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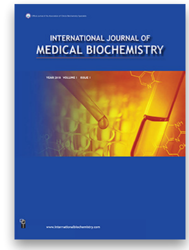

Research Article

Investigation of Prothrombin Time, International Normalized Ratio and Activated Partial Thromboplastin Time reference ranges in children

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Abstract

Objectives: This study aimed to ascertain pediatric age-specific reference ranges for prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT). Retrospective data were obtained from healthy children who had undergone preoperative tests and compared with those obtained from a group of adult patients.

Methods: Reference individuals were determined by the indirect method. A total of 15,179 patients who presented to our hospital in 2022 and 2023 were retrospectively reviewed. Pediatric patients were divided into three age groups: 1–5 years (n=1949), 6–10 years (n=1563), and 11–17 years (n=508). The adult age group consisted of healthy individuals aged 18–50 years (n=11165). The tests were run in a coagulation autoanalyzer with the mechanical coagulometric measurement method. Reference ranges were analyzed using the non-parametric method statistically.

Results: The mean PT, INR, and aPTT values were found to be 14.34±1.99 s, 1.07±0.16, and 31.43±3.47 s, respectively, in children aged 1–5 years; 14.48±1.40 s, 1.08±0.11, and 31.30±2.66 s, respectively, in those aged 6–10 years; and 14.73±1.12 s, 1.10±0.09, and 31.21±2.91 s, respectively, in those aged 11–17 years. Among the adults aged 18–50 years, the mean PT, INR, and aPTT values were 13.95±1.40 s, 1.04±0.11, and 30.21±2.86 s, respectively. The mean PT, INR, and aPTT values of children aged 1–5 years, 6–10 years, and 11–17 years were statistically significantly higher than those of adults aged 18–50 years (for each group $p<0.01$).

Conclusion: It is important for laboratories to employ age-specific reference ranges for coagulation tests performed on children to ensure accurate diagnosis and avoid unnecessary further investigations. In this study, the reference ranges of the PT, INR, and aPTT parameters were determined for pediatric patients and found to be significantly higher than those of adults, which will be useful for clinical evaluation and diagnosis.

Keywords: Age group, coagulation tests, pediatric, reference ranges

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Prothrombin time (PT) is a measure used to examine the extrinsic pathway of coagulation. This test relies on the measurement of fibrin clot formation time after the addition of whole tissue thromboplastin (from lung, brain, or placenta-phospholipid extract) and calcium to citrate-containing plasma samples using the one-step Quick method [1]. PT is used to determine the risk of bleeding before surgical interventions, evaluate coagulation disorders, and assess liver function. In 1983, a model based on the international normalized ratio (INR) was defined for the standardization of PT. This method reports PT results in the form of INR, ensuring consistency across different laboratories, thromboplastins, and devices [2]. Activated partial thromboplastin time (aPTT) is a coagulation parameter used to evaluate the intrinsic coagulation pathway. This test measures the interaction between clotting factors and the conversion of fibrinogen to fibrin.

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aPTT is used to monitor heparin therapy, detect disorders in clotting factors, and diagnose bleeding disorders [3].

Reference ranges are one of the most frequently used decision-making tools to determine whether an individual is healthy. It is recommended that each laboratory determine its own reference ranges, and the reference population that best reflects the society should be carefully selected. Reference individuals are determined by direct or indirect methods. The direct method is a prospective method in which well-defined exclusion criteria are applied before the determination of reference individuals. In the indirect method, retrospective laboratory values from the hospital database are used to determine reference intervals. This method is preferred especially in the pediatric group, where it is very difficult to collect samples from healthy subjects. For an accurate assessment, it is of great importance to establish reference ranges correctly and determine the changes based on factors such as age and gender [4]. The International Society on Thrombosis and Haemostasis (ISTH) has reported that the use of adult reference ranges for the determination of coagulation disorders in pediatric patients may lead to erroneous assessments and recommends that each laboratory establish their own population-specific pediatric reference ranges, considering the combination of the analyzer and reagent used [5, 6].

