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

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Abstract:

***Objective**: To establish a reference range for coagulation parameters in neonates and infants in our Pakistani population*

***Study Design**: Cross sectional study.*

***Place and Duration of Study**: Pathology department, CMH Quetta from January 2021 to December 2021.*

***Materials and Methods**: Blood sample of 470 healthy neonates (birth to 1 month of age) and infants (<1 year age) of both genders (male and female) visiting E.P.I vaccination centre of Combined Military Hospital of Rawalpindi, Quetta, Lahore, Multan and Karachi were taken. 2ml venous blood sample from neonates and infants was drawn under aseptic condition in tube containing anticoagulant Trisodium Citrate.*

***Results**: Out of total 470 patients 312 (66.4%) were male and 158 (33.6%) were females. Mean age of the patients was 27.8 ± 33.2 days. 311 individuals were neonates (<1months of age) and 159 (33.8%) were infants (>1 month but <1 year of age). In males platelet count was higher than females but this difference was not statistically significant. PT, APTT and TT were similar in both genders. Fibrinogen levels were found to be higher in females. The following reference range were found PT (10 – 15sec), APTT (21 – 37sec), TT (13 – 20sec) and Fibrinogen (205 – 410mg/dl).*

***Conclusion**: The results of this study conclude that reference range for coagulation parameters are almost same from the western standards used for adult population. No significant effect of gender is evident on these reference intervals.*

Keywords: Activated partial thromboplastin time (APTT), Blood clot, Bleeding disorder, Coagulation, Fibrinogen, Hemostasis, Prothrombin time (PT), Thrombin time (TT)

INTRODUCTION:

Haemostasis is the natural protective response in the body which works through various mechanisms and leads to control of bleeding after any injury. This usually is the first step in wound healing[1]. Three basic mechanisms are responsible for maintaining hemostasis in the human body, which include contraction of blood vessels, formation of a platelet plug and lastly formation of fibrin mesh. Various components play a complex role in formation of a blood clot. Interaction of platelets, fibrinogen,

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procoagulant and anticoagulation factors at various stages makes this whole cascade possible[2].

There is a marked difference seen in the hemostatic system of neonates and adults[3]. There is no crossover of coagulation factors between the mother and fetus through the placental barrier. They are synthesized independently in the fetus and is usually effected by gestational age and liver maturity. Neonates usually present with platelets which have decreased activity and a considerably lower levels of coagulation factors as compared to adults[4]. Due to this difference a reference range established for adult population cannot be used to diagnose and treat the coagulation disorders in newborns.

Coagulation screening includes various parameters such as Prothrombin time PT, Activated partial thromboplastin time APTT, thrombin time TT, platelets count, fibrinogen and D dimers. APTT depicts the time blood takes to coagulate[5]. Clotting factors and its activity is measured by PT. These two important tests can predict the abnormalities in both extrinsic and intrinsic pathways[6]. To assess the formation of fibrin from fibrinogen present in plasma thrombin time TT can be used. D dimer is a small fragment made up of protein which is released in the body after dissolution of a blood clot. An increase in its levels may be suggestive of an underlying clotting disorder[7].

In order to correctly diagnose and interpret the results of any laboratory tests a reference range is needed. It is basically defined as two test values present between the 25th and 95th percentile of value present in healthy population[8]. The normal values of coagulation screen tests for newborns were identified many years ago, since than a rapid advancement in analyzers, reagents and techniques have occurred. Many studies have been conducted in the past to establish a reference range for Adults and children but the data for newborns and infants is limited especially pertaining to our population. This study was designed with an aim to establish the reference range for coagulation parameters in neonates and infants in our population. This will be helpful in correct diagnosis and interpretation of laboratory tests based on normal reference values taken from our population.

MATERIALS AND METHODS:

It was a multicenter descriptive cross-sectional study, carried out in the Pathology department, CMH Quetta. The total duration for which the study was carried out was one year from January 2021 to December 2021.

Sample size calculation: As per the clinical and laboratory standards institute Guidance Document C28A2A, minimum sample size of 240 (120 males & 120 females) was required to establish a reference interval[9]. Sample size was inflated to 470. Non probabilityconsecutive sampling technique was used.

