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Correlation between Slope 2 in Clot Waveform Analysis of Activated Partial Thromboplastin Time with Factor VIII Activity in Hemophilia A

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ABSTRACT

Hemophilia A is an inherited factor VIII deficiency disease related to the X chromosome. Diagnosis of hemophilia A is made based on a factor VIII assay. Nowadays, hemophilia A therapy gives factor VIII concentrate, so monitoring this therapy must be done by examining factor VIII activity. Still, the examination of factor VIII activity is currently limited and quite expensive. One activated Partial Thromboplastin Time (aPTT) optical method can provide information about every coagulation stage through clot waveform analysis. Factor VIII activity can be described in slope 2 of clot waveform analysis, where deficiency of factor VIII will cause slope 2 to become more than normal because the clot form is not optimal, and the light transmission recorded at clot waves does not decrease maximally. This study aimed to analyze the correlation between slope two on the clot waveform analysis of the optical method on aPTT test with factor VIII activity in hemophilia A subjects. This research was a correlative cross-sectional observational study conducted at Hasan Sadikin General Hospital, Bandung, August 2018-September 2019. The subjects were members of hemophilia A patients of West Java Hemophilia Society. The optical method assessed the research subjects for factor VIII activity and aPTT. Slope 2 was calculated using clot waveform analysis, formed in aPTT examination. This study involved 43 subjects, with a median age of 6 years, an age range of 1-45 years, with 51.2% of patients aged 6-17 years. The results of factor VIII activity in this study had a median of 0% with a range of 0-25.9%, and the value of slope 2 had a median of 1.0%T/sec with a range of 0.5-3.5%T/sec. The correlation test between slope 2 and factor VIII activity with a 95% confidence interval using Spearman's correlation test showed a powerful positive correlation, which was statistically significant ($r=0.854$ and $p < 0.001$). There was a statistically significant, robust positive correlation between slope 2 on the clot waveform analysis of aPTT optical method test with the activity of factor VIII in hemophilia A.

Keywords: Factor VIII activity, hemophilia A, slope 2 of aPTT

INTRODUCTION

Hemophilia is a genetic inherited autosomal recessive deficiency of a coagulation factor, in which mutation occurs in genes associated with the X chromosome. Based on this X-linked factor, hemophilia is more common in males than females. Hemophilia A is factor VIII deficiency, which is the most common hemophilia. Hemophilia A in females usually presents as a carrier, unless a female is born to a father with hemophilia A and the mother is a carrier for hemophilia A, but this is very rare. The incidence of hemophilia A in developing countries is 1:5000 to 1:10000.[1-5]

Hemophilia A is distinguished based on the activity of factor VIII into mild, moderate, and severe. Diagnosis of hemophilia A based on history, physical examination, and laboratory findings. Anamnesis is performed to determine bleeding manifestation, such as spontaneous or traumatic bleeding, and the frequency of bleeding. The most typical bleeding of hemophilia A is joint bleeding, called hemarthrosis.[6-10]

Laboratory assays that are recommended for diagnosing hemophilia A are coagulation assays and specific coagulation factor assays. Activated Partial Thromboplastin Time (aPTT) is a coagulation assay that is used for the initial screening of intrinsic coagulation factor disorder. Activated partial thromboplastin time measures the time needed for fibrin clot formation. The aPTT of a hemophilia A patient will be longer than normal due to factor VIII deficiency, which plays a role in the intrinsic pathway. A specific coagulation factor assay to establish a diagnosis of hemophilia A is the factor VIII activity assay. Still, it is not generally available in all clinical laboratories, relatively expensive, and has short reagent stability (8 hours).[1,3,11-13]

