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ORIGINAL ARTICLE

Some Coagulation Parameters Among Apparently Healthy Medical Laboratory Science Undergraduate Students of Rivers State University, Port Harcourt

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Abstract

Introduction: Prothrombin time (PT), International normalized ratio (INR) and activated partial thromboplastin time (APTT) are screening tests used to evaluate the overall integrity of the intrinsic, extrinsic and common coagulation pathway as well as monitoring and management of coagulation disorders. This study was aimed at assessing the PT, INR and APTT among apparently healthy undergraduate Medical Laboratory Science students of Rivers State University, Port Harcourt.

Materials and Methods: A total of 100 apparently healthy undergraduate Medical Laboratory Science students of Rivers State University, Port Harcourt comprising of 44 males and 56 females within the age range of 17-40 years were recruited for this study. Five milliliters (5ml) of venous blood was collected from each participant using a standard venipuncture technique into a vacutainer bottle containing 3.2% tri-sodium citrate anticoagulant in a ratio of 1:9 of the anticoagulant and the blood and mixed thoroughly. Prothrombin time and activated partial thromboplastin time were determined by clotting method using the Helena C4 semi-automated, photo-optical coagulation analyzer and the Agappe thromboplastin test kit manufactured by Agappe Diagnostics Switzerland while INR was calculated by using the ratio of the study subjects' PT to the mean of control PT raised to the power of the reagent international sensitivity index (ISI). The data obtained was analyzed using Graphpad Prism Software version 6.00. Data was presented as means, median, range and standard deviation.

Results: The mean ± SD of the PT, INR and APTT were 12.9± 1.624s, 1.01±0.1281 and 37.99±8.898s in the same order. The reference intervals obtained for PT, INR and APTT were 9.7-16.2s, 0.8-1.3 and APTT 20.2-55.8s in the same order. The lower limits were within the ranges established by previous researchers while the upper limits were higher. The age range 21-25 had the highest frequency count (54), which was followed by <20 (26), 26-30 (17) and then <30 (3). The

overall mean values for the age groups were within the established normal values and as such no statistically significant difference was observed between the various groups.
Conclusion: This study has established the mean values and reference intervals of PT, INR and APTT for apparently healthy Medical Laboratory Science undergraduate students of Rivers State University, Port Harcourt as well as established that age does not have any significant effect on PT, INR and APTT. Due to geography, lifestyle, and genetic diversity, it is recommended that each laboratory establishes geography-specific reference intervals for PT, INR and APTT.
Keywords: Coagulation parameters, Reference intervals, Prothrombin time, International normalized ratio, Activated partial thromboplastin time, Undergraduates.

Introduction

The mechanism of blood coagulation is a complex and dynamic interaction of platelets, plasma, and blood vessel endothelium. Blood coagulation is an important part of haemostasis process. It is usually initiated through damage to the vessel wall and subsequent activation of protease enzymes and ends with the transformation of soluble fibrinogen into insoluble fibrin (1). A common model used to describe the mechanism of coagulation is the cascade system, which is separated into three areas. The intrinsic system commonly measured by the activated partial thromboplastin time (APTT) is activated by surface contact. The extrinsic system, commonly measured by the prothrombin time (PT) test, is activated by vascular injury (2,3). The common pathway leading to clot formation is activated by the intrinsic and/or extrinsic pathway. The coagulation screening test such as PT, APTT, thrombin time (TT), and fibrinogen are important for the basic assessment of haemostasis.

Haemostatic parameters constitute measurable indices in the haemostatic system used to assess the functionality of the coagulation system of an individual to establish a state of health or disorder. They include bleeding time, clotting time, thrombin test (TT), prothrombin time (PT), fibrinogen level, Activated partial thromboplastin time (APTT) among others (4,5). Prothrombin time (PT) is a laboratory screening test used to detect disorders involving the activity of Factor I (Fibrinogen), Factor II (Prothrombin), Factor V (Proaccelerin), Factor VII (Proconvertin), and Factor X (Stuart factor) of the extrinsic and common pathways. The PT assesses the function of Factor VII, Factor X, Factor V, Factor II (Prothrombin), and Factor I (Fibrinogen) after the addition of thromboplastin and calcium (4,5).

