

# Comparative Evaluation of Whole Blood Clotting Test as a Diagnostic Predictor of Venom Induced Consumption Coagulopathy in Hemotoxic Snake Bite

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## ABSTRACT

**Background:** Venomous snakebites are a significant concern in South-East Asia, with an annual death toll of approximately 45,900 in India, higher in rural areas and during the rainy season. States like Uttar Pradesh, Andhra Pradesh, Bihar, and Odisha report the highest cases. Odisha's venomous snakes include neurotoxic kraits and cobras, myotoxic sea snakes, and hemotoxic species. The aim is to compare WBCT at 20 and 30 minutes as a better predictor of venom-induced coagulopathy and to determine its sensitivity and specificity.

**Methods:** In this study, for each patient before giving ASV 2ml venous blood was drawn for 20 minutes WBCT and 30 minutes WBCT and put in two clean, dry, ordinary test tubes at room temperature. Simultaneously, other blood samples were sent PT, INR, aPTT, D dimer, and serum fibrinogen to the clinical haematology laboratory SCB MCH. These tests were repeated at 6 hours after 1st dose of ASV.

**Results:** Most cases were from rural areas and presented late to the hospital. Fang marks were observed in 41% of cases, with bleeding, oliguria, and haematuria in 5%, 8%, and 6% respectively. Bites mostly occurred during cultivation in the rainy season (73%). WBCT20 showed a sensitivity of 84.72%, specificity of 64.29%, PPV of 85.92%, and NPV of 62.07%. WBCT30 had a sensitivity of 80.28%, specificity of 72.41%, PPV of 87.69%, and NPV of 60%.

**Conclusion:** The study investigates whether WBCT30 offers advantages over the accepted standard WBCT20 for detecting snake venom-induced coagulopathy.

**Key-words:** WBCT 20, WBCT 30, VICC, Hemotoxic, Venomous Snake Bites

## INTRODUCTION

Venomous snakebites are an important medical problem and occupational hazard in South-East Asia.

The annual number of snake bite deaths in India is around 45900, with higher numbers in rural areas than urban ones. Snakebites also occur more often during the rainy season. Annual snake bite deaths were greatest in the states of Uttar Pradesh (8700), Andhra Pradesh (5200), and Bihar (4500) with Odisha reporting (2200) cases.<sup>[1]</sup> Venomous snakes found in Odisha can be hemotoxic, neurotoxicity or myotoxic. Krait and cobra venom is neurotoxicity, sea-snake venom is myotoxic, hemotoxic snakes are Russell's viper, saw-scaled viper, and pit viper. The usual complications of hemotoxic

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snake bites are local cellulitis, hemorrhagic manifestations, DIC, acute renal failure, intravascular hemolysis, pulmonary oedema, shock, acute pituitary insufficiency, cardiac arrhythmia, and death.<sup>[2]</sup>

The venom of these hemotoxic snakes contains serine protease and procoagulant toxins that are factor V activators, factor X activators, prothrombin activators and Thrombin enzymes (TLEs) or fibrinogenases.<sup>[2]</sup> This results in cross-linked fibrin forming in the blood stream, most of which is immediately broken down by the body's own plasmin fibrinolytic system. Eventually, the levels of clotting factors become so depleted by disseminated intravascular coagulation that consumption coagulopathy develops, which is known as venom-induced consumption coagulopathy (VICC)<sup>[3]</sup>.

Viper venom also contains a zinc metalloproteinase, 'hemorrhagin', which damages vascular endothelium and promotes vascular leakage, toxins that impair platelet function and hemolytic phospholipids A2, which has disseminated effects by damaging cell membranes, endothelium, skeletal muscle, nerves and red blood cells. The cytolytic toxins are the digestive hydrolases that destroy cell membranes and tissue, increasing permeability and local swelling. The major treatment for hemotoxic snake bites is anti-snake venom administration, which aims to neutralize the toxins in the venom<sup>[4]</sup>. To detect coagulopathy, it is required to test for PT, apt, INR, fibrinogen, D dimer. For the diagnosis of DIC, a simple scoring system has been developed by the International Society on Thrombosis and Hemostasis. A score of >5 is compatible with DIC.<sup>[3]</sup>

