Evaluation of Thrombin Time among Apparently Healthy Young Adult Females in Enugu Metropolis, Nigeria: A Cross-Sectional Study

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Abstract

This cross-sectional study, conducted at the University of Nigeria, Enugu Campus, Enugu metropolis, Nigeria, aimed to determine the thrombin time (TT) of apparently healthy young adult females and to compare TT between two age groups. Forty-two apparently healthy, non-pregnant females aged 18–30 years were recruited. Citrated venous blood was centrifuged to obtain platelet-poor plasma (platelet count <10,000/µL). TT was measured on a Mindray semi-automated coagulometer using Biosystems reagent, and a full blood count (FBC) was performed. Data are presented as median and interquartile range (IQR). TT was compared between the ≤25-year and ≥26-year age groups using the Mann–Whitney U test (P<.05). The median age was 23.0 years (IQR 22.0–26.0); 26 participants (61.9%) were aged ≤25 years and 16 (38.1%) were aged ≥26 years. The median TT was 17.8 s (IQR 16.6–19.3). Median TT was 17.3 s (IQR 15.9–19.6) in participants aged ≤25 years and 18.1 s (IQR 17.4–19.1) in those aged ≥26 years, a difference that was not statistically significant (P = 0.467). In this sample of 42 healthy young women, TT did not differ significantly between the two age groups. The values are reagent- and instrument-specific and should be regarded as preliminary baseline data. Larger studies that record menstrual phase and hormonal contraceptive use are needed to establish reference intervals for females in this population.

Keywords—*Thrombin time, hemostasis, healthy females, age, Enugu, Nigeria*

1. Introduction

Thrombin is the terminal serine protease of the coagulation cascade. In addition to converting fibrinogen to fibrin, it acts on other cell types and is implicated in processes such as vascular inflammation (Popović et al., 2012). The thrombin time (TT) measures the time taken for a clot to form in citrated plasma after a standardized amount of thrombin is added, and it therefore reflects the final step of coagulation, the conversion of fibrinogen to fibrin. A prolonged TT points to a fibrinogen abnormality, impaired fibrin formation or the presence of a thrombin inhibitor, including direct thrombin inhibitors (Van Cott et al., 2017). Laboratory approaches to inherited fibrinogen disorders such as dysfibrinogenemia continue to be compared and refined (Cunningham et al., 2002; Marchi Cappelletti et al., 2021). The TT has also been re-examined using clot waveform analysis; in that work, the presence and count of platelets strongly influenced the results, which supports testing on platelet-poor plasma (Wada et al., 2021). In women, hemostasis may be modulated by the menstrual cycle, hormonal contraception and pregnancy. A review of the menstrual cycle and hemostasis found evidence of both procoagulant and anticoagulant shifts across the cycle, but with inconsistent findings between phases, and concluded that progesterone plays the central role in the most important changes (Freitas et al., 2016). In 13 healthy ovulatory women followed for six weeks, fibrinogen, factor VIII:C, antithrombin and platelet counts showed no cycle-related change, whereas fibrinolytic activity covaried with progesterone (Siegbahn et al., 1989). More recent work has reported cycle-phase effects on thrombin generation in women with von Willebrand disease, in whom factor VIII and factor X levels were lower in the luteal than in the follicular phase (Govorov et al., 2018), and in seven healthy young women, in whom thrombin generation under central hypovolemia started sooner in the mid-luteal than in the early follicular phase (Goswami et al., 2020). Hormonal and obstetric influences on hemostasis have also been examined in Nigerian women. Among 47 women who used a low-dose combined oral contraceptive for three months, TT and prothrombin time (PT) shortened, and fibrinogen and platelet count rose, whereas 20 age-matched controls showed no change (Babatunde & Olatunji, 2004). In pregnancy, PT shortened and fibrinogen rose with advancing gestation in Ilorin (Durotoye et al., 2012), and PT was shorter in pregnant than in non-pregnant women in Jos (12.82 s vs 14.29 s; Lugos et al., 2018). Among 167 Nigerian women with postpartum hemorrhage, a major obstetric complication addressed by patient blood management strategies (Muñoz et al., 2019), the median TT was 10.4 s (Roberts et al., 2018). The interpretation of TT depends on the reagent and instrument used, so local data are important. A data-mining study of reference intervals for coagulation screening tests, which included TT, found that the intervals varied only slightly with sex and age (Zierk et al., 2019). In contrast, thrombin generation in 22,014 Italian adults showed age- and sex-dependent patterns, and the authors argued for age- and sex-specific reference values (Costanzo et al., 2025). Data on hemostasis in healthy African adults are limited and heterogeneous: a meta-analysis of PT and APTT in Africa reported very high heterogeneity (I^2 of about 97–98%) (Getu et al., 2024), and in a study of 450 healthy Nigerian adults, 33.3% reported a history of bleeding symptoms, with differences between ethnic groups (Ezigbo et al., 2020). In our literature search, we found few Nigerian reports that give TT values for healthy women. This study therefore measured TT and full blood count (FBC) parameters in apparently healthy young adult females in Enugu metropolis and compared TT between two age groups (≤25 years and ≥26 years).

