Original Research Article

Reference Interval of Prothrombin Time and Activated Partial Thromboplastin Time and their association with age among healthy adults in Kumasi, Ghana

ABSTRACT

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are used to access blood coagulation disorders. Use of reference intervals from a different population may result in misinterpretation and misdiagnoses as the reference intervals for the two tests varies from one geographical area to the other. This study established reference intervals for PT and APTT and evaluated their association with age among healthy adults in Kumasi, Ghana. A total of 876 healthy adults, 18-48 years, all residents of Kumasi, Ashanti region, Ghana were recruited for this cross-sectional study conducted at Komfo Anokye Teaching Hospital (KATH). PT and APTT were determined using the Biobase COA series Semi Auto Coagulation Analyzer following the manufacturer's instructions. Reference intervals were established using non-parametric approach: 2.5th-97.5th percentiles. The reference intervals for PT and APTT were 11.4-15.9 seconds and 26.3-44.1 seconds respectively. The reference intervals were wider compared to the reference intervals used at KATH. Participants between the ages of 21-30 years old had significantly higher PT and APTT compared with participants between 18-20 years and 31-48 years old. PT was inversely association with age (β = -1.092, p=.000) among the general population. Upon stratification by gender, PT showed a significant inverse association (β = - .705, p=.000) among males and a direct association (β = .566, p=.004) among females. The association between age and APTT was not statistically significant. There are wider reference intervals for PT and APTT among people in Kumasi. Due to geography, lifestyle, and genetic diversity, it is advisable that each laboratory establishes geography-specific reference intervals for PT and APTT.

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Keywords: Prothrombin time; Activated partial thromboplastin time; Reference interval; Age

1. INTRODUCTION

The Haemostasis is an innate response which involves the complex interplay between platelets, vessel wall and adhesive proteins culminating in the formation of a 'platelet plug'. It represents a delicate balance between the coagulation and the fibrinolytic systems [1, 2].

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In the 1960's, Davie, Ratnoff and Macfarlane described the "waterfall" and "cascade" theories outlining the fundamental principle of cascade of proenzymes leading to the activation of a downstream enzyme, heralding the concept of blood coagulation [2, 3]. The conventional coagulation system is classified into the extrinsic and the intrinsic pathways [4].

Prothrombin time (PT) and activated partial thromboplastin time (APTT) are tests that characterize blood coagulation and detect blood clotting abnormalities. Prothrombin is a 68.7

kD unstable plasma protein produced in the liver that undergoes proteolytic cleavage to produce a smaller protein, thrombin [2]. PT, the time in seconds for patient plasma to clot after the addition of calcium and an activator (thromboplastin), is a measure of the integrity of the extrinsic and final common pathways of the coagulation system. It is prolonged when there are deficiencies or inhibitors of clotting factors within the extrinsic and final common pathways [5]. It is used in the assessment and monitoring of anticoagulant therapy, liver damage, and vitamin K status [6, 7]. PT measures factors I, II, V, VII, and X and is used in conjunction with the APTT, to detect blood clotting abnormalities [7].

The APTT, on the other hand, measures the integrity of the intrinsic and final common pathways of the coagulation cascade. It is the time in seconds for patient plasma to clot after the addition of phospholipid and calcium and it is prolonged when there are deficiencies or inhibitors of clotting factors within the intrinsic and final common pathways [5]. APTT is also used to monitor the effects of the anticoagulants heparin therapy as well as to screen for bleeding risk prior to surgery [5].

