PREDICTION OF THE DECAYING FACTOR VIII LEVELS IN POST FACTOR VIII REPLACEMENT THERAPY USING ACTIVATED PARTIAL THROMBOPLASTIN TIME TEST IN HEMOPHILIA A PATIENTS UNDERGOING TREATMENT AT NATIONAL HOSPITAL OF SRI LANKA

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Hemophilia A is the most common hereditary coagulation disorder characterized by a deficiency in clotting Factor VIII. As an inclusive treatment, Factor VIII replacement therapy is given in the management of Hemophilia A. Prophylaxis has become the primary treatment, focusing on maintaining a baseline Factor VIII level to proactively prevent bleeding episodes. Therefore, predicting the decaying Factor VIII levels, in post Factor VIII replacement therapy is essential to overcome the challenges of managing Hemophilia A. The main aim of the study was to establish a cut-off value for aPTT as an indicator of decaying Factor VIII levels in post Factor VIII replacement therapy. Patients who were attending to Hemophilia clinic at the National Hospital of Sri Lanka were selected for the study (n=61). The patient's age, weight, and latest Factor VIII therapy dose taken were obtained from the records. The laboratory results; aPTT were obtained using the coagulation analyzer Coatron-M4 and elevated Factor VIII levels (EFL) due to the latest dose taken were calculated. The data and obtained aPTT results were analyzed by Microsoft Excel IBM SPSS version 26. In the first step, correlation bivariate analysis was performed to establish an association between aPTT and EFL. Then Receiver Operating Characteristic (ROC) curve analysis was performed to set up the cut-off value for aPTT as an indicator of decaying Factor levels. aPTT showed a weak negative correlation with EFL (Spearmen's correlation coefficient = 0.177). According to the ROC analysis, the cut-off value of aPTT is estimated as 38.8 s with a high sensitivity of 95.2% and specificity of 37.2%. Our results suggest that, once the aPTT is higher than 39 s; the EFL is below 25% and vice versa. We were able to set a cut-off value for aPTT as an indicator of decaying Factor levels in the body using ROC curves, thereby instead of relying on Factor assay, a simple aPTT test could be used as an indicator for the decaying Factor VIII levels in post Factor VIII replacement therapy. However, these initial findings should be validated by carrying out the research with an increasing number of Hemophilia A patients.

Keywords: Hemophilia A, Activated partial thromboplastin time test, Prophylaxis Factor VIII replacement therapy, Elevated Factor VIII level, Receiver Operating Characteristic (ROC) curve analysis

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INTRODUCTION

Hemophilia A is the most common hereditary coagulation disorder characterized by a deficiency in clotting Factor VIII (Benson et al., 2018; Kumar and Carcao., 2013). According to estimates, Hemophilia A affects 1 in 5000 males worldwide and it is estimated that the occurrence rate of Hemophilia is 1 in 20,000 in Sri Lanka. This is triggered by mutations in the genes that produce clotting Factors, which are proteins vital for blood clotting. These mutations can result in reduced or absent production of clotting factors, leading to excessive bleeding and bruising. Current investigations like Complete blood count, PT and aPTT are done as screening tests for Hemophilia A, mixing studies, Factor assays and genetic tests are done to confirm that. aPTT is a vital laboratory test used to screen Hemophilia A patients as it measures the intrinsic pathway of the coagulation cascade. aPTT is typically prolonged due to the deficiency of factor VIII in Hemophilia A patients. As an inclusive treatment, Factor VIII replacement therapy is given in the management of Hemophilia A (Escobar and Key, 2015). Prophylaxis has become the primary treatment, focusing on maintaining a baseline Factor VIII level to proactively prevent bleeding episodes. Since the patients with Hemophilia A are uncertain about the Factor VIII level in their body, they may encounter several poor clinical outputs. To determine the Factor VIII levels in the body, Factor assay has to be done. However, Factor assay involves more investigations, resources, and expenses. In such grounds, establishing a cut-off value for aPTT as an indicator of decaying Factor VIII levels in the body would smooth the way for a new promising diagnosis approach.

