Utility of Clot Waveform Analysis in Russell’s Viper Bite Victims with Hemotoxicity

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Abstract

Introduction: In Russell’s viper bites, due to the lack of a better alternative, whole blood clotting test (WBCT) remains the standard test even though its reliability and sensitivity has been shown to be low. Activated partial thromboplastin time (aPTT)-based clot waveform analysis (CWA) is an optic absorbance assay that can be used as a global clotting test. In this study, the objective was to assess the changes in CWA and to compare CWA to WBCT and aPTT in patients with Russell’s viper envenomation. Methods: The datum was collected prospectively over 2 months as a pilot observational study in a tertiary care center. All proven cases of Russell’s viper-envenomated individuals with preliminary CWA data and WBCT were included in the study. The clot wave (CW) of the five individuals, which met all the stringent inclusion criteria, was analyzed and interpreted. Results: CW absorbance sigmoid waveform was deranged in all 5 cases, of which 4 showed a change in CWA even before an abnormal aPTT. Three of the 5 had a normal WBCT but showed early changes in CWA. Atypical biphasic waveform reported in disseminated intravascular coagulation in other prior studies is seen in venom-induced consumptive coagulopathy also. In all patients where a second derivative was plotted, the second (lower) phase of the second derivative showed a slow rise to baseline. Conclusion: CWA showed changes which provided information earlier than the conventional coagulation studies in the snakebite victims studied. While aPTT or WBCT reflects clotting time, CWA conveys the dynamic process of clot formation and stabilization. CWA may reveal disorders of clotting in snakebite victims before the conventional tests become abnormal. Future research should assess the speed and accuracy of the test in diagnosing hemotoxic envenomation and its potential role in guiding antivenom therapy.

Keywords: Clot waveform analysis, hemotoxic envenomation, snake

INTRODUCTION

Russell’s viper (Daboia russelii) bites are quite common in India and are notorious to cause hemotoxicity.[1,2] In hemotoxic bites, the test recommended to ascertain the development of coagulopathy is a whole blood clotting test (WBCT) as per the current guidelines.[3-5] In Russell’s viper bites, the reliability and sensitivity of WBCT has been shown to be low, but this still remains the standard test.[6] There exists a need to explore other coagulation studies in snakebite to look for a better and efficient alternative.

Clot waveform analysis (CWA) is an activated partial thromboplastin time (aPTT)-based optic absorbance assay that can be used as a global clotting test.[7] Most traditional hemostatic tests assess the clot initiation capacity of the sample but do not evaluate for clot stability which is possible with the use of CWA. It has been shown useful in identifying disseminated intravascular coagulation (DIC) in sepsis with high specificity (97.6%) and sensitivity (98%), and the test is recommended by the British Committee for Standards in Hematology guidelines for the diagnosis and treatment of DIC.[8] The authors in this study aimed to assess the changes in the CW form in Russell’s viper-envenomated victims and to compare CWA with standard tests such as prothrombin time (PT) with the international normalized ratio (INR), aPTT, clotting time (CT) (modified Lee and White method),[9] and WBCT.[3]

METHODS

This observational study was conducted prospectively as a pilot, over 2 months in an academic emergency department (ED) of

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How to cite this article: Abraham SV, Rafi AM, Krishnan SV, Palatty BU, Innah SJ, Johny J, et al. Utility of clot waveform analysis in Russell’s viper bite victims with hemotoxicity. J Emerg Trauma Shock 2018;11:211-6.