At Komfo Anokye Teaching Hospital (KATH), PT and APTT are utilized routinely for the diagnosis of coagulation disorders. However, the use of PT and APTT in diagnosis and the monitoring of therapy depends on a set of predetermined reference intervals. Several studies [5, 8-11] have reported variations in the reference intervals for PT and APTT due to several factors including age, gender and geographical location. However, studies on reference intervals for PT and APTT have not been conducted in Ghana resulting in the reliance of many healthcare facilities in Ghana on the reference intervals established in Caucasian populations. However, due to the variations in lifestyle, physical and genetic characteristics of the different populations, the use of reference intervals established from another population may lead to misdiagnoses, and incorrect therapy. It is against this background that this study was conducted to specifically:

1. Establish the reference intervals for PT and APTT among healthy adults in Kumasi
2. Evaluate the association between PT and APTT with age

2. MATERIAL AND METHODS

2.1 Study design and setting

This cross-sectional study was conducted at Komfo Anokye Teaching Hospital (KATH) located in Kumasi, the capital of the Ashanti Region in Ghana between February to July 2017. Kumasi is Ghana's second largest city located about 300 km from the national capital, Accra. The city of Kumasi lies between latitude 6.35˚N and 6.40˚N and longitude 1.3˚W and 1.35˚W and is 150sq km in size with a population of about 2 million people [12].

2.2 Participants' recruitment

A simple randomized sampling technique was used to recruit a total of 876 healthy adults aged 18-48 years. The sample size was calculated using the Raosoft sample size calculator participants at 95% confidence level, 5% margin of error, and a response distribution of 50% [13]. Previous clinical history of all subjects was assessed through an interview. Basal information includes dietary, medical and family history, use of tobacco, alcohol intake, and recent physical activities. Participants who satisfied the inclusion criteria were identified and included in the study after the aim and objectives had been explained to them.

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2.3 Inclusion and Exclusion criteria

Apparently healthy participants aged 18-48 years, who consented to the study were recruited. Participants who had hypertension, diabetes, liver disease, on any medication (heparin, warfarin, aspirin or any similar drugs), pregnant women, those with history of alcohol and tobacco use and coagulation disorders were excluded.

2.4 Ethics approval and consent to participate

Ethical approval for this study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (CHRPE/AP/219/17) and also from the Research and Development Department of KATH. Informed consent was obtained from all participants after the aim and objectives of the study had been explained to them.

2.5 Sample collection and preparation

Five (5) ml of venous blood was collected from the antecubital fossa with only a light tourniquet to avoid venous stasis, and dispensed into 3.2% sodium citrate tube and the blood was centrifuged immediately at 2500g for 15 minutes at room temperature to obtain platelet-poor plasma. The PT and APTT for each participant was evaluated using the Biobase COA series Semi Auto Coagulation Analyzer (Biobase LLC, 3231 Osgood Common Fremont, CA 94539, USA) within 4 hours of collection following the manufacturer's instructions. Daily calibration and maintenance of the analyzer was performed according to the manufacturer's instructions. In-house method validation was in accordance with the CLSI guidelines [14, 15]. Pre-analytical, analytical, and post-analytical precautions were considered to ensure accuracy and precision. Internal quality control was performed and analyses begun only when all quality controls passed. Precision of analysis was assessed by internal quality control and accuracy was determined based on external quality control performance.

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2.6 Statistical analysis

All categorical data were presented as frequencies (percentages). The data was not normally distributed using the Kolmogorov-Smirnov test ($p<0.05$) and presented as median (interquartile ranges). Reference intervals were determined using the non-parametric method at 2.5th and 97.5th percentiles, according to and the CLSI guidelines on defining, establishing and verifying reference intervals in the clinical laboratory (CLSI C28-A3) [16]. Outliers were retained in the distribution if D/R <0.33, where D is the absolute difference between the most extreme distribution and the next value and R is the Range (maximum – minimum). The Mann-Whitney U test was used to test for significance of differences between genders. Kruskal-Wallis test followed by Bonferroni multiple comparison test was used to determine the significance of differences between variables by age groups. Linear regression was used to evaluate the association between PT and APTT with age. All statistical analyses were performed at 95% confidence level using Stata/MP version 13.0 (StataCorp LP, Texas, USA).