METHODOLOGY

Ethical clearance was granted by the Ethical Review Committee of the National Hospital of Sri Lanka (ERC NO: AAJ/ETH/COM/2023). A total of 61 volunteer participants attending to Hemophilia clinic at NHSL were selected for the study. Data on patients' age, weight, amount of latest Factor VIII therapy dose were obtained from medical records. Blood sample was collected in to 3.2% Tri sodium citrate tube from each participant for aPTT test and performed the test in semi-automated method using the coagulation analyser Coatron-M4 (Licon, S.A.) and elevated Factor VIII levels (EFL) due to the latest dose taken were calculated. The data and obtained aPTT results were analysed by Microsoft Excel IBM SPSS version 26. In the first step, correlation bivariate analysis was performed to establish an association between aPTT and EFL. Then Receiver Operating Characteristic (ROC) curve analysis was performed to set up the cut-off value for aPTT as an indicator of decaying Factor levels.

RESULTS AND DISCUSSION

A total of 61 patients were selected for the study according to the inclusion and exclusion criteria mentioned below.

Inclusion criteria;

- Patients aged 18-65 years
- Hemophilia A diagnosis confirmed by medical records and lab tests
- Patients with records of the last time and the amount of the dose of Factor VIII replacement therapy
- Patients undergoing Hemophilia A treatment at the Hematology Clinic of the National Hospital Sri Lanka
- Patients who are willing to provide blood samples for the aPTT test as part of the study

Exclusion criteria;

- Patients with a known history of other bleeding disorders or coagulation disorders
- Pregnant individuals or nursing mothers and minors (age below 18 years)
- Patients with significant medical conditions that might interfere with the assessment of treatment efficiency

The EFL was calculated using the formula below (Escobar and Key, 2015),

$$EFL = \frac{\text{Amount of dose}}{Plasma\ volume(5\%\ of\ Body\ Weight)} \times 100\%$$

Table 1: Hemophilia A patients data used in the study.

No	Age	Gender	TFL	aPTT	Time-Day	No	Age	Gender	TFL	aPTT	Time-Day
1	52	M	23.1	67.1	3	**32**	65	F	22.0	109.3	4
2	24	M	8.1	86.3	2	**33**	34	F	8.3	112.3	1
3	40	M	25	109.5	7	**34**	26	M	27.7	103.6	26
4	38	M	7.4	90.7	3	**35**	29	M	18.1	88.1	21
5	26	M	18.2	112.2	7	**36**	41	M	32.7	79.3	30
6	30	M	11.8	109.7	2	**37**	61	M	43.9	64.2	3
7	42	M	20	96.5	1	**38**	57	M	25.4	68.3	6
8	36	M	26.6	98.5	28	**39**	51	M	17.2	70.1	2
9	28	M	7.4	92.1	21	**40**	46	M	25	69.3	1
10	54	M	28.6	84.4	30	**41**	57	M	23.4	70.3	1
11	33	M	8.3	105.3	3	**42**	46	M	8.3	42.4	1
12	65	M	31.7	83.2	7	**43**	27	M	8.3	53.9	2
13	60	M	41.6	130.9	2	**44**	30	M	8.4	55.5	0.15
14	24	M	16.4	90.2	7	**45**	18	M	28.0	36.2	0.13
15	31	M	16.6	116.5	30	**46**	27	M	18.8	39.3	0.5
16	57	M	7.3	79.1	2	**47**	61	M	29.8	38.3	0.04
17	36	M	10	53.6	1	**48**	32	M	19.6	36.3	0.54
18	30	M	29.4	97.1	7	**49**	45	M	26.3	33.4	0.42
19	25	M	24.1	125.6	30	**50**	32	M	28.8	37.1	0.21
20	41	M	28.3	120	30	**51**	29	M	13.8	43.3	0.17
21	35	M	15.3	95.6	14	**52**	32	M	22.7	41.3	0.25
22	28	M	20	90.2	7	**53**	66	M	34.4	37.2	0.25
23	24	M	16.6	80.5	7	**54**	61	M	42.3	32.3	0.29
24	26	M	13.8	82.3	14	**55**	30	M	19.6	43.2	0.46
25	26	M	22.2	57.4	5	**56**	23	M	15.3	49.2	0.54
26	22	M	20.8	112.2	5	**57**	38	M	18.1	40.6	0.42
27	48	M	38.4	65.2	5	**58**	28	M	19.6	32.7	0.58

28	30	M	8.3	115.4	7	**59**	49	M	28.8	36.4	0.33
29	38	M	28.5	87.3	5	**60**	35	M	15.6	83.1	0.08
30	22	M	20	112.4	3	**61**	22	M	13.3	101.2	0.023
31	40	M	23.2	64.6	7						

Statistical analysis

The entire statistical analysis was performed using IBM SPSS version 26.