Since the physiology of hemostasis in pediatric patients is very different from that in adults, reference ranges vary depending on the age of the children. Therefore, the use of adult reference ranges makes it harder to interpret pediatric coagulation tests accurately, and laboratories are required to develop age-specific reference ranges for children to ensure accurate diagnosis and avoid unnecessary further investigations [7, 8].

The objective of this study was to ascertain pediatric age-specific reference ranges for PT, INR, and aPTT. For this purpose, retrospective data were obtained from children aged between 1–17 years who had undergone preoperative tests and compared with reference values obtained from a group of adult patients. It is considered that the establishment of age-specific reference ranges for pediatric patients will be useful for clinical evaluation and diagnosis.

Materials and Methods

Approval was received for this study from Istanbul Medipol University Ethics Committee (No: E-10840098-772.02-7732, Date: 12/12/2023). Reference individuals were determined by the indirect method. The data on coagulation tests (PT, INR, and aPTT) performed at Medipol Mega University Hospital from January 2022 to December 2023 were retrospectively reviewed. The study was carried out based on the data of a total of 15,179 patients of whom 61.1% were female (n=9279) and 38.9% were male (n=5909). Pediatric patients were divided into three age groups: 1–5 years (n= 1949), 6–10 years (n=1563), and 11–17 years (n=508) [5]. The adult age group consisted of healthy individuals aged 18–50 years (n=11165).

The PT, INR, and aPTT results of preoperative tests required for minor surgical procedures (inguinal and umbilical hernia operations, excision of rectal polyps, diagnostic cystoscopy, tonsillectomy, septoplasty, dental treatment, etc.) were evaluated. The exclusion criteria included having a history of bleeding or thrombus, receiving anticoagulant therapy, and having an acute infection, malignancy, or cirrhosis.

The plasma obtained after centrifugation at 3000 rpm for 10 minutes in citrated tubes was analyzed using the mechanical coagulometric measurement method in a Stago Compact Max 3 coagulation autoanalyzer. The PT test relies on the use of calcium thromboplastin to measure the clotting time of the plasma obtained from the patient and its comparison to the normal standard. In principle, the aPTT test involves the recoagulation of plasma in the presence of a standard amount of cephalin and kaolin.

PT was analyzed as PT (sec), PT activity (%), and INR using a STA NeoPTimal kit. INR was calculated using the ratio of the patient's PT value to the mean value of the normal reference range raised to the power of the reagent international sensitivity index (ISI). The ISI for the PT reagent used was 1.01. aPTT was analyzed as aPTT (sec) using a STA C.K PREST kit.

Calibration and control samples were assayed using the standard methods of the manufacturer. These tests were evaluated with normal and abnormal controls on a daily control basis, as well as by following a monthly ECAT external quality control program.

Statistical Analyses

The Statistical Package for the Social Sciences (SPSS) 2020 program was used for statistical analyses when assessing the findings obtained from the study. Quantitative variables were represented using mean, standard deviation, median, minimum, and maximum values, and qualitative variables were represented using descriptive statistical methods, such as frequencies and percentages. A non-parametric data distribution was observed for the PT, INR, and aPTT values. The Shapiro-Wilk test and box plots were used to evaluate the conformity of the data to the normal distribution. The Mann-Whitney U test was conducted to compare the variables that did not display a normal distribution between paired groups. The chi-square test was used to compare qualitative data. The results of reference ranges were evaluated at a 95% confidence interval and a significance level of $p<0.05$.

Results

The reference range values for PT, PT activity, INR, and aPTT were analyzed for the three pediatric age groups. These values were compared to the reference values obtained from the adult group. The comparison of the data by age groups is shown in Table 1. The age distributions of PT, PT activity, INR, and aPTT are presented in Figures 1, 2, 3, and 4, respectively.