2. Materials and Methods

2.1 Study design and area

This was a cross-sectional study carried out among students of the University of Nigeria, Enugu Campus, Enugu metropolis, in south-eastern Nigeria.

2.2 Participants
Participants were apparently healthy adult females aged 18–30 years. Random sampling was used to select participants.

2.3 Inclusion criteria
(i) healthy adult females; (ii) no blood transfusion in the preceding three months; (iii) not taking medications, such as anticoagulants or antiplatelet agents, that affect blood coagulation; and (iv) no history of a bleeding or other coagulation disorder.

2.4 Exclusion criteria
(i) history of a blood disorder such as sickle cell anemia or thalassemia; (ii) blood transfusion in the preceding three months; (iii) history of liver or kidney disease; (iv) history of a bleeding disorder or abnormal bleeding tendency; and (v) pregnancy.

2.5 Sample size
The sample size was estimated with the formula $n = Z^2P(1-P)/d^2$, where $Z = 1.96$ (95% confidence level), $P = 0.020$ (expected proportion) and $d = 0.05$ (precision). This gave $n = (3.8416 \times 0.020 \times 0.98)/0.0025 = 30.12$, i.e., a minimum of 30 participants. Forty-two participants were recruited.

2.6 Ethical considerations
Ethical approval was obtained from the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla (NHREC/05/01/2008B). Written informed consent was obtained from all participants before enrolment. Participant identity was protected and the study followed the approved ethical guidelines.

2.7 Blood collection
After site preparation with an alcohol swab, venous blood was collected from the cubital fossa or wrist with a sterile 5 mL syringe and needle and dispensed into a container with trisodium citrate anticoagulant. The container was labeled with the participant identifier and sent to the laboratory for processing.

2.8 Plasma preparation
A volume of 2.7 mL of blood was added to 0.5 mL of 3.2% trisodium citrate. The sample was centrifuged within 1–2 hours of collection at 1500 rpm for 15 minutes. The supernatant plasma was centrifuged again at 1500 rpm for 15 minutes until a platelet count <10,000/µL was obtained. Platelet-poor plasma was transferred with a pipette into a cryovial labeled with the participant identifier.

2.9 Thrombin time assay
TT was measured on a Mindray semi-automated coagulometer (model: C200/4) using commercially prepared thrombin reagent from Biosystems. In this test, a standardized concentration of thrombin is added to citrated plasma and the time to fibrin clot formation is recorded in seconds. A cuvette containing a bead was placed in the instrument chamber, 50 µL of plasma was added, and the sample was incubated at 37 °C for 2 minutes. Then 50 µL of working reagent was added with a clean pipette tip, timing started automatically, and the clotting time displayed on the screen was recorded as the TT.

2.10 Full blood count
FBC parameters (white cell count and differential, red cell indices, hematocrit, and platelet indices) were determined with an automated hematology analyzer.

2.11 Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. Continuous variables are presented as median and interquartile range (25th–75th percentiles), and categorical variables as frequency and percentage. TT was compared between participants aged ≤25 years and ≥26 years using the Mann–Whitney U test. A P value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Results

A total of 42 apparently healthy females were recruited. As shown in Table 1, the median age was 23.0 years (IQR 22.0–26.0). Twenty-six participants (61.9%) were aged ≤25 years and 16 (38.1%) were aged ≥26 years.