Testing parameters for normalization

In the first step, a normality of the data was tested separately using SPSS. Kolmogorov-Smirnov method was used to test the normality as the sample size was above 50. The data was considered in normal distribution when $p>0.05$. It was observed that the distribution of Weight followed a normal distribution. However, the variables aPTT Value, age and amount of dose did not exhibit a normal distribution. Accordingly, both the parametric and non-parametric analysis were carried out.

Correlation bivariate analysis

Correlation bivariate analysis was performed to establish an association between aPTT and EFL. Spearmen Bivariate analysis was used as one set of data was not followed the normal distribution. The aPTT showed a weak negative correlation with EFL. (Spearmen's correlation coefficient = 0.177)

Receiver Operating Characteristic (ROC) curve analysis

As the final step, Receiver Operating Characteristic (ROC) curve analysis was performed to set up cut-off values of aPTT as an indicator of decaying Factor levels in the body, by utilizing information on elevated Factor VIII levels (EFL) resulting from administered doses and their corresponding aPTT values.

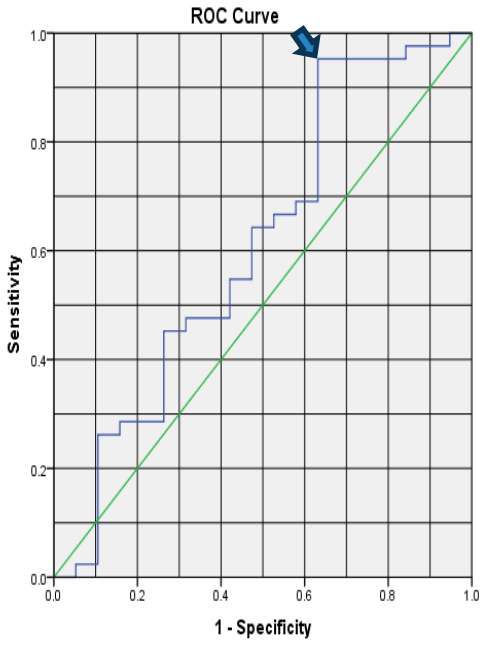

Figure 1: ROC curve analysis of aPTT based on two groups of EFL; EFL=<25% and EFL>25%.

The data was grouped based on Factor levels, creating two distinct groups with one containing Factor levels similar or less than 25% (Group 1) and another comprising Factor

levels exceeding 25% (Group 2). As an illustration, the corresponding empirical ROC curve was drawn using SPSS software (Figure 05).

According to the Figure 05, the cut off aPTT value is estimated as 38.8 s with the high sensitivity of 95.2% and specificity of 37.2% (AUC=0.603). Since the aPTT has the weak negative correlation with EFL the result could be interpreted as, Cut-off value of for aPTT =38.8 s (approximately 39 s). Once the aPTT is higher than 39 s; EFL is below than 25%, conversely, aPTT is lesser than 39 s; EFL is higher than 25%.

Similar studies were carried out using ROC curve analysis to establish a predictive relationship between Factor XII levels and aPTT (Bachler et al, 2019) and to determine the cut-off value of aPTT for Factor VIII inhibitor (Kumano and Leko, 2019). Even though a strong correlation was not established between EFL and the aPTT we were successful in obtaining a cut-off value for aPTT as an indicator of elevated Factor VIII levels in post factor VIII therapy. Thereby instead of relying on factor assay, a simple aPTT test could be used as an indicator for the elevated Factor VIII levels due to dose taken. However, the number of patients should be widened, and accurate monitoring of the patients are required to validate the initial findings before implementing them.

CONCLUSIONS/RECOMMENDATIONS

Even though a strong correlation was not established in between EFL and the aPTT we were successful in obtaining a cut-off value with high sensitivity of 95.2% for aPTT as an indicator of decaying Factor VIII levels in post Factor VIII replacement therapy. By prioritizing sensitivity, we ensure that all patients with elevated Factor levels are correctly identified, which is crucial in guiding therapeutic decisions. Although this approach may lead to more false positives, the primary goal is to avoid missing any cases of elevated Factor levels, ensuring effective and timely treatment adjustments. Thereby instead of relying on Factor assay, a simple aPTT test could be used as an indicator for the decaying Factor VIII levels due to dose taken. However, the number of patients should be widened, and accurate monitoring of the patients are required to validate the initial findings before implementing them.

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ACKNOWLEDGMENTS

We acknowledge General Sir John Kotelawala Defence University and NHSL for providing facilities for this research study.