Table 1. Data comparison by age

	Age groups						
	1–5 years (n=1949)	6–10 years (n=1563)	11–17 years (n=508)	18–50 years (n=11165)	p (1–5/ 18–50)	p (6–10/ 18–50)	p (11–17/ 18–50)
Gender, n, (%)							
Female	790 (40.5)	574 (36.7)	204 (40.2)	7711 (69.1)	[a]0.001**	[a]0.001**	[a]0.001**
Male	1162 (59.5)	989 (63.3)	304 (59.8)	3454 (30.9)			
PT(s)							
Mean±SD	14.34±1.99	14.48±1.40	14.73±1.12	13.95±1.40	[b]0.001**	[b]0.001**	[b]0.001**
Median (min-max)	14.2 (11–80)	14.3 (10.6–47.9)	14.6 (11.6–23.3)	13.8 (8–53.5)			
95% CI	14.25–14.42	14.41–14.55	14.63–14.82	13.91–13.97			
PT activity (%)							
Mean±SD	91.47±10.53	89.66±9.81	87.08±9.43	95.39±11.12	[b]0.001**	[b]0.001**	[b]0.001**
Median (min-max)	92 (11–120)	90 (19–120)	88 (46–120)	96 (17–120)			
95% CI	91.00–91.94	89.17–90.15	86.26–87.90	95.21–95.62			
INR							
Mean±SD	1.07±0.16	1.08±0.11	1.10±0.09	1.04±0.11	[b]0.001**	[b]0.001**	[b]0.001**
Median (min-max)	1.06 (0.89–6.47)	1.07 (0.9–3.89)	1.09 (0.9–1.8)	1 (0.9–4.1)			
95% CI	1.07–1.08	1.08–1.09	1.10–1.11	1.039–1.043			
aPTT (s)							
Mean±SD	31.43±3.47	31.30±2.66	31.21±2.91	30.21±2.86	[b]0.001**	[b]0.001**	[b]0.001**
Median (min-max)	31.2 (18.4–74.9)	31.1 (20.4–68.7)	31 (19.6–66.0)	30 (14.9–115.4)			
95% CI	31.28–31.59	31.17–31.43	30.96–31.46	30.16–30.26			

**: p<0.01. [a]: Pearson Chi-Square; [b]: Mann-Whitney-U Test. PT: Prothrombin time; SD: Standard deviation; CI: Confidence interval; INR: International normalized ratio; aPTT: Activated partial thromboplastin time.

The mean PT, INR, and aPTT values of children aged 1–5 years, 6–10 years, and 11–17 years were statistically significantly higher than those of adults aged 18–50 years (p=0.001, p=0.001, p=0.001, and p<0.01, respectively for PT; p=0.001, p=0.001, p=0.001, and p<0.01, respectively for INR; and p=0.001, p=0.001, p=0.001, and p<0.01, respectively, for aPTT). The mean PT activity values of children aged 1–5 years, 6–10 years and 11–17 years were significantly lower than those of adults 18–50 years (p=0.001, p=0.001, p=0.001, p=0.001 and p<0.01 respectively for PT activity).

The PT, PT activity, INR, and aPTT values were also evaluated in each age group by gender (Table 2). The mean PT, PT activity, and INR values did not show any statistically significant differences according to gender in the pediatric group aged 1–5 years (p>0.05), while the mean aPTT value of the male children was statistically significantly higher than that of the female children in this group (p=0.038; p<0.05). In the pediatric group aged 6–10 years, there were no statistically significant differences in PT, PT activity, INR, or aPTT values according to gender (p>0.05). The PT, PT activity, and INR values did not statistically significantly differ between the male and female children in the 11–17-year-old group (p>0.05); however, the mean aPTT value of the male children in this group was sta-