Sample collection: Prior Approval was taken from the ethical review committee board. (Reference number of ethical review committee form: CMH-QTA-IRB / 042). Informed consent was taken from the parents prior to enrolling the patients into the study.Demographic details and history were recorded.Blood sample of healthy neonates (birth to 1 month of age) and infants (<1 year age) of both genders (male and female) visiting the E.P.I vaccination centres of combined military hospitals of Rawalpindi,

Quetta, Lahore, Multan and Karachi were taken and considered for inclusion into the study. Those infants who were premature, with any history of congenital disease, blood loss, transfusion of blood or blood products, any known systemic illness or with any drug history were excluded from the study.

2ml venous blood sample from new-borns and infants was taken under aseptic conditions in tube containing anticoagulant Trisodium Citrate. All the samples were manually analysed within 1 hour of blood collection by incubation in water bath at optimum temperature (37ºC)and results were recorded by taking the mean of two readings. Repeated on control plasma to check validity of procedure. 40 samples were reanalysed in order to check for any errors.

Statistical analysis: Data was analysed using SPSS version 25.0. Mean, SD and 2.5th and 97.5th percentiles were calculated for variables such as Age, weight,PT, APTT, TT, D dimer, platelet count and fibrinogen. Percentage and Frequency was calculated for variables (categorical) such as gender and Age group (new-born vs infants). Comparison of coagulation parameters was carried out among male and female subjects and between infants and new-bornsusing independent samples T test.2.5th and 97.5th percentiles were calculated for establishment of reference range. Outliers (10% out of manufacturers range) were identified and removed from the study samples by visually inspecting the data and using the method proposed by Dixon. p value of ≤0.05 was considered to be significant.

RESULTS:

Out of total 470 patients 312 (66.4%) were male and 158 (33.6%) were females. Mean age of the patients was 27.8± 33.2 days. Age range of the patients included in the study fell between1– 231 days. 311 individuals were neonates (<1months of age) and 159 (33.8%) were infants (>1 month but <1 year of age)Basic characteristics of the study population are listed in table I:

Table I: Basic characteristics of study population (n=246)

Variables	Mean ± SD	Minimum	Maximum
Weight (kg)	4.2 ± 1.4	2.7	9.9
Platelets (x10^9/l)	169.4 ± 65.5	105	379
PT (sec)	12.9 ± 1.2	10	15
APTT (sec)	28.8 ± 4.0	21	37
TT (sec)	16.4 ± 1.8	13	20
Fibrinogen (mg/dl)	294.8 ± 52.3	205	412

In males platelet count was higher than the females but this difference was not statistically significant. PT, APTT and TT were similar in both genders. Fibrinogen levels were found to be higher in females. Table II

Table II: Comparison of Coagulation parameters among Male and Female population

Variables	Male (Mean ± SD)	Female (Mean ± SD)	p value
Platelets (x10^9/l)	170.8 ± 67.8	166.7 ± 60.9	0.08
PT (sec)	12.9 ± 1.2	12.9 ± 1.1	0.07
APTT (sec)	28.9 ± 4.1	28.6 ± 4.0	0.5
TT (sec)	16.4 ± 1.8	16.4 ± 1.7	0.8
Fibrinogen (mg/dl)	291.5 ± 52.2	301.3 ± 52.0	0.8

With increasing age Platelet count, PT, APTT, TT were seen to show an increasing trend. Fibrinogen levels were lesser in infants as compared to neonates. Table III.

Table III: Comparison of Coagulation parameters among Neonates and Infants

Variables	Neonates (Mean ± SD)	Infants (Mean ± SD)	p value
Platelets (x10^9/l)	150.3 ± 47.0	206.8 ± 79.2	0.001
PT (sec)	12.97 ± 1.3	12.98 ± 1.1	0.8
APTT (sec)	28.8 ± 4.0	28.9 ± 4.1	0.6
TT (sec)	16.45 ± 1.8	16.41 ± 1.7	0.9
Fibrinogen (mg/dl)	295.1 ± 52.7	294.3 ± 51.6	0.7

Reference range of Coagulation parameters for male/female and infants/neonates were calculated and tabulated using the 2.5th and 97.5th percentile. Table IV and Table V.