An abnormal INR result indicating coagulopathy is 1.2 or above, and it is an indication for the administration of 10 vials of ASV according to WHO guidelines.<sup>[5]</sup> In developing countries, particularly India, most envenomations occur in rural areas. They are managed in peripheral health centers that cannot perform automated lab tests to diagnose or monitor readmission of envenomed patients' ASV<sup>[6]</sup>. ASV is expensive, difficult to obtain in some parts of the world and associated with a significant risk of systemic hypersensitivity reactions. It is essential to determine which patients have envenoming rapidly and accurately and will require ASV<sup>[7]</sup>. The 20-minute WBCT has been used in hemotoxic snake bites for several decades to determine if patients have clinically significant coagulopathy. The first evidence of systemic envenoming might appear at any

time from the time of bite and therefore, the patients should be monitored continuously to detect it.

Positive WBCT 20 indicates administering the first dose of ASV therapy. After that, successive WBCT 20s are performed at 6 hr after every dose of ASV to determine the need for repeated doses of ASV. However, its effectiveness has not been critically analyzed. Some data show low sensitivity (40%) and good specificity (100%) of 20-minute WBCT.<sup>[4]</sup> A study conducted in SCB MCH in 2017 with an INR of 1.5 or above showed the sensitivity of 67.19. % and specificity 91.4% of the 20-minute WBCT.<sup>[5]</sup> Another study with an INR 1.4 or above showed a sensitivity 82% and a specificity of 98%<sup>[7]</sup>. The quality of the test tube and room temperature also affect the 20 min WBCT. The study performed in ceftriaxone bottles showed a sensitivity 83.3% and a specificity 90%.<sup>[8]</sup> Despite the widespread reliance on this test and it is regarded as the standard of care for the treatment of snake envenoming in resource-poor settings, there have been very few studies that have determined the conditions under which the test can be performed accurately and validated it against standard tests or demonstrated that it is accurate in the field. According to recent studies, expanding the interpretation of the WBCT to include evaluation at both 20 and 30 minutes has resulted in a reduction in false positives and negatives during the initial assessment and a better correlation with coagulopathy.<sup>[9,10]</sup>

## MATERIALS AND METHODS

This hospital-based case comparative study was done among hemotoxic snake bite cases admitted to the Medicine department, SCB MCH Cuttack.

All patients fulfilling inclusion criteria were recruited for the study after receiving informed consent, providing a detailed history, and performing a physical examination through preset proforma.

Ethical clearance was taken from the Institutional Ethical Committee SCB MCH Cuttack before starting the study. For each patient, before giving ASV 2 ml venous blood will be drawn each for 20 minutes WBCT and 30 minutes WBCT and put in two clean, dry, ordinary test tubes at room temperature. Simultaneously, other blood samples will be sent PT, INR, aPTT, D dimer, and serum fibrinogen to the clinical haematology laboratory SCB MCH. These patients should be followed up with routine investigations like Complete blood count, Comment on

peripheral smear, Urine routine examination, Renal Function Test (serum urea, serum creatinine, serum sodium and serum potassium), Blood glucose level and Liver Function Test. These tests were repeated at 6 hours after 1st dose of ASV to consider the requirement of repeated doses of ASV. The result of 20 minutes WBCT was compared with 30-minute WBCT and correlated with PT, INR, aPTT, serum fibrinogen, D dimer to assess the validity of 2 ml WBCT in early detection of coagulopathy in hemotoxic snake envenoming and reaching endpoint of ASV treatment in venom-induced consumption coagulopathy.