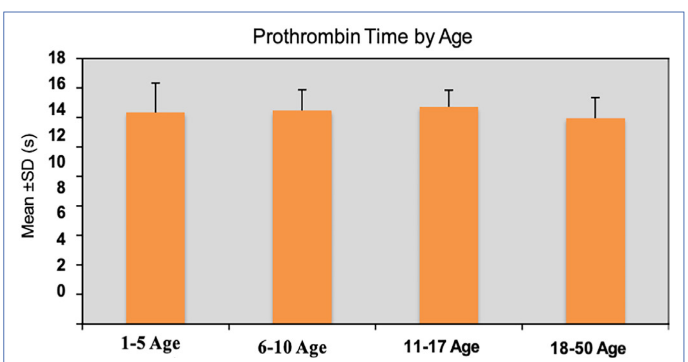

Figure 1. Distribution of prothrombin time by age.

SD: Standard deviation.

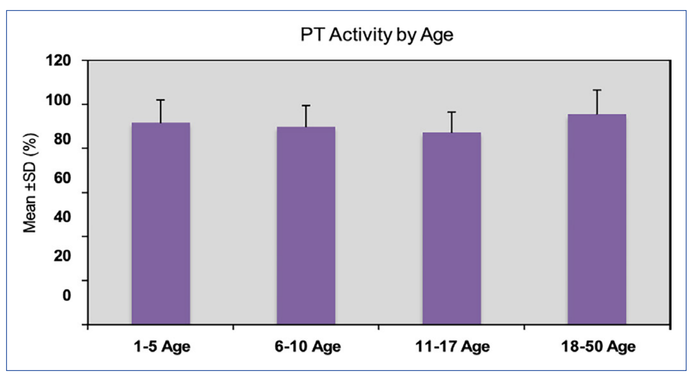

Figure 2. Distribution of PT activity by age.

PT: Prothrombin time.

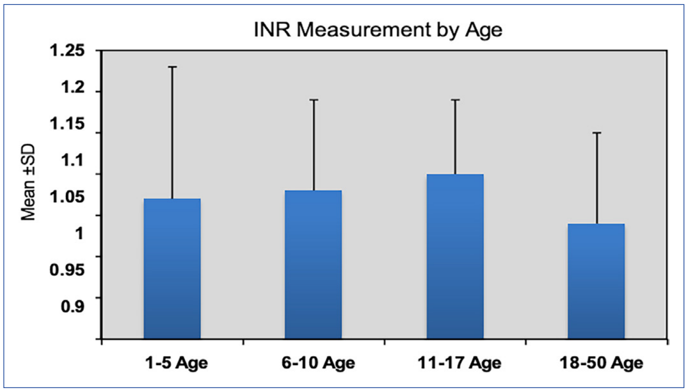

Figure 3. Distribution of INR measurement by age.

INR: International normalized ratio.

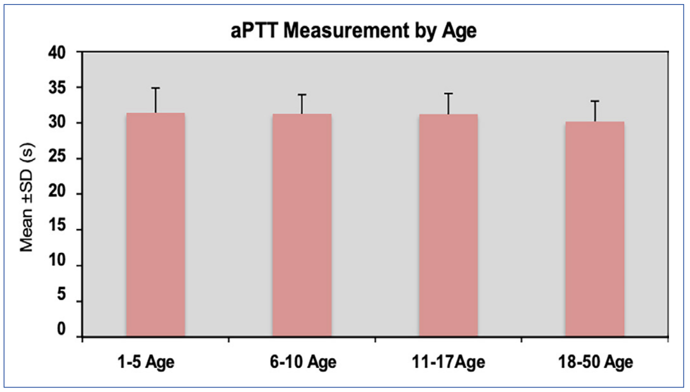

Figure 4. Distribution of aPTT measurement by age.

aPTT: Activated partial thromboplastin time.

tistically significantly lower than that of the female children ($p=0.007$; $p<0.01$). Lastly, no gender-related statistically significant differences were observed in the PT, PT activity, INR, or aPTT values of the adult group aged 18–50 years ($p>0.05$).