The Factor VIIa/tissue factor complex activates Factor Xa and through the action of prothrombinase complex, prothrombin is converted to thrombin. The time in seconds for the conversion of fibrinogen to insoluble fibrin by thrombin is reported as PT(6). Activated partial thromboplastin time (APTT) is an assay used to screen for abnormalities of the intrinsic and common clotting systems and to monitor the anticoagulant effect of circulating heparin. It measures the activities of Factors I, II, V, VIII, IX, X, XI, and XII of the intrinsic and common pathways (6).

Reference intervals (RI) are required in coagulation to evaluate results in relation to a patient haemostatic disorder. This becomes one of the most important tasks conducted in the laboratory as up to 80% of medical decisions are made based on laboratory results (7). Reference intervals are required to be reported out with every laboratory result to aid in interpreting a normal versus an abnormal coagulation result. The guidelines of British Committee for standards in Haematology recommend that either reference ranges or clinical cutoffs values should be validated for the instrument reagent combination used in the laboratory (8). These reference values are of great importance as they are geared towards making the life of the patient better and as such are required for every laboratory to obtain. The relevance of accessing the PT, INR and APTT in apparently healthy subjects is to evaluate their risk of developing coagulation disorders and thus the need for this research.

Materials and Methods

Study Design

Stratified randomized study design was employed for this study. Twenty apparently healthy undergraduate students were randomly selected from each of the five levels of the Medical Laboratory Science Department of Rivers State University.

Study Area

The study was conducted among the Medical Laboratory Science undergraduate students of Rivers State University, located in the Diobu area of Port Harcourt, Rivers State, Nigeria. Rivers State University is a non-profit public higher-education institution located in the urban setting of the metropolis of Port Harcourt. It was established in 1980 from the College of Science and Technology which was itself established in 1972. It is located in Diobu area of Port Harcourt, at latitude 4.7974°N and longitude 6.9803° E. The University has a staff strength of 1,870 and a student population of 22,400 as at 2017(9). The university has 12 faculties and a total of 63 departments (9).

Study Population

A total of one hundred (100) apparently healthy volunteer subjects aged between 17 and 40 years were recruited. A well-structured questionnaire was used to obtain the demographic information of the study participants. A well written and signed informed consent was obtained from each subject before sample collection.

Inclusion and Exclusion Criteria

Apparently healthy Students of Medical Laboratory Science Department of Rivers State University, Port-Harcourt within the age range of 17-40 years were included while 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 all excluded.

Sample Collection and Processing

Five milliliters (5ml) of venous blood were collected with the use of vacutainer needle from each subject as described by (10), into a tri-sodium citrate anticoagulant tube. A ratio of 1:9 of the 3.2% tri-sodium citrate and the blood respectively were mixed in the tube gently. The samples were then centrifuged for fifteen minutes at 3000rpm to obtain platelet poor plasma. The supernatant plasma was then transferred into another test tube immediately. The samples were analyzed within two hours of collection.

Sample Analysis/Methodologies

Samples for APTT and PT were analyzed using plasma clotting method with Helena C4 Semi-automated, photo-optical coagulation analyzer using Agappe thromboplastin test kit manufactured by Agappe Diagnostics Switzerland (Lot number: 301101769; Expiry date: March 2023).

The detection of plasma clotting is based on a photometric principle. No mechanical aids like mixing bars are required. Blood plasma is filled into a cuvette. Special reagents are added, which initiate the blood coagulation. The cuvette is transmitted by ultraviolet light during the coagulation process. When the sample starts to clot a change of light absorbance is measured. The time from measurement start, to change of light (turning point) is called clotting time and expressed in seconds(s).

The INR was calculated by using the ratio of the Patient PT to the mean 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.

$$INR = \left(\frac{PT\ patient}{PT\ control}\right)^{ISI}$$

Data Analysis

Results were presented as mean ± SD. Where appropriate, statistical analyses were performed using frequency distribution (GraphPad Prism Software Version 5.03, San Diego, CA). Statistical significance was set at 95% confidence interval ($p \leq 0.05$).