At the same time, examining aPTT with clot waveform analysis (an optical method) can be performed, providing information about every coagulation stage. Clot waveform analysis is an analysis of clot formation providing more details

than the aPTT value. The indication of clot waveform analysis is suspected coagulation factor disorder that requires analysis of every coagulation stage. Some studies said that through the clot waveform analysis, the coagulation factors disorder can be seen by the clot wave pattern. Parameters assessed in clot waveform analysis is the slope of the precoagulation phase (slope-1), maximum velocity of first derivatives (Min1), the maximum velocity time of first derivatives (TMin1), maximum acceleration of second derivatives (Min2), a maximum acceleration time of second derivatives (TMin2), maximum deceleration of second derivatives (Max2), maximum deceleration time of second derivatives (TMax2), the slope of the coagulation phase (slope 2), and slope of the post coagulation phase (slope 3).[12-13]

Clot waveform analysis results can be obtained automatically by the aPTT test without the need to carry out two-stage examinations, so the clot waveform analysis is an effective and efficient analysis to find coagulation kinetics. The study of clot waveform analysis is mostly done to assess the deficiency of coagulation factors, especially in Hemophilia patients. Clot waveform analysis in hemophilia A will show a prolonged pre-coagulation phase and a slightly sloped second curve due to factor VIII deficiency. The use of clot waveform analysis in clinical practice can maximize the aPTT test, which currently only provides information about the time of clot formation. The advantages of clot waveform analysis are that it's easier and faster because the results will be obtained simultaneously with the results of the aPTT test, and there is no need for additional costs.[12-16]

METHODS

This research was a cross-sectional observational study conducted at Hasan Sadikin General Hospital, Bandung, from August 2018-September 2019. The subjects were a member of Hemophilia A sufferers of West Java Hemophilia Society who clinicians had diagnosed. Inclusion criteria were patients (male) who had been diagnosed with hemophilia A by clinicians. Exclusion criteria were hemophilia A patients who had received factor VIII concentrate therapy within ≤ 24 hours before blood sampling because factor VIII activity will increase for 24 hours after the administration of the concentrate, so it will not describe the true factor VIII activity in subjects. Blood samples were collected in a blood collection tube containing 3.2% sodium citrate as an anticoagulant. The aPTT and factor VIII activity assay were analyzed using CS2500 automatic coagulation device. The result of slope 2 will be obtained along with aPTT and expressed as %T/second. Factor VIII activity was analyzed using the automatic coagulation device CS2500 with the chromogenic method, and the assay was expressed as a percentage (%). Data were analyzed using Statistical Package for the Social Science (SPSS). The normality test was performed using Saphiro Wilk's test. A correlation test was performed using Spearman's correlation test. The study was approved by Dr. Hasan Sadikin General Hospital's Ethic Committee with number 567/UNG.KEP/EC/2019 and conducted in Dr. Hasan Sadikin General Hospital from August 2018 to September 2019.

Additional data study used in this study are normal subjects' data from "Clot waveform analysis on coagulation examination" research (unpublished data), which has been categorized as normal subjects based on the following examinations: Hematology (hemoglobin, hematocrit, erythrocytes count, leukocytes count, platelets); Clinical chemistry (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT)): excludes coagulation factor disorders due to liver dysfunction; Immunoserology (qualitative C-reactive protein): excludes infections; Thromboelastography (TEG): excludes other coagulation factor disorders; Hemostasis (fibrinogen, Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT)): excludes hemostasis disorders. The slope 2 results from aPTT test in normal subjects of that study will be used to compare with the slope 2 results from this study.

RESULTS AND DISCUSSIONS

There were 43 subjects that fulfilled inclusion criteria and gave informed consent. Subjects' characteristic data are shown in Table 1.

