Article

Establishment of Reference Ranges for Coagulation Parameters in Neonates and Infants in Our Population

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ABSTRACT

Background: Coagulation reference intervals vary with developmental age, laboratory method, reagent system, and population characteristics; therefore, adult-based reference values may not be appropriate for interpreting haemostatic parameters in neonates and infants. Objective: To establish local reference intervals for selected coagulation parameters among healthy Pakistani neonates and infants. Methods: This multicentre descriptive cross-sectional study was conducted from January to December 2021 through Combined Military Hospital Quetta, with recruitment from EPI vaccination centres of Combined Military Hospitals in Rawalpindi, Quetta, Lahore, Multan, and Karachi. Healthy neonates and infants aged from birth to less than one year were enrolled after parental consent. Venous blood samples were collected in trisodium citrate tubes and analysed for platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen. Reference intervals were estimated using the 2.5th and 97.5th percentiles. Results: A total of 470 participants were included, comprising 312 males and 158 females. The overall reference intervals were 10–15 seconds for prothrombin time, 21–37 seconds for activated partial thromboplastin time, 13–20 seconds for thrombin time, and approximately 205–410 mg/dL for fibrinogen. Platelet count showed the clearest age-related difference, with higher mean values among infants than neonates. Conclusion: Locally derived coagulation reference intervals may improve interpretation of neonatal and infant haemostatic screening in Pakistan. Keywords: Activated partial thromboplastin time; Coagulation; Fibrinogen; Neonates; Prothrombin time; Reference intervals; Thrombin time

INTRODUCTION

Haemostasis is a complex physiological process that prevents excessive bleeding after vascular injury while maintaining blood fluidity within the circulation. It depends on coordinated vascular constriction, platelet plug formation, activation of coagulation factors, conversion of fibrinogen into fibrin, and subsequent clot stabilization and regulation (1,2). In neonates and infants, this system differs substantially from that of older children and adults because coagulation factor concentrations, platelet function, fibrinolytic activity, hepatic maturity, and developmental haemostatic balance continue to evolve after birth (3,4). These age-related physiological differences are clinically important because interpretation of coagulation screening tests using adult-based reference values may lead to misclassification, unnecessary treatment, delayed diagnosis, or inappropriate transfusion decisions in early life.

Routine coagulation assessment commonly includes prothrombin time, activated partial thromboplastin time, thrombin time, platelet count, and fibrinogen concentration. Prothrombin time primarily reflects the extrinsic and common coagulation pathways, while activated partial thromboplastin time reflects the intrinsic and common pathways and is used to detect abnormalities involving multiple clotting factors (5,6). Thrombin time assesses the conversion of fibrinogen to fibrin, and fibrinogen concentration provides information about a key plasma glycoprotein required for clot formation and stability (7). Because these parameters are influenced by age, analytical method, reagents, instruments, sample handling, and population characteristics, locally derived reference intervals are essential for accurate clinical interpretation.

A reference interval is generally defined as the central 95% distribution of values observed in an appropriately selected healthy reference population, commonly estimated using the 2.5th and 97.5th percentiles (8). Although reference intervals for coagulation parameters have been studied in adult and paediatric populations, data for neonates and infants remain limited, particularly in Pakistan. This gap is relevant because inherited bleeding disorders, consanguinity-related haematological conditions, neonatal illness, and laboratory-dependent diagnostic variation can create substantial clinical uncertainty when locally validated values are unavailable. In addition, advances in laboratory techniques, reagents, and quality-control procedures mean that older reference values may not be directly applicable to current practice.

Establishing population-specific coagulation reference intervals in healthy neonates and infants can support more accurate diagnosis of bleeding and clotting abnormalities, reduce unnecessary investigations, and guide safer clinical decision-making in paediatric and neonatal care. Therefore, this study was conducted to establish reference intervals for prothrombin time, activated partial thromboplastin time, thrombin time, platelet count, and fibrinogen among healthy Pakistani neonates and infants, and to examine whether these parameters differed according to sex and age group.

MATERIAL AND METHODS

This multicentre descriptive cross-sectional study was conducted over one year, from January 2021 to December 2021, through the Pathology Department of Combined Military Hospital Quetta, with participant recruitment from EPI vaccination centres of Combined Military Hospitals in Rawalpindi, Quetta, Lahore, Multan, and Karachi. The study was designed to establish coagulation reference intervals among healthy Pakistani neonates and infants using a reference population approach. Neonates were defined as children from birth to less than one month of age, while infants were defined as children aged more than one month and less than one year.