**Inclusion criteria-** Patients admitted to the Medicine department of SCB Medical College from September 2018 to September 2019 with a definite history of snake bites has to be selected. The patient was diagnosed as a case of snake bite by presenting features such as :

- ✓ The patient gave definite history of snake bite
- ✓ Swelling at the site of bite with or without fang marks, bruising and necrosis
- ✓ Hypotension and shock
- ✓ Bleeding manifestations include hematemesis, malena, hematuria, epistaxis, continuous blood oozing from the bite site, etc.
- ✓ Decreased urine output, AKI
- ✓ Ptosis, dysphasia, single breath count less than 30.
- ✓ Description of snake by the patient
- ✓ Patients with a history of snake bite with signs of envenoming were included in the study after obtaining ethical committee clearance and informed consent from all patients.
- ✓ Patients with a history of unknown bites with signs of hemotoxic snake envenoming are also included in the study

#### **Exclusion criteria**

- ✓ Patient of non-poisonous snake bite, neurotoxic snake bite
- ✓ Exclude those who are on hormonal contraceptives or hormonal therapy for any disease or anticoagulant/ antiplatelet therapy.
- ✓ Exclude those with chronic medical conditions like chronic liver disease, chronic kidney disease, coronary artery disease, coagulopathy, leukemia, and connective tissue diseases.

**Statistical Analysis-** In this study, sensitivity, specificity, positive predictive value (PPV) and Negative predictive Value (NPV), 95% Confidence Interval (95% CI) analysis of 20min Whole Blood Clotting Test (WBCT20) and WBCT 30 was performed. Sensitivity was defined as the proportion of cases with VICC where the WBCT20 was positive. Specificity was defined as the proportion of non-envenomed patients with negative WBCT20. Positive Predictive Value (PPV) is the probability that the patients with a positive WBCT20 Test truly envenomed and having VICC. Negative Predictive Value (NPV) is the probability that the patients with a negative WBCT20 Test are truly non-envenomed.

**Ethical Approval-** The study has obtained the required Ethical Approval from the Ethical Committee of SCB Medical College & Hospital, Cuttack, Odisha, India.

#### **RESULTS**

Table 1 shows the distribution of cases by age group, showing a predominance of patients aged 31-40 (29 cases) and 21-30 (25 cases), with 100 cases included in the study. The second table outlines the snakebite site, with many bites occurring on the right lower limb (43 cases) and left lower limb (34 cases). Clinical features such as local swelling/cellulitis were common among all patients, with other symptoms like fang marks, oliguria, and bleeding manifestations also present, albeit less frequently.

Table 2 evaluates the sensitivity and specificity of the Whole Blood Clotting Test (WBCT) at admission (T0) and 6 hours post-ASV (T6), using INR as the standard. The test demonstrates high sensitivity but relatively low specificity.

Table 3 assesses the sensitivity and specificity of WBCT 6 hours post-ASV, considering both PT and serum fibrinogen as standards. Results indicate moderate sensitivity and low specificity.

Table 4 compares the performance of WBCT20 and WBCT30 concerning various coagulation parameters, demonstrating comparable sensitivity and specificity between the two tests, with slight variations in predictive values and p-values.

**Table 1:** Age Wise Distribution of Cases

Age (years)	Total cases
15 - 20	8
21 -30	25
31- 40	29
41-50	16
51-60	12
>60	10
TOTAL	100
Site of Snake Bite	
Site of Snake Bite	No. of Patients
Right upper limb	12
Left upper limb	9
Right lower limb	43
Left lower limb	34
Face	1
Scrotum	1
Total	100
Clinical Features	
Symptomatology	No. of patients
Local swelling /cellulitis	100
Fang mark	31
Oliguria	8
Haematuria	6
Bleeding manifestations	5
Gangrene	18
pain Abdomen	1

**Table 2:** Determination of sensitivity and specificity of WBCT20 during admission i.e. T0 (by considering INR as standard) and WBCT20 6 hours after receiving first dose of ASV i.e. T6 (by considering INR as standard)

	WBCT20 during admission i.e. T0		WBCT20 6 hours after receiving first dose of ASV i.e. T6	
WBCT20	INR $\leq$ 1.2	INR>1.2	INR $\leq$ 1.2	INR>1.2
Clotted	61	10	32	2
Not clotted	11	18	4	1
Total	72	28	36	3
Diagnostic Utility of WBCT20	Value	Confidence Interval	Value	Confidence Interval
Sensitivity	84.72	74.31- 92.12	8.89	73.94-96.89
Specificity	64.29	44.07-81.36	33.33	0.84-97.29
Positive Predictive Value(PPV)	85.92	78.61-91.01	94.12	87.7-97.29
Negative Predictive Value(NPV)	62.07	47.07-75.07	20	3.79-61.34
Accuracy	79	69.7-86.51	84.62	69.47-94.14
p-value	<0.001			