Table 1. Age distribution of the study participants (N = 42)

Characteristic	Result
Age (years), median (IQR)	23.0 (22.0–26.0)
Age group ≤25 years, n (%)	26 (61.9)
Age group ≥26 years, n (%)	16 (38.1)

IQR: interquartile range (25th–75th percentiles).

The median TT of the 42 participants was 17.8 s (IQR 16.6–19.3). The FBC parameters are shown in Table 2.

Table 2. Thrombin time and full blood count parameters of the participants (N = 42)

Parameter	Median (IQR)
TT (s)	17.8 (16.6–19.3)
WBC ($\times 10^9$/L)	4.9 (4.1–5.9)
Lymphocytes ($\times 10^9$/L)	2.3 (2.0–2.6)
Granulocytes ($\times 10^9$/L)	2.2 (1.8–2.9)
MID cells ($\times 10^9$/L)	0.4 (0.3–0.4)
RBC ($\times 10^{12}$/L)	4.4 (4.1–4.5)
HCT (%)	35.0 (34.3–36.2)
MCV (fL)	84.2 (79.5–87.2)
MCH (pg)	26.1 (23.9–27.2)
MCHC (g/dL)	30.9 (30.3–32.2)
RDW-CV (%)	14.0 (13.6–14.3)
RDW-SD (fL)	37.8 (35.4–39.3)
PLT ($\times 10^9$/L)	203.0 (165.8–259.5)
MPV (fL)	9.0 (8.8–9.9)
PDW (fL)	15.7 (15.4–15.9)
PCT (mL/L)	1.9 (1.6–2.3)

IQR: interquartile range; TT: thrombin time; WBC: white blood cells; MID cells: mid-sized cells (white cells not classified as lymphocytes or granulocytes); RBC: red blood cells; HCT: hematocrit; MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW-CV: red cell

distribution width, coefficient of variation; RDW-SD: red cell distribution width, standard deviation; PLT: platelets; MPV: mean platelet volume; PDW: platelet distribution width; PCT: plateletcrit.
Median TT was 17.3 s (IQR 15.9–19.6) in participants aged ≤25 years and 18.1 s (IQR 17.4–19.1) in those aged ≥26 years (Table 3). The Mann–Whitney U test showed no statistically significant difference between the two age groups (P = 0.467).

Table 3. Thrombin time by age group (N = 42)

Age group	n	Thrombin time (s), median (IQR)
≤25 years	26	17.3 (15.9–19.6)
≥26 years	16	18.1 (17.4–19.1)
P value (Mann–Whitney U)		0.467

IQR: interquartile range. Differences were considered significant at P < 0.05.

3.2 Discussion

This cross-sectional study measured TT and FBC parameters in 42 apparently healthy young adult females in Enugu metropolis. The median TT was 17.8 s (IQR 16.6–19.3), and it did not differ significantly between participants aged ≤25 years and those aged ≥26 years (17.3 s vs 18.1s; P = 0.467).

3.2.1 Interpreting the absolute TT value

Absolute TT values depend on the reagent, its thrombin concentration and the instrument, so they should be compared only with values obtained by the same method. Our median of 17.8 s was obtained with Biosystems reagent on a semi-automated coagulometer. Roberts et al. (2018) reported a median TT of 10.4 s in Nigerian women with postpartum hemorrhage, but that is a different clinical group, and the report does not allow a like-for-like comparison with our method. Kusfa et al. (2018) measured TT in 50 adults with sickle cell anemia and 25 healthy HbAA controls in Zaria and reported TT as higher in the sickle cell group, but the control values could not be extracted reliably from the available abstract and the participants differed from ours. For these reasons, we do not interpret the 17.8 s median against an external reference; the manufacturer's reference interval for the reagent used, or a locally derived interval, should be used. Our results can contribute to the latter.

