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A STUDY IN A TERTIARY CARE HOSPITAL ANALYZING APTT-BASED CLOT WAVEFORM PARAMETERS

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ABSTRACT

The Activated Partial Thromboxane Time (APTT) and Prothrombin Time (PT) are two coagulation times that can be measured by automated coagulation analyzers. APTT and CWA parameters are generated using the newer fully automated analyzers. The objective of this study was to analyze the morphology of clot waveforms in cases with abnormal APTT as well as their first and second derivative values. 125 patients with 20 normal controls are included in the 125 ACL TOP 300 curves for APTT. The second derivative showed early and late shoulders, biphasic peak, delayed deceleration, and biphasic peak in addition to the first derivative and second derivative. This study examined wave clot forms from 125 patients. There were 46.9 women out of 2.2 men, with a mean age of 46.1 years old. Covid (20%), liver disease (23%), polytrauma (10.4%), cardiac diseases (8.8%), sepsis/DIC (7.2%), thromboembolism (7.2%), renal disease (7.2%) were some of the clinical conditions observed. Six percent are diseases, four percent are bacterial infections, four percent is dengue, six percent is snake bite, and six percent are factor deficiencies. In terms of acceleration and deceleration peaks, liver and heart disease were significantly different, followed by sepsis, dengue, polytrauma, and sepsis/DIC. Among Covid patients, the peak of deceleration was prolonged ($p<0.05$). The first derivative peak was prolonged in patients with sepsis and liver disease (p 0.05). Automated coagulation analyzers all offer CWA. With a fast turnaround time, it is inexpensive. The wave pattern was tracked both quantitatively and qualitatively, using velocity, acceleration, and clot formation details. In order to identify quantitative and qualitative parameters of CWA, we performed an APTT test on automated analyzers.

Key words: Velocity acceleration, APTT, clot waveforms

INTRODUCTION

When measuring coagulation assays such as Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT), Clot Waveform Analysis (CWA) is performed on the curve that is generated by an optical detection system. The absorbance of the light is used to detect light transmittance. A global hemostasis test is used to determine the overall hemostasis factor [1]. The first and second derivative curves (First and Second DCs) linked to clot reactions are displayed on automated photo-optical coagulation analyzers [2]. A DC's height can be used to reflect the hemostatic ability of the thrombin burst in the APTT-CWA. It appears that there is a bleeding risk in the first DC of APTT-CWA because of its low height. [3] For detecting coagulation factor deficiency in APTT-CWA, measuring the second DC's height is useful. A different pattern of information can be found by using thromboelastography (TEG), but this method is slightly more costly and time-consuming [4]. It has a cascade-based mechanism, thrombin burst, and phospholipids (PLs) that enhance the activation of clotting by the coagulation system. Nowadays, thrombin generation test (TGT), activated partial thromboplastin time (aPTT), thromboelastography (TEG) and thromboelastography (PT) are available as tests to measure the coagulation system.

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It evaluates how fibrin forms during tests for aPTT or PT by measuring the kinetics of fibrin formation. The clot waveform provides information regarding light transmission during clot formation. The clot reaction curves of both the PT and APTT are immediately visible with the optical end-point coagulation analyzer. In addition, the thrombin burst is visually visible with enhanced clotting activation due to PLs.

The slope generated by optical detection is analyzed using a plot waveform when routine coagulation tests, like PT and aPTT, are performed. The optical detection system detects clots by analyzing the change in transmittance and absorbance as the light beam passes through the sample.

Continuous APTT and PT assays display waveform data as a function of light transmission or absorbance. During the clotting process, light transmittance or absorbance decreases or fibrin forms as the clotting process progresses. Coagulation produces a sloped waveform. A linear segment is seen after coagulation in light transmission or absorbance.

As both TGT and CWA correlate with thrombin formation velocity and rate, they provide a similar picture of thrombin synthesis. There are different clot waveform morphologies for patients with DIC/Sepsis, Factor VIII deficiency [3], bacterial infections [11], Covid 19 [12], and patients taking anticoagulant therapy. We analyzed and compared the characteristics of clot waveforms in patients with abnormal APTT by analyzing the morphology and second derivative values.

MATERIALS AND METHODS

During the course of six months, the study will be conducted prospectively and cross-sectionally. Twenty age-matched controls and 125 patients participated in the study. All the abnormal APTT samples were thoroughly selected through the analyzer. A study aims to determine the efficacy and safety of low-molecular-weight heparin (LWMH) and unfractionated heparin (UFH). Anticoagulant was diluted with 3.2% trisodium citrate solution in the collection tube. In order to obtain platelet poor plasma, blood samples were centrifuged for 15 minutes at 3000 rpm for 15 minutes. Platelet counts in plasma should be less than 10000/microliter using a cell counter. An ACL Top 300 CTS Coagulation Analyzer was used to conduct the PT/APTT tests. In all abnormal APTT conditions, the morphology of the clot waveform was studied.

During clot wave formation, the maximum acceleration and deceleration were studied using the first derivative (clot velocity maximum), and the second derivative (clot wave maximum and minimum acceleration). SPS15 software was used for statistical analysis. For the APTT, first derivative and second derivative, we calculated the mean and standard deviation. P values were calculated using the Mann Whitney U test. An alpha level of 0.05 was set as the level of statistical significance.

RESULTS

Study participants included 145 patients and 20 controls. This study had a mean age of 46.9 years with a male:female ratio of 2.2:1. Controls and cases were compared in terms of APTT, second derivative, and first derivative range.