**Table 3:** Determination of sensitivity and specificity of WBCT20 6 hours after receiving first dose of ASV i.e. T6 (by considering INR as standard and Serum fibrinogen as standard)

WBCT20	PT as standard		Serum fibrinogen as standard	
	PT <15.9	PT ≥15.9	Sr F <150	Sr F ≥ 150
Clotted	32	2	16	18
Not clotted	4	1	3	2
Total	36	24	19	20
Diagnostic Utility of WBCT20	Value	Confidence Interval	Value	Confidence Interval
Sensitivity	88.89	73.94-96.89	84.21	60.42-96.62
Specificity	33.33	0.84-90.57	10	1.23-31.70
Positive Predictive Value(PPV)	94.12	87.7-97.29	47.06	11.10-78.07
Negative Predictive Value(NPV)	20	3.79-61.34	40	11.10-78.07
Accuracy	84.62	69.47-94.14	46.15	30.09-62.82

**Table 4:** Comparative Evaluation of WBCT20 and WBCT30 Test by considering different coagulation parameters

		INR	PT	Sr.Fibrinogen
Sensitivity & (95% CI )	WBCT20	84.72 (74.31-92.12)	85.53 (75.58-92.55)	78.69 (66.32-88.14)
	WBCT30	80.28 (69.14-88.78)	82.89 (72.53-90.57)	75.41 (62.71-85.54)
	WBCT20 and WBCT30	80.28 (69.14-88.78)	80.82 (69.62-89.10)	75 (62.34-85.28)
Specificity & (95% CI)	WBCT20	64.29 (44.07-81.36)	75 (53.29-90.23)	38.46 (23.36-55.38)
	WBCT30	72.41 (52.76-87.27)	87.50 (67.64-97.34)	51.28 (34.78-67.58)
	WBCT20 and WBCT30	72.41 (52.76-87.27)	88.89 (70.84-97.65)	50 (33.8-66.2)
Positive Predictive Value (PPV) & (95% CI)	WBCT20	85.92 (78.61-90.1)	91.55 (84.34-95.61)	66.67 (60.17-72.58)
	WBCT30	87.69 (79.62-92.85)	95.45 (87.88-98.38)	70.77 (62.99-77.50)
	WBCT20 and WBCT30	87.69 (79.62-92.85)	95.16 (87.06-98.29)	69.23 (61.5-76.02)
Negative Predictive Value (NPV) & (95% CI)	WBCT20	62.07 (47.07-75.07)	62.07 (47.48-74.76)	53.57 (38.19-68.30)
	WBCT30	60 (47.13-71.62)	61.76 (49.04-73.05)	57.14 (43.84-69.49)
	WBCT20 and WBCT30	60 (47.13-71.62)	63.16 (51.24-73.66)	57.14 (43.84-69.49)
p-value	WBCT20	<0.001	<0.001	<0.062
	WBCT30	<0.001	<0.001	<0.006
	WBCT20 and WBCT30	<0.001	<0.001	<0.010