3. RESULTS

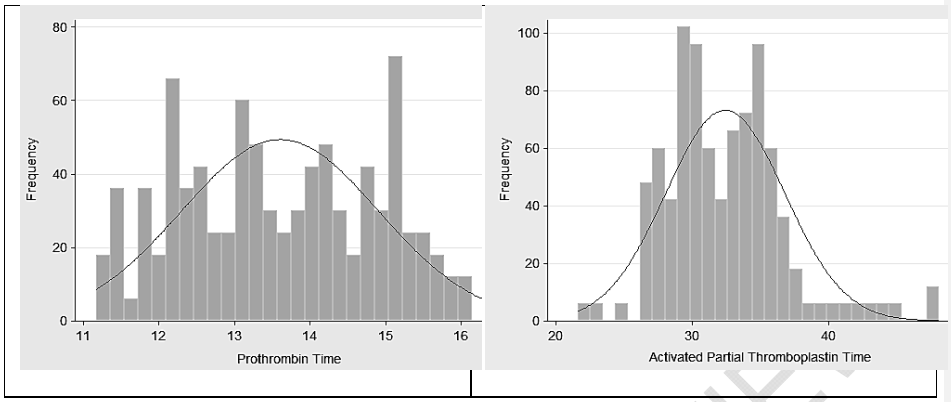

Figure 1. Distribution of PT and APTT in the general study population

Figure 1 shows the distribution of values for PT and APTT among the entire study population.

Table 1 shows the demographic characteristics and the reference interval for PT and APTT among the study population. Out of a total of 876 healthy adults recruited for this study, 54.1% were females while 45.9% were males. Most of the participants were 21-30 years old (73.3%). The median age for the entire study population was 24.0 years. The average PT and APTT was 13.5 seconds and 32.1 seconds respectively and their corresponding reference intervals established from the 2.5th – 97.5th percentile was 11.4-15.9 seconds and 26.3-44.1 seconds respectively (Table 1).

Table 1. Demographic characteristics and the reference interval for PT and APTT among the study population

Variables	Median (IQR)	Reference intervals	
		2.5th(95% CI)	97.5th(95% CI)
Age (years)	24.0(22.0-39.0)	NA	NA
	n(%)		
18-20	120(13.7%)	NA	NA
21-30	642(73.3%)	NA	NA
31-48	114(13.0)	NA	NA
Gender	n(%)		
Male	402(45.9%)	NA	NA
Female	474(54.1%)	NA	NA

PT	13.5(12.5-14.8)	11.4(11.2-11.4)	15.9(15.7-15.9)
APTT	32.1(29.5-34.9)	26.3(25.0-26.3)	44.1(41.9-44.9)

PT; Prothrombin Time, APTT; Activated Partial Thromboplastin Time, NA; Not applicable

The average age for both males and females was 24.0 years. The median PT for male and female participants was 13.4 seconds and 13.6 seconds respectively and the median APTT for male and female participants was 32.1 seconds and 31.6 seconds respectively. However, the difference was not statistically significant. Participants between the ages of 21-30 years old had significantly higher PT and APTT compared with participants between 18-20 years and 31-48 years old (Table 2).

Table 2. Age and PT and APTT among the study population stratified by gender

Variables	PT		APTT	
	Median (IQR)	2.5th-97.5th	Median (IQR)	2.5th-97.5th
Male: Female Age (years)	1:1 (24.0:24.0)			
Gender				
Male	13.4(12.2-14.7)	11.2-15.9	32.1(28.8-25.1)	23.0-44.9
Female	13.6(12.7-14.8)	11.4-16.0	31.6(29.7-34.7)	26.8-44.1
p-value	.185		.624	
Age				
18-20 (1)	13.2(12.3-15.0)	11.2-16.0	30.5(28.8-35.3)	21.6-40.3
21-30 (2)	14.0(12.7-15.0)	11.4-15.9	32.3(29.7-34.9)	26.3-44.9
31-48 (3)	12.6(12.0-13.5)	11.4-14.6	30.4(28.8-36.3)	26.3-41.7
p-value	**.000**		**.000**	
Significant pairs	2&1; 2&3		2&1; 2&3	

The Mann-Whitney U test was used to test for significance of differences between genders. Kruskal-Wallis test followed by Bonferroni multiple comparison test was used to determine the significance of differences between variables by age groups. $p < .05$ was considered statistically significant (p-values of significant variables in bold print).