The minimum sample size requirement was determined according to reference interval guidance recommending adequate numbers of healthy reference individuals for estimation of percentile-based reference limits (9). A minimum sample size of 240 participants, including 120 males and 120 females, was required, and the sample was increased to 470 to improve the stability of reference interval estimates and allow subgroup comparisons by sex and age group. Participants were selected using a non-probability consecutive sampling technique from healthy neonates and infants attending EPI vaccination centres during the study period.

Healthy neonates and infants of either sex, from birth to less than one year of age, were considered eligible for inclusion. Participants were excluded if they were premature; had a history of congenital disease, systemic illness, blood loss, blood transfusion, transfusion of blood products, or relevant drug exposure; or had any clinical condition that could affect haemostatic parameters. Written informed consent was obtained from parents or guardians before enrolment. Demographic information and relevant clinical history were recorded before sample collection.

A 2 mL venous blood sample was obtained from each participant under aseptic conditions and collected in a tube containing trisodium citrate anticoagulant. Samples were processed within one hour of collection. Coagulation parameters were manually analysed by incubation in a water bath at 37°C, and results were recorded using the mean of two readings. Control plasma was used to validate the procedure, and 40 samples were reanalysed to assess procedural reliability and identify analytical error. The measured variables included platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen concentration.

Data were analysed using IBM SPSS Statistics version 25.0. Continuous variables, including age, weight, platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen concentration, were summarized using mean, standard deviation, minimum, maximum, and percentile values where appropriate. Categorical variables, including sex and age group, were summarized using frequency and percentage. Reference intervals were calculated using the 2.5th and 97.5th percentiles. Outliers were identified by visual inspection and Dixon's method before final calculation of reference intervals. Comparisons of coagulation parameters between males and females and between neonates and infants were performed using independent-samples t-tests. A p-value of ≤ 0.05 was considered statistically significant.

Ethical approval was obtained from the institutional review board before commencement of the study, with approval reference number CMH-QTA-IRB/042. Written informed consent was obtained from parents or guardians of all participants. Data integrity was maintained through standardized sample collection, duplicate reading of coagulation results, use of control plasma, reanalysis of selected samples, and statistical screening for outliers before final reference interval estimation.

RESULTS

A total of 470 healthy neonates and infants were included in the study. Of these, 312 participants were male and 158 were female. The mean age of the study population was 27.8 ± 33.2 days, with an age range of 1 to 231 days. Neonates constituted 311 participants, while 159 participants were infants aged more than one month and less than one year.

Table 1. Demographic Characteristics of the Study Population

Variable	n	%
Total participants	470	100.0
Male	312	66.4
Female	158	33.6
Neonates	311	66.2
Infants	159	33.8

The study population was predominantly male, with males accounting for 312 of 470 participants. Neonates represented approximately two-thirds of the cohort, while infants constituted about one-third of the study sample.

Table 2. Distribution of Anthropometric and Coagulation Parameters

Variable	Mean ± SD	Minimum	Maximum
Weight, kg	4.2 ± 1.4	2.7	9.9
Platelet count, $\times 10^9$/L	169.4 ± 65.5	105	379
Prothrombin time, seconds	12.9 ± 1.2	10	15
Activated partial thromboplastin time, seconds	28.8 ± 4.0	21	37
Thrombin time, seconds	16.4 ± 1.8	13	20
Fibrinogen, mg/dL	294.8 ± 52.3	205	412

The mean platelet count was 169.4 ± 65.5 $\times 10^9$/L, while the mean prothrombin time, activated partial thromboplastin time, and thrombin time were 12.9 ± 1.2 seconds, 28.8 ± 4.0 seconds, and 16.4 ± 1.8 seconds, respectively. The mean fibrinogen concentration was 294.8 ± 52.3 mg/dL. Platelet count was numerically higher in males than females, with mean values of 170.8 ± 67.8 $\times 10^9$/L and 166.7 ± 60.9

$\times 10^9$/L, respectively. Prothrombin time and thrombin time were similar between male and female participants. Activated partial thromboplastin time was 28.9 ± 4.1 seconds in males and 28.6 ± 4.0 seconds in females. Fibrinogen concentration was numerically higher in females than males, with mean values of 301.3 ± 52.0 mg/dL and 291.5 ± 52.2 mg/dL, respectively.