Received: 30.04.17. Accepted: 20.02.18.
a single tertiary care teaching institute in South India with the approval of the Institutional Ethics Committee and Institutional Research Board (Ref No. 42/1b/IEC/JMMC and RI). Only adults (≥18 years of age) consenting to be part of the study, with confirmed Russell’s viper bites, where the specimen was brought and positively identified by the treating physician were included in the study [Figure 1]. The specimens were preserved for further confirmation by an expert, and all live specimens brought were later released into the wild after identification. The victims were managed as per the Institutional Snakebite Treatment Protocol which adheres to the World Health Organization (WHO) and the National Snakebite Treatment Protocol.[13-15] Unless specified, the institutional policy is to draw a blood sample for bedside coagulation tests, WBCT, and CT at admission, 1st, 2nd, 3rd, and 6th h postbite and then every 6th h for the next 24 h. Derangement of bedside coagulation study served as a trigger to commence anti-snake venom (ASV). Additional blood investigations were sent as per the clinical requirement of the treating physician, which included PT with INR, aPTT, D-dimer, and serum fibrinogen levels. The individuals who had a deranged WBCT at admission or treated outside with ASV were excluded from the study. If there was no simultaneously drawn aPTT sample, their datum was also excluded since it defeated the purpose of this research. All pertinent data including the clinico-epidemiological profile of the victims, coagulation parameters obtained, and treatment administered including ASV and blood products were chronologically noted and patient followed up for the course of their hospital stay.

Materials

Bedside coagulation tests: Blood clotting test and clotting time

WBCT was done as per the WHO recommendation[13] and CT as per the modified Lee and White method.[9]

To do the bedside coagulation tests, as per the institutions snakebite protocol, 2 ml of atraumatic venepuncture blood samples was collected in four clean, dry, properly labeled, glass test tubes.

The first test tube was left undisturbed for 20 min at ambient temperature. At the end of 20 min, the test tube was tipped once, and if the blood was still not clotted, the test was taken as “abnormal WBCT.”

The second, third, and fourth test tubes were kept on a rack at ambient temperature, and every 30 s, the second tube was tilted approximately 45° checking for clot formation while the other tubes were left undisturbed. After the blood in the first tube clotted, the second tube was tilted every 30 s and examined. Following its clotting, the third tube was examined. Since agitation and handling accelerate clotting, CT of the third tube is recorded as the CT value. If clotted, then the sample in the third test tube was assessed for 30 min to look for breakup or lysis of the clot. A CT of more than 20 min was taken as prolonged CT and those samples that lysed within 30 min of clotting as “unstable clot.”

Automated coagulation analyzer

The aPTT test was performed using ACL TOP® 300 automated coagulation analyzer. The ACL TOP® is a fully automated random-access multiparameter coagulation analyzer equipped with a photo-optical clot detection unit. It is designed to perform coagulation, chromogenic, and immunologic assays with continuous loading capabilities for samples, reagents, and disposables.[10] The APTT reagent used was SynthASil®, a colloidal silica-activated APTT reagent.[11]

Clot waveform analysis

Waveform analysis of the optical data obtained from the modified aPTT assay was performed on the “ACL TOP® 300” coagulation analyzer as described previously and the data automatically processed by algorithms built into the software. The raw datum was procured by eliminating any smoothing of the curves by the machine.

CWA is based on the traditional aPTT assay. On assessing aPTT with light transmission, a change in the light absorbance is observed as the clot stabilizes by fibrin polymerization.[12] Plotting the milliabsorbance (mAbs) of the sample to time, a curve is obtained which reflects the optical profile that is generated as a clot is formed.

This tracing against time gives a qualitative assessment of fibrin polymerization.[13] The delta in absorbance (dAbs) is based on the change in mAbs value from the baseline to the endpoint (plateau) along the Y-axis of the clot curve. The dAbs values of <100 mAbs denote low fibrinogen samples.[14]

The normal clot waveform

The normal clot waveform has five main phases: delay, baseline, acceleration, deceleration, and end point [Figure 2].[12-14] The “delay” period begins at 0 and precedes the “baseline,” which denotes the mixing of the reagents and system optimization of light intensity. The “baseline” is the portion of the curve which appears after all reagents have been added to the time and the clot formation begins. The aPTT value is noted at this point (vertical red line). The “acceleration” phase denotes fibrin clot formation, whereas “deceleration” denotes decreasing rate of clot formation.[13,14]

Figure 1: Flow diagram: Inclusion and exclusion of participants
Figure 2: Clot waveform of normal plasma by monitoring of absorbance. (a) Normal “S” clot reaction curve showing the phases of the clotting reaction, change in absorbance (Y-axis) versus acquisition time (X-axis). Indicates the activated partial thromboplastin time value on X-axis. The upper blue-colored trace shows the changes in absorbance observed during the performance of activated partial thromboplastin time with normal reference plasma. The clot waveform is comprised of five main phases: delay (0-A), baseline (A-B), acceleration (B-C), deceleration (C-D), and end point (D-E). (b) The purple-colored curve is the first derivative of the absorbance corresponding to the coagulation velocity. The light blue-colored biphasic curve is the second derivative of the absorbance corresponding to the coagulation acceleration.