Discussion

PT and aPTT are generally considered routine tests to assess preoperative bleeding risk. PT and its mathematical derivative, INR, allow for monitoring vitamin K antagonist therapy. Age-related reference ranges may vary due to differences in physiologic hemostasis in children. Therefore, reference ranges for pediatric age groups should be used [8, 9]. Population, reagent, and analyzer-specific differences in reference ranges have been previously shown [5, 6].

Currently, there are many coagulation analyzers and reagents available for the analysis of PT, INR, and aPTT. Some earlier studies used different analyzers and reagents and established pediatric and adult reference ranges for PT, INR, and aPTT tests.

Liu et al. [10] found age-related differences between children and adults for PT. For aPTT, all pediatric age groups had higher median values than adults. Aral et al. [11] reported that PT levels differed according to age and gender, but there were no age-related differences in aPTT levels. Greenway and Monagle [7] suggested that many children might be misdiagnosed with von Willebrand disease due to prolonged aPTT values compared to adults. Sivrikaya et al. [12] reported that the average PT level of the 0-14-year-old group was 0.4 seconds longer than that of adults. In contrast, Ercan et al. [13] determined that the PT reference ranges were similar between the pediatric and adult groups. On the other hand, the determined reference ranges for aPTT were significantly higher in all pediatric groups compared to adults. In a study by Flanders et al. [14], the PT values were found to be higher in the 7-17-year-old group compared to the adult group, and they showed no difference in aPTT values between the groups.

In our study, the reference ranges of PT, INR, and aPTT were determined for all pediatric age groups, including 1–5 years, 6–10 years, and 11–17 years, and all three parameters were found to be significantly higher when compared to the adult reference ranges (for each group $p<0.01$).We think that low levels of coagulant proteins (II, V, VII, IX, X, XI, XII) in children caused prolonged PT, INR and low levels of Vitamin K–dependent coagulation factor IX concentrations and von Willebrand Factor caused prolonged aPTT [7, 9]. Our study is one of the rare studies in the literature that is compatible with physiological hemostasis in children.The study was conducted with a large reference population. Age-related reference ranges will be very useful in terms of accurate diagnosis in the clinics.

The discrepancies in age-related reference ranges between studies may be due to the differences in the coagulation analyzer and reagent used, as well as the reference intervals of the population and analyzer. This may also result from the variance of pediatric age groups in each study.

Some studies have evaluated differences in PT, INR, and aPTT by gender in various age groups. Sultana et al. [15] reported that male and female individuals had significantly different PT and INR values. The authors noted that men and women aged 15–50 years were more likely to have increased PT, INR, and aPTT readings than the pediatric group aged 0–14 years.

Based on the results of our study, the pediatric group aged 6–10 years and the adult group (aged 18–50 years) showed no significant differences in the PT, INR, or aPTT values when evaluated according to gender ($p>0.05$).

The PT and INR values also did not statistically significantly differ by gender in the 1-5-year-old and 7-11-year-old groups ($p>0.05$); however, the aPTT value of the male children in the 1-5-year-old group was higher than that of the female children ($p=0.038$; $p<0.05$), while the aPTT value of the male children in the 11-17-year-old group was significantly lower than that of the female children ($p=0.007$; $p<0.01$). The difference in aPTT levels in the 1-5-year-old group may be due to the numerical difference between male and female