Table 3. Comparison of Coagulation Parameters According to Sex

Variable	Male, Mean ± SD	Female, Mean ± SD	p-value
Platelet count, $\times 10^9$/L	170.8 ± 67.8	166.7 ± 60.9	0.08
Prothrombin time, seconds	12.9 ± 1.2	12.9 ± 1.1	0.07
Activated partial thromboplastin time, seconds	28.9 ± 4.1	28.6 ± 4.0	0.5
Thrombin time, seconds	16.4 ± 1.8	16.4 ± 1.7	0.8
Fibrinogen, mg/dL	291.5 ± 52.2	301.3 ± 52.0	0.8

Table 4. Comparison of Coagulation Parameters According to Age Group

Variable	Neonates, Mean ± SD	Infants, Mean ± SD	p-value
Platelet count, $\times 10^9$/L	150.3 ± 47.0	206.8 ± 79.2	0.001
Prothrombin time, seconds	12.97 ± 1.3	12.98 ± 1.1	0.8
Activated partial thromboplastin time, seconds	28.8 ± 4.0	28.9 ± 4.1	0.6
Thrombin time, seconds	16.45 ± 1.8	16.41 ± 1.7	0.9
Fibrinogen, mg/dL	295.1 ± 52.7	294.3 ± 51.6	0.7

Platelet count differed between age groups, with infants showing a higher mean platelet count than neonates. The mean platelet count was 150.3 ± 47.0 $\times 10^9$/L among neonates and 206.8 ± 79.2 $\times 10^9$/L among infants. Prothrombin time activated partial thromboplastin time, thrombin time, and fibrinogen values were closely comparable between neonates and infants.

Table 5. Reference Intervals for Coagulation Parameters According to Sex

Variable	Male, 2.5th–97.5th Percentile	Female, 2.5th–97.5th Percentile
Platelet count, $\times 10^9$/L	105–352	105–333
Prothrombin time, seconds	10–15	10.9–15
Activated partial thromboplastin time, seconds	21–37	21–37
Thrombin time, seconds	13–20	13–20
Fibrinogen, mg/dL	205–410	205–410

The sex-specific reference intervals showed similar ranges for most coagulation parameters. Prothrombin time ranged from 10 to 15 seconds in males and 10.9 to 15 seconds in females. Activated partial thromboplastin time and thrombin time had identical reference intervals in both sexes. Fibrinogen ranged from 205 to 410 mg/dL in both male and female participants.

Table 6. Reference Intervals for Coagulation Parameters According to Age Group

Variable	Neonates, 2.5th–97.5th Percentile	Infants, 2.5th–97.5th Percentile
Platelet count, $\times 10^9$/L	105–315	105–367
Prothrombin time, seconds	10–15	10–15
Activated partial thromboplastin time, seconds	21–37	21–37
Thrombin time, seconds	13–20	13–20
Fibrinogen, mg/dL	205–410	205–401

The age-specific reference intervals showed a wider upper limit for platelet count among infants than neonates. Platelet count ranged from 105 to 315 $\times 10^9$/L among neonates and from 105 to 367 $\times 10^9$/L among infants. Reference intervals for prothrombin time, activated partial thromboplastin time, and thrombin time were identical between neonates and infants. Fibrinogen ranged from 205 to 410 mg/dL among neonates and from 205 to 401 mg/dL among infants.

Overall, the established reference intervals for the study population were 10–15 seconds for prothrombin time, 21–37 seconds for activated partial thromboplastin time, 13–20 seconds for thrombin time, and approximately 205–410 mg/dL for fibrinogen. Platelet count demonstrated the clearest age-related difference, with higher mean values and a wider upper reference limit among infants compared with neonates.