There are two more curves that are plotted in CWA, the first derivative and the second derivatives. They are both derivatives of the absorbance curves. The first derivative reflects the coagulation velocity, whereas the second derivative reflects the acceleration of coagulation.\textsuperscript{[14]}

Results

Over a period of 2 months, 207 snakebite victims were brought to the ED, of which 20 had brought specimen which was initially identified as Russell’s viper, of which two turned out to be Hump-nosed pit vipers [Figure 1]. In nine individuals, the coagulation parameters were completely deranged at the time of admission and hence were excluded. Three had delayed (>12 h) development of the signs of envenomation, of which none had simultaneously drawn aPTT sample. One had no signs of envenomation. Therefore, only five Russell’s viper-envenomated individuals had usable CWA data which were done simultaneously with the initial WBCT.

These five were individually analyzed [Table 1].

Patient 1 was bitten at 8:30 am and he reached ED by 9:15 am (75 min postbite), with a tight tourniquet in place. The CT was 13 min at admission and WBCT normal. Simultaneously drawn sample for aPTT was 21.1 s with a normal CWA and slower second phase of the second derivative. The dAbs was low (=63 mAbs). The baseline of CW was very short, and then, a sudden sharp increase in clot was noted [Figure 3a]. WBCT for this patient at 10:15 am was again normal and CT 13 min, but he started complaining of slight loin pain. The decision was made to commence antivenom in view of clinically worsening symptoms. The third WBCT sample collected at 11:15 am just before the administration of ASV was abnormal, and CT showed that it clotted at 15 min but lysed after 2 min.

Patient 2 [Figure 3b] was a nurse bitten at a nearby hospital premise at 7:30 pm. WBCT within 5 min of bite was recorded as normal at that hospital and he was referred over. The admission WBCT done at our department, 1 h and 15 min after the bite (08:45 pm), was abnormal. The CT sample clotted at 15 min but the sample lysed after 3 min. Simultaneously sent aPTT was 27.4 seconds(s), D-dimer 12800 ng/ml (positive), INR1.72, and fibrinogen 69 mg% (low). The CW showed a delayed plateauing (delayed “acceleration” and prolonged “deceleration phase”) of the sigmoid absorbance curve and delayed second phase of the second derivative peak (33.892). The change in optical absorbance (dAbs) was low (=35). He was subsequently started on ASV.

Patient 3 [Figure 3c] was bitten at 7:30 am over her left foot, and she reached the ED by 8:00 am (30 min postbite). The initial WBCT at admission (8:10 am) was normal and the CT was 14 min. The second blood WBCT sample taken at 9:10 am was also normal. The simultaneously drawn CT was 14 min, aPTT was 30.4 s, fibrinogen 76 mg%, and INR2.03. Baseline of the CW was deranged. It showed a delayed plateauing of the sigmoid absorbance curve (delayed acceleration and prolonged deceleration phase), and the first derivative was wider. The second derivative had a notched upstroke (twin-peaked) and a delayed second phase of the second derivative. The peak value of second derivative (33.721) and dAbs (≈40) were low.

Patient 4 [Figure 3d] was envenomated at 8:30 pm following which he presented to a hospital nearby. They recorded a CT of 4 min and referred him over since they noticed swelling and discoloration at the bite site. Reaching the ED at 9:20 pm (50 min postbite), he had tender inguinal lymphadenopathy and his initial WBCT at 9:30 pm was abnormal, and the sample for CT clotted at 15 min but lysed after 3 min. Simultaneously drawn aPTT was 28.2 s, fibrinogen 99 mg%, PT17.7 s, and INR1.67. All three waves plotted on CWA were deranged. The peak of the second derivative was low (37.459). The first derivative base was broader and the dAbs was low (=25 mAbs).