3.2.2 Age

We found no significant difference in TT between the two age groups. This agrees with the finding of Zierk et al. (2019) that reference intervals for coagulation screening tests, including TT, varied only slightly with sex and age. In contrast, Costanzo et al. (2025) found age-dependent changes in thrombin generation parameters in 22,014 adults, but that assay measures a different aspect of coagulation, and their participants spanned a much wider age range. All our participants were young adults (18–30 years; median 23 years), so the two groups differ by only a few years, and the older group contained only 16 participants. The study therefore had little power to detect a small difference, and the 0.8 s difference between group medians should not be interpreted as a trend. Durotoye et al. (2012) found higher fibrinogen in older than in younger participants in a pregnancy study, which suggests that age-related changes could be more evident in other hemostatic variables or in other life stages; we did not measure fibrinogen.

3.2.3 Female-specific influences on hemostasis

The hormonal environment is a reason to study hemostasis in women separately. The evidence on cycle-related change is mixed. Siegbahn et al. (1989) found no cycle-related change in fibrinogen, factor VIII:C or

antithrombin in 13 women, while Freitas et al. (2016) described inconsistent findings across phases in their review. Govorov et al. (2018) and Goswami et al. (2020) reported phase-related differences in thrombin generation, but in a small group of women with von Willebrand disease and in seven healthy young women studied under a physiological stressor, respectively. Hormonal contraception may be more relevant to TT specifically: in the Nigerian study of Babatunde and Olatunji (2004), TT shortened significantly after three months of a low-dose combined oral contraceptive. Pregnancy also changes coagulation tests (Durotoye et al., 2012; Lugos et al., 2018). We excluded pregnant women, but we did not record menstrual phase or contraceptive use. These factors could contribute to the spread of TT values we observed (IQR 16.6–19.3 s) and could not be assessed.

3.2.4 Full blood count parameters

The FBC results are reported to describe the sample and are not the main finding. The median platelet count was 203 × 10 9/L, which is of a similar order to the platelet count of about 230 × 10 9/L reported for healthy HbAA controls in Zaria (Kusfa et al., 2018). We did not compare FBC parameters with reference intervals, and hemoglobin concentration was not reported.

3.2.5 Implications

TT is a simple test that is used to evaluate the final step of coagulation, so a local baseline for healthy women can help when TT is interpreted in similar settings, for example in the work-up of a prolonged TT in suspected fibrinogen disorders or thrombin inhibition. Because TT depends on the method, any clinical use of our values must be tied to the same reagent and instrument. Global-assay approaches, such as thrombin generation (Hemker et al., 2003) or clot waveform analysis of TT (Wada et al., 2021), may provide additional information in women, but neither was assessed here and our data cannot support recommendations about them. The very high between-study heterogeneity in African coagulation data (Getu et al., 2024) and the bleeding-symptom differences between Nigerian ethnic groups (Ezigbo et al., 2020) support the generation of locally derived reference data.

3.2.6 Strengths and limitations

Strengths of this study include clear exclusion of participants with recent transfusion, anticoagulant or antiplatelet use, bleeding or liver and kidney disease, and sickle cell disease or thalassemia, and the preparation of platelet-poor plasma by repeated centrifugation. The main limitations are as follows. The sample was small (n = 42) and drawn from one university campus, so the results cannot be generalized to all females in Enugu metropolis. The age range was narrow (18–30 years), and the older group was small. There was no male comparison group, so we cannot comment on sex differences. Menstrual phase, hormonal contraceptive use and body mass were not recorded, and no other coagulation tests (PT, APTT, fibrinogen) were done. All TT measurements used a single reagent and instrument, and the results are not transferable to other methods. The sample size was estimated with a formula for a single proportion, which is not designed for a continuous outcome such as TT; the precision of the TT estimate is therefore not guaranteed by that calculation.

4. Conclusion

In 42 apparently healthy young adult females in Enugu metropolis, the median TT was 17.8 s (IQR 16.6–19.3), with no significant difference between participants aged ≤25 years and ≥26 years. These findings are preliminary, are specific to the reagent and instrument used, and are limited by sample size and a narrow age range. We recommend larger, multi-site studies that include women across a wider age range, record menstrual phase and hormonal contraceptive use, and measure PT, APTT and fibrinogen alongside TT, so that method-specific reference intervals for females in this population can be established.

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Competing Interests
The authors have declared that no competing interests exist.
Authors' Contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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