Results

Table 1 shows the demographic data of the study participants. A total of 100 subjects (Males 44 and females 56) were recruited for the study. The age distribution of the study population was <20, 21-25, 26-30, and >30. Ninety-nine (99) of the study participants were single while only one(1) participant was married.

Table 2 shows the mean, standard deviation, standard error of men, median, range, minimum and maximum levels of PT, APTT, INR and age of the study participants. The mean±SD of the PT, APTT, INR and age were, 12.9±1.624s, 37.99±8.898s, 1.01±0.1281 and 23.02±3.977years in the same order. The median values obtained for PT, APTT, INR and age of the study participants were 12.90s,1.010, 39.00s and 22.00years in the same order. Details of other results are as shown in Table 2.

Table 3: shows the mean, standard deviation, mean normal (Mean ±2SD) and range of the coagulation parameters PT, INR and APTT. The Mean ±2SD of PT, INR and APTT were 12.9±3.248s, 1.011±0.2562 and 37.99±17.796s in the same order The reference ranges were thus; PT(s) was 9.7-16.2, INR was 0.8-1.3 and APTT(s) was 20.2-55.80.

The mean value and range for those <20years was 13.46s with a range of 11.06-15.9s for PT; INR 1.05 with a range of 0.9-1.3 for INR and 42.19s with a range of 28.8-58.6s for APTT. For the age range 21-25years, PT(s) was 12.60 (range 9.3-15.9), INR was 0.98 (range 0.7-1.3) and APTT(s) was 36.34 (range 17.6-55.0) while 26-30years had PT(s) of 13.25 with a range 9.45-17.05, INR of 1.03 (range 0.7-1.3) and APTT(s) of 37.34 (22.5-52.1) while for those >30years, PT(s) was 12.40 (range 8.1-16.7), INR was 0.97 (0.7-1.3) and APTT(s) was 33.70 (23.1-44.3) as shown in Table 4.

Table 1: Demographic Characteristics of Study Participants.

Parameters		Total number (%)
Gender	Males	44 (44)
	Females	56 (56)
	Total	**100 (100)**
Age distribution (years)		
	<20	26 (26)
	21-25	54 (54)
	26-30	17 (17)
	>30	3 (3)
	Total	**100 (100)**
Marital status		
	Single	99 (99)
	Married	1 (1)
	Total	**100 (100)**

Table 2: Measurement of Prothrombin Time (PT), International Normalized Ratio(INR)and Activated Partial Thromboplastin Time (APTT) of the Study Participants.

	Age(years)	PT(s)	INR	APTT(s)
Valid (*n*)	100	100	100	100
Missing (*n*)	0	0	0	0
Mean	23.02	12.9	1.01	37.99
SD	3.977	1.624	0.1281	8.898
SEM	0.3977	0.1624	0.01281	0.8898
Median	22.00	12.90	1.010	39.00
Range	28	9.400	0.7400	42.10
Minimum	17	9.900	0.7700	21.10
Maximum	45	19.30	1.510	63.20

KEY: SD= Standard deviation, SEM= Standard error of mean, APTT= Activated partial thromboplastin time, INR= International normalized ratio.

Table 3 Reference Ranges of Prothrombin Time, International Normalized Ratio and Activated Partial Thromboplastin Time of Study Subjects.

Parameters	Mean	SD	Mean normal (mean±2SD)	Range
PT(s)	12.93	1.624	12.9±3.248	9.7-16.2
INR	1.011	0.1281	1.011±0.2562	0.8-1.3
APTT(s)	37.99	8.898	37.99±17.796	20.2-55.8

KEY: SD= Standard deviation, SEM= Standard error of mean, APTT= Activated partial thromboplastin time, INR= International normalized ratio.