Patient 5 [Figure 3e] reached ED in 15 min after being bitten by a Russell’s viper at 9 pm. The first WBCT sample taken at 9:20 pm was normal and the CT was 13 min. The second WBCT at 10:15 pm was also normal. Simultaneously drawn sample showed CT of 16 min, PT >180 s, aPTT >180 s and...
a completely deranged CW. The decision to commence antivenom was made. WBCT sample drawn at 11:15 pm; before commencing the antivenom, was abnormal and CT prolonged more than 30 min.

**DISCUSSION**

Recognizing the heavy economic, physical, and psychological toll on snakebite victims, the WHO categorized snakebite as a neglected tropical disease in 2009. The magnitude of the issue is often underestimated by the developing nations probably because of the complacency of both the victim and the physician tending to the ailment. Mohapatra et al., in 2011, estimated that annually 46,000 people (99% confidence interval 41,000–51,000) die of snakebite in India.

In the South Indian state of Kerala, coagulopathy following viper envenomation is quite common. Conventionally, although this coagulopathy is likened to DIC, in the recent years, it has been described as venom-induced consumptive coagulopathy (VICC). It would be interesting to note that VICC defers from DIC in its rapidity of both onset and resolution. The triggers in VICC also differ from DIC. The snake venom contains multiple coagulation cascade activators, which unlike the tissue factor trigger in DIC, triggers the cascade at different levels. This makes it a more complex phenomenon than DIC. However, for more than a decade, WBCT remains the gold standard recommendation to detect coagulopathy in snakebite victims. It would be of interest to note that in bite victims it serves not only as a clotting test but also as a detector of envenomation and a guide to trigger antivenom administration. The financial feasibility of this test, requiring just a glass tube and a sample of the patients’ blood, in a developing nation like India is unparalleled.

Antivenom is a precious commodity due to its scarcity and price in developing nations like India. Dry bites by venomous snakes are not uncommon either. Antivenom administration itself may result in life-threatening anaphylaxis. Therefore, we do not administer antivenom to victims of a bite, even if the snake has been identified as venomous, unless and until they show signs or symptoms of envenomation. In this context, it is evident that the coagulation studies play a key role in the management of victims of snakebite. Even though WBCT plays such significant role in bite victims, it is remarkable that no attempts at standardization have been made for this test. The glassware used and the procedure per se including the duration of the test varies from 10 to 30 min in various studies. Even the frequency at which it should be repeated is not standardized. In clinical practice, the WBCT is known to have a low sensitivity for detecting coagulopathy in snakebites. An initial negative WBCT invariably delays the administration of ASV in envenomated individuals. Snakebite victims need better measures of coagulation to assure that patients who need antivenom will receive it as quickly as possible while avoiding unnecessary risk and cost of giving antivenom to a patient who will not benefit from it.

An ideal coagulation test should not only be easy to perform but also provide us with a highly reliable result. It should also be able to allow an accurate estimation of the thrombotic risk and clot stability. There are several coagulation tests that assess the clotting process in its entirety. However, the utility of such global clotting tests is yet to be evaluated in snakebite victims. CWA can detect the DIC earlier than conventional methods in up to 19% of the cases. Hence, the test is recommended by the guidelines for diagnosis and treatment of DIC. Coagulopathy resulting from Russell’s viper bite has been shown to cause depletion of fibrinogen, factor V, and factor X. CWA is sensitive even to minor factor deficiencies, making it useful in the management of hemophilia. CWA is shown to denote changes in fibrin- and factor-depleted plasma. If validated in a large sample study, CWA has the potential to act in lieu for the factor assays and could help to guide the blood product administration in snakebite victims.