Table IV: Reference range of Coagulation parameters among Male and Female population

Variables	Male (2.5th- 97.5th percentile)	Female (2.5th- 97.5th percentile)
Platelets (x10^9/l)	105 – 352	105 – 333
PT (sec)	10 – 15	10.9 – 15
APTT (sec)	21 – 37	21 – 37
TT (sec)	13 – 20	13 – 20
Fibrinogen (mg/dl)	205 – 410	205 – 410

Table V: Reference range of Coagulation parameters among Neonates and Infants

Variables	Male (2.5th - 97.5th percentile)	Female (2.5th - 97.5th percentile)
Platelets (x10^9/l)	105 – 315	105 – 367
PT (sec)	10 – 15	10 – 15
APTT (sec)	21 – 37	21 – 37
TT (sec)	13 – 20	13 – 20
Fibrinogen (mg/dl)	205 – 410	205 – 401

DISCUSSION:

Coagulation disorders are among the highly prevalent conditions presenting a greater challenge to the health authorities in Pakistan. The reason of this high incidence is the prevalence of consanguineous marriages in our region[10]. The first step in providing accurate treatment to the patient with such disorders is the establishment of a correct diagnosis of the underlying disease. The laboratory results are compared with some set standards and reference ranges. No significant work has been done in our population regarding the establishment of reference ranges for coagulation parameters in our population especially those of infants and neonates. The haemostatic system of newborn is drastically different from the adults, thus the reference intervals set by examining the adults cannot be used in the neonates.

The time taken by the clot to form is assessed by using PT and APTT. In PT a specialised substance called thromboplastin is added where as in APTTplatelet substitute kaolin mixture along with calcium chloride is added andthe clot formation is assessed under normal conditions. Abnormalities in the clotting pathways (intrinsic and extrinsic) both are assessed by these tests. PT usually is used to detect disorders of the extrinsic pathway including activity of clotting factor I, II, V, VII and X. Abnormalities of intrinsic pathways are seen by APTT including activity of clotting factor I, II, V, VIII, IX, X, XI, XII, XIII.[11,12]. In our study the reference ranges for coagulation parameters showed no difference among male and female subjects. Platelet count was however, slightly greater in females. The reference interval of PT was found

to be 10- 15 seconds which was slightly greater than the adults (11-13.5 sec). No difference in APTT was found between neonates and adults values.

The inactive zymogen, prothrombin circulating in plasma gives rise to Thrombin. It is one of the most strong platelet agonist. It has a proteolytic activity which removes fibrin peptidase A and B from fibrinogen and results in the polymerization of fibrin mesh to form a clot. One of the most important glycoprotein of coagulation cascade, having its origin in liver cells is fibrinogen[13].

Arsalan et el in his study revealed that PT and APTT values were significantly higher in children as compared to adults[14]. The reference interval set by weidmer et al in his study gave a slightly wider range for platelet count using the 5th and 95th percentiles. This range was 104- 750 x10^{9}/l[15]. Appel et al established the need for having age related ranges for coagulation parameters as it widely varies with increasing age in childhood and puberty[16]. Zhang et al established reference intervals for coagulation parameters in Chinese children and concluded that Ranges for children among these parameters differed widely as compared to the adults[17]. Kiran et al conducted a study in Pakistan on healthy infants and revealed that PT showed a range of 12-15 seconds in 95% population and APTT was found to be between 32-35 seconds[18]. In another study conducted on healthy volunteers revealed that mean PT was 11.95± 0.7 sec and APTT was 40.5 ± 5.30 sec. The reference ranges (at 5th and 95th percentile) were 10.8 – 13.3 sec for PT and 31.4- 48 sec for APTT[19]. Neary et al in their large cross sectional study formulated normal coagulation indices for preterm infants. Range for PT (12.7-26.6 sec), APTT (48.7-134.3 sec) and fibrinogen (0.72-3.8 g/l) were quite different as found in our study on full term healthy infants[20]. Rachel et al performed a study on parameters of thromboelastographic in healthy newborns and concluded that the clot formation beginning, firmness of clot and platelet function analysis were quite different among neonates when compared to adults standards.

Obtaining a reference interval in the coagulation laboratory for neonates presents the clinician with many challenges, some of which include the lack of time and expertise of sample collection, non-standardized reagents that are being used, and samples which are least stable[21]. There is a need of conducting large scale studies and design a reference range based on age keeping in mind the concept of developmental haemostasis. This is highly significant to understand in order to ensure optimum diagnosis, prevention and treatment of thrombotic and haemorrhagic diseases in new-borns and children.

CONCLUSION:

The results of this study conclude that reference range for coagulation parameters are almost same from the western standards used for adult population. No significant effect of gender is evident on these reference intervals.

Conflict of interest: The author declares no conflict of interest.

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