Table 4 Measurement of Prothrombin Time, International Normalized Ratio and Activated Partial Thromboplastin Time of Study Subjects According to Age Groups

Age	Parameter	Mean	SD±2	Range	Maximum	Mini-mum	Count
<20	PT(s)	13.46	2.4	11.06-15.9	16.30	10.90	26
	INR	1.053	0.2	0.9-1.3	1.280	0.8500	26
	APTT(s)	42.19	16.4	28.8-58.6	63.20	29.10	26
21-25	PT(s)	12.60	3.3	9.3-15.9	19.30	9.900	54
	INR	0.9846	0.3	0.7-1.3	1.510	0.7700	54
	APTT(s)	36.34	18.7	17.6-55	61.20	21.10	54
26-30	PT(s)	13.25	3.8	9.45-17.05	16.60	9.900	17
	INR	1.036	0.3	0.7-1.3	1.300	0.7700	17
	APTT(s)	37.34	14.8	22.5-52.1	55.30	26.40	17
>30	PT(s)	12.40	4.3	8.1-16.7	14.80	10.60	3
	INR	0.9700	0.3	0.7-1.3	1.160	0.8300	3
	APTT(s)	33.70	10.6	23.1-44.3	39.80	30.60	3

KEY-SD= Standard deviation, SEM= Standard error of mean, APTT= Activated partial thromboplastin time, INR= International normalized ratio.

Discussion, Conlusion and Recommendations

Discussion

Prothrombin time and activated partial thromboplastin time are primary screening tests for coagulopathies whereas international normalized ratio is simply a mathematical processing of the PT that permits standardization of test results across different laboratories. 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 the use of pre-established reference intervals from other countries is inappropriate. This study, therefore, established the reference intervals for PT, INR and APTT and evaluated their association with age among apparently healthy undergraduate Medical Laboratory Science students of Rivers State University, Port Harcourt.

This study established a mean PT, INR and APTT values of 12.9± 1.624s, 1.01±0.1281 and 37.99±8.898s in the same order. The reference intervals obtained for PT, INR and APTT from this study were 9.7-16.2s, 0.8-1.3 and APTT 20.2-55.8s in the same order. The lower limits were within the ranges established by previous researchers while the upper limits were higher. This finding could be because of the relatively small sample size used for this study. This study is not in tandem with work done by (15) who established the reference interval in the coagulation laboratory as; PT=11.6-13.8s and the APTT=22-36s. It is also not consistent with the mean APTT of 34s obtained by (16) among apparently healthy males in India but in agreement with the mean PT value of 13s obtained by the same authors. The findings from in this study is also slightly at deviant with the International Standard Laboratory reference interval for PT and APTT reported to be 11-14s and 25-35s respectively (17). Also the reference ranges obtained from this study for PT and APTT are not consistent with that obtained by (18) among 876 apparently healthy adults in Ghana. The reference intervals obtained in this study is wider than that obtained by them.

There was no effect of age on the coagulation parameters assayed. A plausible reason could be because of the narrow age range of the study participants. It could also be due to the fact that the subjects used in this study were all physically active as many of them were preparing for their second semester examination. Increased activity has been shown to improve coagulation activity. The age range 21-25years had the highest frequency which was followed by <20yeares, 26-30years and then <30years. This finding is in disagreement with the study by (18) where participants within the age group of 21-30 years were higher compared to 18-20 years and 31-48 years.

Conclusion

The mean values and reference intervals for the PT, and APTT for apparently healthy undergraduate Medical Laboratory Science students of Rivers State University, Port Harcourt were 12.9± 1.624s, 1.01±0.1281 and 37.99±8.898s in the same order while the reference intervals obtained were 9.7-16.2s, 0.8-1.3 and APTT 20.2-55.8s in the same order. There are wider reference intervals for PT and APTT among people in the study participants. There was no significant association between age and coagulation parameters measured. Due to geographical, lifestyle, and genetic diversity, it is advisable that each laboratory establishes their own reference intervals for PT, and APTT. This study has established the mean values and reference intervals for PT, INR and APTT for apparently healthy undergraduate students of Rivers State University. It has also established that age does not have any significant effect on PT, INR and APTT.

Recommendations

It is recommended that further studies should be carried out on a larger population of Rivers State University undergraduate students. Other tests such as D-dimer, platelet factors, and protein assays that would better show the haemostatic profile of study participants should also be carried out.

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