142

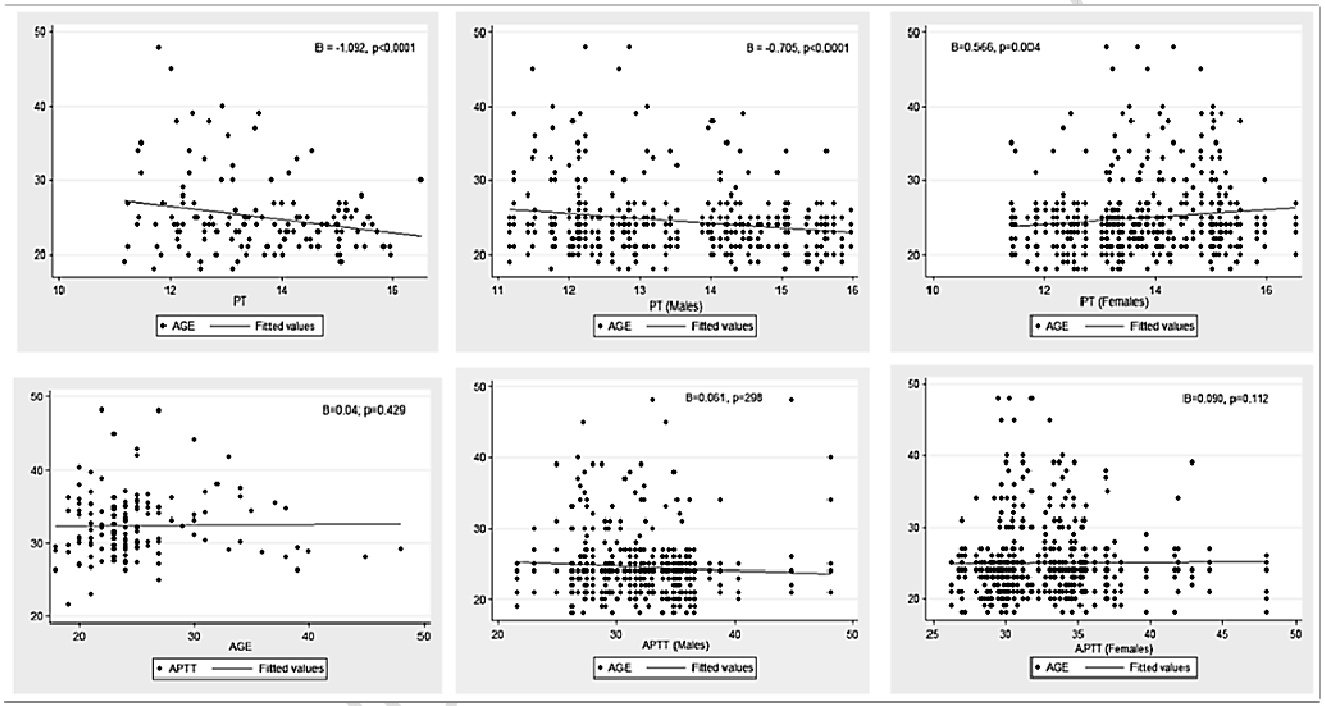

Figure 2. The association between PT and APTT with age

Among the entire study population, PT had a significant inverse association with age (β = -1.092, p=.000). Upon stratification by gender, PT showed a significant inverse association (β = - .705, p=.000) among males and a direct association (β = .566, p=.004) among females. The association between age and APTT was not statistically significant.

4. DISCUSSION

Reference intervals for most clinical parameters used in many African countries are those established among Caucasian populations. Diversity in geography, lifestyle, physical and genetic factors affect the normal physiological processes of a people, and hence variations in the measurement of 'normal' functions among and between populations are expected. Thus, making the use of pre-established reference intervals from other countries inappropriate. This study, therefore, established the reference intervals for PT and APTT and evaluated their association with age among healthy adults in Kumasi, Ghana.