CWA sigmoid waveform was deranged in all five cases in this study. Four of the five cases (patients 1, 2, 3, and 4) showed a change in CWA even before an abnormal aPTT being reported. The remaining one (patient 5) already had a deranged aPTT with a normal WBCT and a CT at 16 min, at presentation. In patient 1, the baseline is very short, and then, a sudden sharp rise in the CW is noted. A shortened aPTT time is shown to have increased thrombin generation and a higher risk for thromboembolism. The clot curve shows an atypical “biphasic waveform” (BPW), in patient 2, with a slow rising

### Table 1: Comparison of coagulation parameters of individuals

| Patient | CT (mins) | WBCT (s) | INR | Fibrinogen (mg/dl) | dAbs (milli abs) | Pre Coagulation phase change | Coagulation phase change | Peak of second derivative | Slow rise second derivative | Deranged CWA with Normal aPTT | Deranged CWA with Normal WBCT |
|---------|-----------|----------|-----|--------------------|-----------------|-----------------------------|--------------------------|----------------------------|----------------------------|------------------------------|------------------------------|
| 1       | 13        | Normal   | 21.1 | !                  | !               | 63 Early activation         | Slow rise                | 182 (normal)              | +                          | +                            | +                            |
| 2       | 15        | Abnormal | 27.4 | 1.72               | 69              | 35 BPW                      | Slow rise                | 33.8 (low)                | +                          | +                            | -                            |
| 3       | 14        | Normal   | 30.4 | 2.03               | 76              | 40 Downsloping              | Slow rise                | 33.7 (low)                | +                          | +                            | -                            |
| 4       | 15        | Abnormal | 28.2 | 1.67               | 99              | 28.2 Downsloping            | Slow rise                | 37.5 (low)                | +                          | +                            | -                            |
| 5       | 16        | Normal   | >18  | >18                | ^               | Deranged                    | Downsloping ^            | ^                          | -                          | -                            | +                            |

*Clotted at 15 min, but lysed after 3 min. # sample not sent for analysis. + Present, - Absent. WBCT: Whole blood clotting test, Mins: Minutes, aPTT: Activated partial thromboplastin time, S: Seconds, INR: International normalization ratio, dAbs: Delta absorbance, Milliabs: Milliabsorbance, CWA: Clot wave form analysis*
baseline, instead of the flat line before the commencement of clotting. BPW is a known sensitive indicator of DIC. BPW is hypothesized to be due to an immediate and increasing light absorbance following activation and recalcification of the plasma sample before formal clot formation. Simultaneously done fibrinogen in the sample denotes that it is very low which probably denotes consumption. In this case, it is interesting to note that the atypical BPW earlier reported in DIC is also seen in VICC. In patients 3 and 4, the baseline of CW is downsloping. The peak of the second derivative is low and it shows changes with second derivate pattern. Simultaneously drawn blood sample shows low fibrinogen in both. Whether this pattern is unique to factor- or fibrin-depleted plasma needs to be further studied.

In patient 5, CWA denotes complete depletion of fibrinogen in the victim while the WBCT showed an increasing trend.

Figure 3: Clot waveforms of individual patients. (a) Patient 1, (b) Patient 2, (c) Patient 3, (d) Patient 4, (e) Patient 5

Limitations
The reasons why the WBCT is the standard test are that it requires no sophisticated laboratory testing and resource-limited hospitals can easily perform this at the bedside. CWA requires a laboratory analyzer. CWA datum though is inexpensive and extremely easy to perform optical tracing in aPTT analyzers is not universally available in all machines. Currently, there are only two systems, which are able to assess the light transmittance or absorbance tracings. However, analyzers, which work with the same principles, should be able to create the graphs after updating with the necessary software. Another problem while using CWA is that these analyzers also need to use clear agents. This might also form a problem in case of colored plasma (hyperbilirubinemia, hyperlipidemia, or hemolysis). Finally and most importantly,
there is no much experience with these assays. Most data and literature available with regard to its interpretation are in the experimental phase, let alone have clinical validation.

Another limitation is that our exclusion criteria excluded 97% of the snakebite victims who came to our hospital. It may be possible that these five patients are not reflective of the larger group of patients. The comparison data with patients bitten by venomous snakes were not included making it not possible for us to comment whether CWA can distinguish between venomous and nonvenomous bites.

**Conclusion**

While aPTT reflects CT, the CWA conveys the dynamic process of clot formation and stabilization. CWA may reveal disorders of clotting in snakebite victims before standard tests such as WBCT or aPTT becomes abnormal. Future research should assess the speed and accuracy of the test in diagnosing hemotoxic envenomation and its potential role in guiding antivenom therapy.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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