In all 125 cases, the shape of the clot waveform was examined. In addition to the sigmoid clot wave pattern of delayed deceleration, there were also biphasic, prolonged precoagulation phases, slow or steep slopes, and second-derived morphologies. Two of the most prevalent conditions among 125 patients in this study were liver disease and Corona Virus Disease 2019 (Covid-19). Sigmoid patterns and prolonged precoagulation phases were most common. A variety of clot waveform morphologies are shown in different clinical conditions. Based on the means of APTT and its first and second derivatives in each clinical condition and those in the control group, the first and second derivatives have been calculated.

According to the first derivative, liver disease and sepsis are significantly associated ($p<0.05$). In relation to second derivative cases of Covid-19, sepsis, heart disease, and liver disease (p0.05), a significant association was found (p0.05).

Table 1: In different clinical conditions, the mean and second derivative values for APTT are presented

	APTT Mean	**It can be calculated as the first derivative of the mean velocity**	**The second derivative of the mean acceleration**	**Inflation (Second derivative) plus mean deceleration**
A control system	34.2	218.94	743.04	363.37
In COVID-19	58.2	258.77	625.71	254.04
Diseases of the heart	46.2	201.33	514.28	230.29
Diseases that are chronic	50.8	210.06	570.15	265.74

Infections caused by bacteria	48.4	345.80	866.01	271.28
Symptoms of liver disease	43.5	167.61	431.29	203.70
Adenovirus	43.4	152.38	385.33	147.67
Deficiency of factors	53.5	257.19	668.97	233.25
Diseases of the kidneys	63.4	284.28	641.50	264.03
Asthma/Diabetes	74.5	145.23	295.31	122.38
Bites by snakes	118.3	12.42	22.28	9.94
Thromboembolism of the veins	56.2	255.33	451.46	200.87

Table 2: Different clinical conditions associated with APTT and its derivatives

Situation clinically		**APTT**	**Derivative one**	**(+)2nd derivative**	**(-)2nd derivative**
	Whitney Mann University test	14.000	207.000	186.000	128.000
Covid					
	P value	0.000*	0.575	0.284	0.013*
	Whitney Mann University test	0.000	41.000	16.000	12.000
Deficiency of oxygen					
	P value	0.000*	0.047*	0.001*	0.001*
	Whitney Mann University test	0.000	88.000	58.000	53.000
Having a heart condition					
	P value	0.000*	0.364	0.032*	0.019*
	Whitney Mann University test	6.000	185.000	113.000	90.000
Inflammation of the liver					
	P value	0.000*	0.033*	0.000*	0.000*

A clot waveform with abnormal morphology was observed in two snakebite cases. DIC was present after the precoagulation phase, as evidenced by deformation of the first derivative and deformation of the second derivative. A second derivative curve with early and late shoulders was observed in two cases of factor deficiencies (Factor V and Factor VIII).

DISCUSSION

In the coagulation cascade, there are multiple components, including blood vessel walls, plasma proteins, platelets, and coagulation factors. A PT or an aPTT test gives information about hemostasis, a test that is routinely performed. In this new era, coagulation tests are extremely important. [13].

CWA is based on global coagulation tests, APTT and PT, and is studied using an automated coagulation analysis system based on optical detection. There are a variety of ACL series available, and these analyzers work on the principle of light absorption to help study the entire hemostasis process as represented by waves [14].

Hemostatic alterations and abnormal patterns were identified in the current study in various clinical cases, including Covid-19, bacterial infections, liver diseases, DIC/sepsis, hemophilia and venous thromboembolism. Covid-19 with severe hypercoagulability has been associated with an increase in thrombotic events, including pulmonary embolism [15].

MF Rubereto et al. [16] studied the CWA of 191 patients with liver cirrhosis and found that those with liver cirrhosis had lower maximum acceleration and deceleration values than those with control livers. A study by Takuya et al. [12] examined clot waveforms of APTT clots

According to the results, Covid-19 patients with abnormal second derivative morphology (early shoulder

type and late shoulder type) exhibited similar patterns as ours.

A study [1] compared 101 patients with and without bacterial infections found significantly higher CWA parameters among patients with infections. Compared to patients with dengue infection, those with CWA parameters were significantly lower. Our study also found a similar result.

According to [3] first and second derivatives of clot waveforms could be used for diagnosing and predicting bleeding risks in patients with sepsis. The second derivative of the disease condition was significantly associated with the disease condition.

Based on a study [17], bleeding severity in patients with Hemophilia A can be correlated with clot waveforms and thrombin generation tests, but not always. A CWA also provided new insight into the potential use of CWA to detect hypercoagulability or bleeding risk in various clinical conditions [17] as we had two cases of factor deficiency with abnormal clot wave formation.

The clotting waveforms of patients with LA-positive APS were shown [18] to be significantly atypical in peak and deceleration/acceleration ratios. [18] analyzed the same waveforms.

Using the CWA, [19] explored the relevance of anticoagulation from a fibrinolytic perspective to therapeutic efficacy and bleeding risk.

Using preexisting test protocols and assay equipment, CWA extends the routine aPTT test. A clot waveform curve is presented by clotting analyzer software that measures changes in optical properties over time as clots form, capturing these optical changes over time as clots form.

CONCLUSION

Studies that have shown correlation between clot wave patterns illustrate the necessity of utilizing the automation machine data without added cost or turn-around time.

Similar to TGT, clot waveform analysis reflects the whole process of thrombin generation, as it correlates with thrombin formation rate and velocity. By contrast, clotting time is only indicative of coagulation initiation in routine coagulation assays.

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