The reference intervals for the PT, and APTT established in this study were 11.4-15.9 seconds and 26.3-44.1 seconds respectively. On the other hand, the reference intervals for PT and APTT currently being used at Komfo Anokye Teaching Hospital-KATH (the study site), adopted from reference interval established in Caucasian population, is 10-14 seconds and 23.4-36.2 respectively. This shows a disparity, where the reference intervals developed in this study is wider compared to the reference intervals currently being used at KATH. This finding is consistent with previous studies [5, 9-11], where differences in geographical locations have led to reports of varying reference intervals; for instance, the international standard laboratory reference interval for PT and APTT are 11-14 seconds and 25-35 seconds respectively. Again, a review by Bajaj and Joist reported the reference interval for PT and APTT to be 10-14 seconds and 20-35 seconds respectively [17]. Additionally, Tietz [18] recommends that a reference interval of 8.15-16.13 seconds for PT and 24-45 seconds for APTT may be considered acceptable. It is therefore evident that, there is variabilities in reference intervals from one geographical region to another. Thus, reinforce the need for reference intervals for specific geographical location.

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The median PT for female participants was marginally higher than their male counterparts (13.6 seconds vs 13.4 seconds respectively). Likewise, a slightly lower APTT was observed in females compared to males (31.6 seconds vs 32.1 seconds respectively). However, in both instances, the differences were not statistically significant. This may be due to the effects of sex hormones on coagulability of plasma [19, 20].

This study reports a wider reference interval for the PT and APTT. Furthermore, participants within the age group of 21-30 years had higher PT and APTT compared to 18-20 years and 31-48 years. A plausible reason for this is the fact that majority of the study participants were between 21-30 years. Another reason may be due to the fact that, individuals with 21-30 are more active in the study setting and increased activity (exercise) have been shown to improve coagulation activity [21-23].

This study also reports a significant inverse association between PT and age (β = -1.092, p=.000). After grouping by gender, PT showed a significant inverse association (β = - .705, p=.000) among males and a direct association (β = .566, p=.004) among females. The inverse association between PT and age is consistent with a study by Ujiie et al. [24] among blood donors between 20–62 years old in Japan. However, our finding among females is similar to a study by Kurachi and Kurachi [25] who reported that coagulation factors increased with age. Nonetheless, it is evident that PT and APTT are influence by many factors due to the highly scattered PT and APTT values when regressed with age.

The reference intervals for PT and APTT developed for the Kumasi study area will be of immense benefit to clinical trials and therapies that require monitoring of coagulation profile and general patient care. However, a limitation to be addressed by future studies is the fact that this present study was conducted in an urban setting and might not be generalizable to

other areas because behavioral and lifestyle patterns which may influence coagulation are different across different settings in Ghana.

5. CONCLUSION

The reference intervals for the PT, and APTT for Kumasi are 11.4-15.9 seconds and 26.3-44.1 seconds respectively. There are wider reference intervals for PT and APTT among people in Kumasi. Generally, PT is inversely association with age among healthy adults in Kumasi. There is no significant association between age and APTT. Due to geographical, lifestyle, and genetic diversity, it is advisable that each laboratory establishes their own reference intervals for PT and APTT.

COMPETING INTERESTS

Authors have declared that no competing interests exist

CONSENT

Written approval was for this study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (CHRPE/AP/219/17) and also from the Research and Development Department of KATH. Informed consent was obtained from all participants after the aim and objectives of the study had been explained to them.

REFERENCES

1. Marieb E, Hoehn K: **Human Anatomy & Physiology**, 8th edn. San Francisco: Benjamin Cummings; 2010.
2. Palta S, Saroa R, Palta A: **Overview of the coagulation system**. *Indian journal of anaesthesia* 2014, **58**(5).
3. Achneck H, Sileshi B, Parikh A, Milano C, Welsby I, Lawson J: **Pathophysiology of bleeding and clotting in the cardiac surgery patient: from vascular endothelium to circulatory assist device surface**. *Circulation* 2010, **122**(20):2068-2077.
4. Mackman N, Tilley R, Key N: **Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis**. *Arteriosclerosis, thrombosis, and vascular biology* 2007, **27**(8):1687-1693.
5. Kamal A, Tefferi A, Pruthi R: **How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults**. *InMayo Clinic Proceedings* 2007, **82**(7):864-873.
6. Blanchard R, Furie B, Jorgensen M, Kruger S, Furie B: **Acquired vitamin K-dependent carboxylation deficiency in liver disease**. *New England journal of medicine* 1981, **305**(5):242-248.
7. Malloy P, Grassi C, Kundu S, Gervais D, Miller D, Osnis R, Postoak D, Rajan D, Sacks D, Schwartzberg M *et al*: **Consensus guidelines for**