Table 2: Data comparison by gender

	Age groups							
	1–5 years		6–10 years		11–17 years		18–50 years	
	Female	Male	Female	Male	Female	Male	Female	Male
PT (s)								
Mean±SD	14.35±1.57	14.33±2.23	14.53±1.83	14.45±1.08	14.77±1.09	14.7±1.15	13.93±1.38	13.98±1.44
Median (min-max)	14.2 (11.8–42)	14.2 (11–80)	14.4 (12.1–47.9)	14.3 (10.6–21.7)	14.7 (12.1–17.9)	14.5 (11.6–23.3)	13.8 (8–53.5)	13.8 (11.3–48.6)
^{b}p	**0.761**		**0.603**		**0.255**		**0.093**	
PT activity (%)								
Mean±SD	91.37±10.6	91.54±10.48	89.61±9.99	89.69±9.71	86.71±9.47	87.33±9.41	95.53±11.13	95.07±11.09
Median (min-max)	92 (22–120)	92 (11–120)	90 (19–120)	90 (50–120)	86 (65–115)	88 (46–120)	96 (17–120)	95 (19–120)
^{b}p	**0.920**		**0.839**		**0.308**		**0.171**	
INR								
Mean±SD	1.07±0.13	1.07±0.18	1.09±0.15	1.08±0.09	1.11±0.1	1.1±0.09	1.04±0.11	1.04±0.11
Median (min-max)	1.1 (0.9–3.4)	1.1 (0.9–6.5)	1.1 (0.9–3.9)	1.1 (0.9–1.7)	1.1 (0.9–1.8)	1.1 (0.9–1.8)	1 (0.9–4.1)	1 (0.9–3.9)
^{b}p	**0.914**		**0.740**		**0.269**		**0.125**	
aPTT (s)								
Mean±SD	31.33±3.83	31.50±3.2	31.31±2.41	31.29±2.79	31.53±2.48	31.00±3.15	30.18±2.88	30.28±2.8
Median (min-max)	31.1 (18.4–74.9)	31.2 (21.3–55.3)	31.1 (23.6–40.2)	31 (20.4–68.7)	31.4 (25.3–44)	30.6 (19.6–66)	30 (14.9–115.4)	30.1 (21.3–108.6)
^{b}p	**0.038***		**0.585**		**0.007****		**0.061**	

*: $p<0.05$; **$p<0.01$. ^{b}: Mann-Whitney-U test. PT: Prothrombin time; SD: Standard deviation; INR: International normalized ratio; aPTT: Activated partial thromboplastin time.

children. The difference in aPTT levels at puberty (11-17-year-old group) according to gender may be due to the menstrual cycle. Von Willebrand Factor levels vary according to the days of the menstrual cycle, and estrogen increases the synthesis of coagulation factors such as II, VII, IX, X, which are dependent on vitamin K [16]. Therefore, the menstrual cycle may affect the results.

The varying results reported by studies in terms of gender comparisons may be due to the different groupings of individuals included in each sample.

Laboratory findings should be interpreted in light of the child's age, the analyzer used, and the appropriate reagent reference ranges to provide accurate treatment of hemostatic disorders in the pediatric population. Erroneous test findings are likely to result in the need for additional diagnostic examinations and consultations, inappropriate treatment, cancellation of surgical operations, and an extra financial burden on patients.

Laboratories cannot always rely on adapting literature data on the combination of analyzers and reagents to align with their own needs. It is also often not possible for each laboratory to develop their own reference ranges. It can therefore be concluded that using adult reference ranges in the pediatric population has the potential to result in false-positive assessments in distinguishing if individuals are healthy. It is evident that our study will provide benefits for the clinical evaluation of coagulation tests in pediatric patients. It will also serve as a guide for laboratories using the same kit and analyzer.

Limitations

There are two limitations of the study. First, reference individuals were determined by the hospital database retrospectively. There may be unhealthy individuals in this data. Second, menstrual cycle information for girls aged 11–17 is not available. Therefore, multicenter prospective studies can be conducted in the future.

Conclusion

In conclusion, the hemostatic system in children continues its maturation until adulthood. Pediatric reference ranges of PT, INR, and aPTT are necessary in laboratories and they are very important for clinical diagnosis. Our study revealed age-specific reference ranges to be used in order to avoid erroneously high PT, INR, and aPTT results in children. It is considered that the study will contribute to the literature by presenting data obtained from a large case group.

Ethics Committee Approval: The study was approved by The Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (No: E-10840098-772.02-7732, Date: 12/12/2023).

Conflict of Interest: The authors declare that there is no conflict of interest.

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