The median age of the subjects was six y.o with a range of 1-45 y.o, mostly 5-13 y.o (46.5%). This finding was following data from the World Federation of Hemophilia (WFH) 2017, which stated that aged 5-13 y.o was the second most prevalent age group in Indonesia (31%). Most people with severe hemophilia A are diagnosed in the neonatal period. Providing adequate factor VIII concentrate therapy and good monitoring of the quality of life will allow hemophilia A patients to have a better quality of life and a higher survival rate. The study by Prasetyawaty et al. stated that the survival rate of hemophilia A increased to 60-70 y.o with adequate factor VIII concentrate therapy.[17] The data in the United States in 2014 showed an increase in average life expectancy, from 49 y.o to 63 y.o. A national

Table 1. Characteristic of subjects (n=43)

Variable	n (%)	Median (range)
Age group (years)		6 (1–45 years)
0 – 4	16 (37.2)	
5 – 13	20 (46.5)	
14 – 18	6 (13.9)	
19 – 44	0 (0.0)	
≥ 45	1 (2.4)	
Factor VIII activity categories		0 (0–25.9%)
> 5-40% (mild hemophilia A)	8 (18.6)	
1-5% (moderate hemophilia A)	7 (16.3)	
< 1% (severe hemophilia A)	28 (65.1)	

study performed in the Netherlands between 1992 and 2001 demonstrated that the life expectancy in the non-severe hemophilia population was 67 and 73 years for moderate and mild hemophilia.[18,19]

The data in this study showed that 28 (65.1%) subjects were severe hemophilia A with factor VIII activity < 1%. This result was following data from WFH 2017 that shows the highest percentage of hemophilia A categories in the world was severe hemophilia A (45.79%).[20]

Slope 2 is expressed as %T/second, which shows the percentage of light transmission over the time of clot formation. The data of slope 2 and factor VIII activity were shown in Table 2.

Table 2. Slope 2 and factor VIII activity (n=43)

Variable	Median (range)
Factor VIII activity (%)	0 (0–25.9)
Slope 2 (%T/second)	1.0 (0.5-3.5)

The median of slope 2 in this study was 1,00%T/second with a range of 0.5-3.5%T/second. The average value of slope 2 obtained from "Clot waveform analysis on coagulation examination" in normal subjects was 4.2%T/second (unpublished data). It showed that slope 2 of hemophilia A subjects is lower than slope 2 in normal subjects.

The correlation test between slope 2 and factor VIII activity was performed with Spearman's correlation test. The correlation result between slope 2 and factor VIII activity is shown in Table 3.

Table 3. Correlation between slope 2 with factor VIII activity (n=43)

Variable	r-value	p-value
Slope 2 with factor VIII activity	0.854	< 0.001*

* = statistically significant (p < 0.05) according to Spearman's correlation test

This study showed a solid positive correlation, which was statistically significant (r =0.854 and p < 0.001) between slope 2 and factor VIII activity. This positive correlation showed that the higher slope 2, the higher factor VIII activity. There is currently no literature correlating slope 2 and factor VIII activity. Still, the results of this study are that factor VIII deficiency in hemophilia A patients cause coagulation phase disorder, which can affect slope 2 in clot waveform analysis of aPTT test. Shima *et al.'s* study showed a strong correlation between the factor VIII activity and clot waveform analysis curve of aPTT in hemophilia A patients, especially in the Min2 parameter (r=0.922, p<0.001). The results of that study indicate that a minimum increase of factor VIII activity may affect the acceleration of coagulation and then cause slope 2 to slope slightly more than normal. However, it is still possible that coagulation factors other than factor VIII affect the kinetics of clot waveform.[12,14,21]

Additional analysis was performed by manually observing data of factor VIII activity and slope 2. The range of slope 2 in factor VIII activity < 1% (severe hemophilia A) was 0.5-1.2%T/second, and slope 2 in factor VIII activity ≥1% was 1.3-3.5%T/second.

Nevertheless, this study had limitations. In this study, no medical record research was conducted regarding the patient's recent history of receiving factor VIII concentrate therapy. Instead, all the data was obtained from the questionnaire.

CONCLUSIONS AND SUGGESTIONS

This study showed a robust positive correlation, which is statistically significant, between slope 2 and factor VIII activity in hemophilia A patients. If slope 2 <1.3%T/second, there may be no need for additional tests other than aPTT because factor VIII activity is likely 0%. Further study with more subjects is needed to determine the cut-off for slope 2 in hemophilia A.

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