periprocedural management of coagulation status and hemostasis risk in percutaneous image-guided interventions. *Journal of Vascular and Interventional Radiology* 2009, **20**(7):S240-249.

8. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L: **Maturation of the hemostatic system during childhood**. *Blood* 1992, **80**:1998-2005.
9. Ejlersen E, Melsen T, Ingerslev J, Andreasen R, Vilstrup H: **Recombinant activated factor VII (rFVIIa) acutely normalizes prothrombin time in patients with cirrhosis during bleeding from oesophageal varices**. *Scandinavian journal of gastroenterology* 2001, **36**(10):1081-1085.
10. Lippi G, Favaloro E: **Activated partial thromboplastin time: new tricks for an old dogma**. *Semin Thromb Hemost* 2008, **34**(7):604-611.
11. Shahangian S, Stankovic A, Lubin I, Handsfield J, White M: **Results of a survey of hospital coagulation laboratories in the United States, 2001**. *Arch Pathol Lab Med* 2005, **129**:47-60.
12. Service GS, Ghana, Research NMIfM, Legon GaOm, Calverton, Maryland, USA: **Ghana demographic health Survey**. In.; 2003.
13. Shanti R, Potluri M: **Sample Size Calculator**. *Raosoft Inc* 2009.
14. Carey N, Anderson P, George H, Hartmann A, Janzen V, Kallner A: **User verification of performance for precision and trueness; Approved guideline**. *CLSI* 2006.
15. McEnroe R, Burritt M, Powers D: **Interference testing in clinical chemistry; approved guideline**. *CLSI* 2005.
16. Wayne P: **Defining, establishing, and verifying reference intervals in the clinical laboratory: approved guideline. CLSI documalet C28-A3c**. *Clinical and Laboratory Standards Institute* 2008.
17. Bajaj S, Joist J: **New insights into how blood clots: implications for the use of APTT and PT as coagulation screening tests and in monitoring of anticoagulant therapy**. *InSeminars in thrombosis and hemostasis* 1999, **25**(4):407-418.
18. Tietz N: **Clinical guide to laboratory tests**: WB Saunders Co.; 1995.
19. Bonnar J: **Coagulation effects of oral contraception**. *American Journal of Obstetrics & Gynecology* 1987, **157**(4):1042-1048.
20. Rosendaal F, Helmerhorst F, Vandenbroucke J: **Oral contraceptives, hormone replacement therapy and thrombosis**. *Thrombosis and haemostasis* 2001, **86**(1):112-123.
21. Patelis N, Karaolanis G, Kouvelos G, Hart C, Metheiken S: **The effect of exercise on coagulation and fibrinolysis factors in patients with peripheral arterial disease**. *Experimental Biology and Medicine* 2016, **241**(15):1699-1707.
22. Menzel K, Hilberg T: **Blood coagulation and fibrinolysis in healthy, untrained subjects: effects of different exercise intensities controlled by individual anaerobic threshold**. *European journal of applied physiology* 2011, **111**(2):253-260.
23. Hilberg T, Menzel K, Wehmeier U: **Endurance training modifies exercise-induced activation of blood coagulation: RCT**. *European journal of applied physiology* 2013, **113**(6):1423-1430.

24. Ujiie H, Kawasaki M, Suzuki Y, Kaibara M: **Influence of age and hematocrit on the coagulation of blood**. *Journal of biorheology* 2009, **23**(2):111-114.
25. Kurachi K, Kurachi S: **Genetic mechanisms of age regulation of blood coagulation; factor IX models**. *Arterioscler Thromb Vasc Biol* 2000, **20**:902–906.