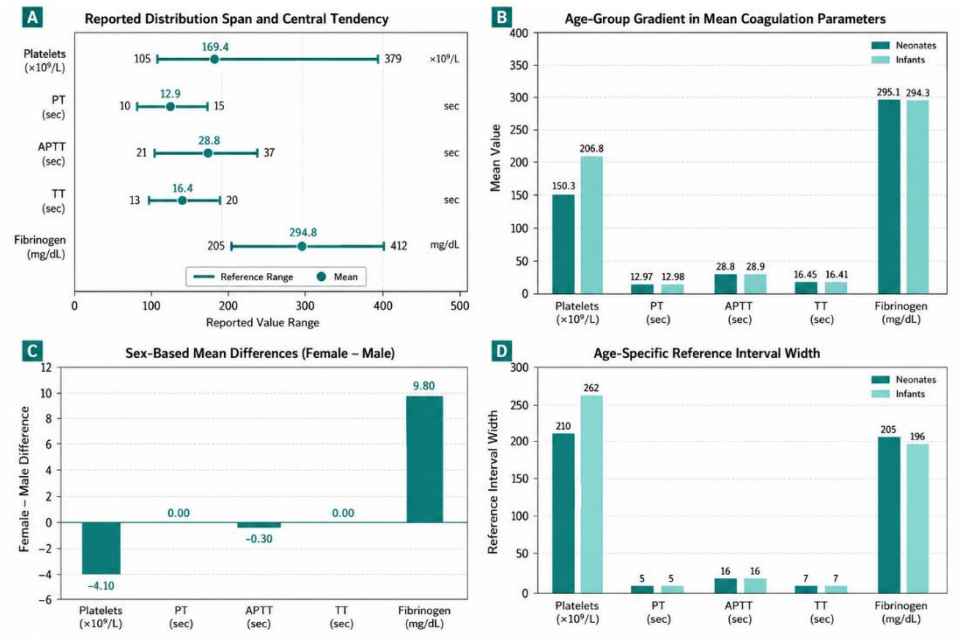

Figure 1 Coagulation Reference Profile in Healthy Pakistani Neonates and Infants. The panelled figure summarizes clinically relevant patterns across the reported coagulation parameters. The overall reported ranges were 105–379 ×10⁹/L for platelet count, 10–15 seconds for prothrombin time, 21–37 seconds for activated partial thromboplastin time, 13–20 seconds for thrombin time, and 205–412 mg/dL for fibrinogen. Age-group comparison showed the clearest gradient for platelet count, increasing from 150.3 ± 47.0 ×10⁹/L in neonates to 206.8 ± 79.2 ×10⁹/L in infants, while prothrombin time, activated partial thromboplastin time, thrombin time, and fibrinogen remained closely comparable across age groups. Sex-based differences were small for most coagulation parameters, although fibrinogen was numerically higher in females than males. Age-specific reference interval width was widest for platelet count, particularly among infants, supporting the need to interpret platelet values with age-specific reference limits in early life.

DISCUSSION

This multicentre cross-sectional study established reference intervals for selected coagulation parameters among healthy Pakistani neonates and infants and examined differences according to sex and age group. The overall reference intervals observed in the study population were 10–15 seconds for prothrombin time, 21–37 seconds for activated partial thromboplastin time, 13–20 seconds for thrombin time, and approximately 205–410 mg/dL for fibrinogen. These findings provide locally relevant laboratory values for early-life coagulation assessment and may support more accurate clinical interpretation in neonatal and infant care. The study also showed that sex-related differences were minimal for most parameters, whereas age-group differences were most evident for platelet count, which was higher among infants than neonates.

The need for local coagulation reference intervals is clinically important because haemostatic parameters in neonates and infants are influenced by developmental physiology, liver maturation, platelet function, coagulation factor concentrations, laboratory reagents, and pre-analytical procedures. In Pakistan, this issue is particularly relevant because inherited bleeding disorders and consanguinity-related conditions may contribute to paediatric haematological presentations, increasing the need for accurate laboratory interpretation (10). Reliance on adult reference values for neonates and infants can result in inappropriate classification of normal developmental variation as abnormal, particularly when local paediatric reference intervals are unavailable.

In the present study, prothrombin time and activated partial thromboplastin time showed narrow and clinically stable intervals across sex and age groups. Prothrombin time reflects the extrinsic and common coagulation pathways, while activated partial thromboplastin time reflects the intrinsic and common pathways; therefore, both tests remain central to screening for coagulation abnormalities in clinical practice (11,12). The observed similarity of PT and APTT between males and females suggests

that sex-specific interpretation may not be necessary for these parameters in the studied age range. Similarly, the small differences between neonates and infants for PT, APTT, and TT indicate relative stability of these screening values within the included early-life age groups.

Platelet count demonstrated the clearest age-related difference. Mean platelet count was higher in infants than neonates, and the upper reference limit was wider among infants. This pattern is consistent with the concept that haematological and haemostatic indices evolve during early development and may require age-specific interpretation. Previous large-scale neonatal platelet reference data have also shown that platelet distributions vary across neonatal populations and clinical contexts, reinforcing the need for age-appropriate reference intervals rather than uniform adult thresholds (15). In the present study, platelet count was numerically higher among males than females; therefore, discussion statements suggesting higher platelet counts in females should be corrected to maintain consistency with the reported results.

Fibrinogen values showed minimal age-related variation but were numerically higher among females than males. Fibrinogen is a key plasma glycoprotein synthesized by the liver and is essential for fibrin formation, clot stability, and haemostatic integrity (13). Although the sex-based fibrinogen difference in this study was small, the reported p-value should be rechecked against the original statistical output because the mean difference appears larger than several other parameters reported as statistically non-significant. Before publication, all p-values should be verified from the original dataset, particularly for sex-based comparisons of PT and fibrinogen.

The findings are broadly aligned with previous paediatric coagulation literature showing that coagulation parameters vary with age and may differ from adult values depending on population, analytical system, and age category. Arslan et al. reported age-dependent reference ranges for coagulation tests in children and highlighted the importance of paediatric-specific interpretation (14). Appel et al. also emphasized age dependency of coagulation parameters during childhood and puberty, supporting the need for age-stratified reference data (16). Zhang et al. established coagulation reference intervals in Chinese children and reported that paediatric ranges may differ substantially from adult intervals (17). These studies collectively support the rationale for developing local reference intervals rather than applying universal adult-derived cut-offs to neonatal and infant populations.

Comparison with studies in very preterm infants must be made cautiously because gestational maturity strongly influences coagulation indices. Neary et al. reported coagulation indices in very preterm infants that differed considerably from values observed in healthy term or near-term populations, particularly for PT, APTT, and fibrinogen (19). Similarly, studies assessing thromboelastographic and thromboelastometry parameters in healthy newborns have shown that neonatal clot formation and clot firmness may differ from adult haemostatic patterns, further supporting the concept of developmental haemostasis (20,21). The present study contributes population-specific data for Pakistani neonates and infants but should not be interpreted as evidence that neonatal and infant values are interchangeable with adult western standards.

This study has several strengths, including multicentre recruitment, inclusion of both neonates and infants, and calculation of percentile-based reference intervals. The study also attempted quality assurance through duplicate readings, control plasma testing, reanalysis of selected samples, and outlier screening. However, important limitations must be acknowledged. First, the manuscript contains an unresolved discrepancy between the reported total sample size of 470 and the original continuous-variable table labelled as n=246. This must be reconciled before publication. Second, details of laboratory reagents, analyser or manual clot-detection method, calibration, sample transport, centrifugation, and inter-centre standardization are insufficient and should be expanded to ensure reproducibility. Third, outlier removal requires clearer reporting, including the number of excluded observations and final analytical denominator for each parameter. Fourth, subgroup reference intervals should be interpreted cautiously if subgroup sample sizes were insufficient for robust percentile estimation. Finally, the study

did not include comparison with an independently validated local paediatric laboratory dataset, limiting external validation of the proposed intervals.

Overall, the study provides clinically useful preliminary reference intervals for coagulation parameters among healthy Pakistani neonates and infants. The most consistent finding was the relative stability of PT, APTT, TT, and fibrinogen across sex and age groups, while platelet count showed a clearer age-related difference. Before final publication, the authors should correct sample-size inconsistencies, verify p-values, clarify laboratory methodology, correct table labelling, and revise the conclusion to avoid inappropriate comparison with adult standards.

CONCLUSION

This study established local reference intervals for selected coagulation parameters among healthy Pakistani neonates and infants, including prothrombin time, activated partial thromboplastin time, thrombin time, platelet count, and fibrinogen. The findings suggest minimal sex-related variation in most coagulation parameters, while platelet count showed the clearest age-related difference, with higher mean values and a wider upper reference limit among infants compared with neonates. These locally derived values may support more accurate interpretation of coagulation screening tests in early life; however, the reference intervals should be applied with attention to age group, laboratory methodology, and final verified analytical sample size.

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