## DISCUSSION

Patients with hemotoxic snake bites were found in all age groups. A maximum number of patients (29) were found in the age group of 31 to 40. This indicates that snake bite affects people mostly in the working age group. The number of cases in the elderly age group (>60 years) was less since people in this age group are less involved in outdoor activities. According to the results of studies reported by Thang *et al.* <sup>[6]</sup>, 71% of cases were found in the age group of 11 to 50 years. In this study, 78% of cases were in the same age group. Our observation is corroborated by Bragazzi <sup>[11]</sup> study, where 80% of the cases were in the same age group. In this study, most cases were in the age group of 31 to 40 years. Laloo reported 335 patients with a mean age of presentation 25.2 years. <sup>[12]</sup> In our study, rural patients comprised 88% of the population, and urban patients comprised 12%. Similar observations were made in the study conducted by Kumar *et al.* <sup>[13]</sup>, where 85% of patients were from rural areas. The maximum number of patients was from the Balasore district of Odisha (21%). The most common site of the bite was in the lower limb (77%). Kumar *et al.* <sup>[14]</sup> reported that most bites in their study group were inflicted in the lower extremities (96%). In their study, Mahmood *et al.* <sup>[15]</sup> and Biradar SK *et al.* <sup>[16]</sup> also reported the lower extremity as the most frequent site of bite. The majority of bites occurred when the subjects were working in the cultivation field i.e. 50% and total bites during outdoors activities were 93 %. This matches with the observation of the study done by Wedasingha *et al.* <sup>[17]</sup>, where nearly 75% of bites were outdoors. All of the patients had local cellulitis, indicating the hemotoxic nature of envenomation. Similar findings were reported in the study by Costa *et al.* <sup>[18]</sup>. In viperine bites, the earliest symptom is pain and swelling due to cellulitis, which can spread over the whole extremity and lead to compartment syndrome, threatening the viability of the limb or its part. This can have important consequences if it leads to loss of digits due to ischemia and gangrene. The other common symptoms were Fang marks (31%), and bleeding manifestation (5%). The symptoms like oliguria (8%) and haematuria (6%) were consequences of renal failure in the patients. Similar observations have been reported in the study by Costa *et al.* <sup>[18]</sup> and Resiere *et al.* <sup>[19]</sup>. According to Patil *et al.* <sup>[20]</sup>, the sensitivity and specificity of WBCT20 were 50% and 89.13%, respectively, which does not match our study.

According to Wedasingha *et al.* <sup>[17]</sup> and Costa *et al.* <sup>[18]</sup> the sensitivity and specificity of WBCT20 were 40% and 100%, which does not match our study. In a recent study by Patil *et al.* <sup>[20]</sup>, where using 1 ml of whole blood for the WBCT20 Test and INR cut-off was > 1.4, the sensitivity and specificity of WBCT20 were found to be 82% and 98%, respectively. A study conducted in SCB MCH in 2017 with an INR of 1.5 or above showed a sensitivity of 67.19% and a specificity of 91.4% of the 20-minute WBCT. <sup>[17,19]</sup>

The present study reveals that WBCT20 is very sensitive, though have a low specificity. The sensitivity of WBCT 30 is less than that of WBCT20, but its specificity is better than WBCT20 <sup>[21]</sup>. The probability that the patients with a negative WBCT20 are truly non-envenomed is less (NPV=62.07%) and that of WBCT30 is 60%. Hence, by this test, the negative WBCT20 and WBCT30 cannot exclude systemic coagulation disease in all cases. Therefore, the doctors recommend an immediate ASV administration despite a negative result. Test sensitivity is lower when reassessing the patient after 6 hours compared to the INR. The present study has observed that the sensitivity and specificity of WBCT20 and WBCT30 are not satisfactory in detecting systemic coagulation disorder at the initial examination of the patient for considering ASV & at 6hr for assessing the requirement of further ASV. Since the study was performed in a tertiary care hospital, most patients had already received ASV. There was a discrepancy between WBCT20 and WBCT30 in 7 cases out of 100. Among them, in 6 cases, WBCT was clotted at 20 minutes but did not clot when observed after 30 minutes; out of these 6 cases, INR >1.2 was seen in 3 cases. So WBCT should be continued for 30 minutes to prevent missing cases of coagulopathy due to hemotoxic snake bite. The present study used borosil test tubes due to the unavailability of ordinary test tubes. By using branded test tubes, the sensitivity and specificity of the test were not satisfactory. So, further testing is required using ordinary test tubes. However, given easy bedside testing that less experienced health care personnel can do in the peripheral health care centres, both WBCT20 and WBCT30 should be done without a good alternative bedside test. PT/INR is an essential laboratory test besides aPTT, serum Fibrinogen for increasing the detection of coagulation disorder in hemotoxic snake bites at initial examination and re-evaluation. Clinicians should be made aware of the limited sensitivity of the

Test (WBCT20), and all the patients with clinical features of envenomation should receive antivenom. The serial evaluations of standard laboratory tests have good prognostic value. This study shows that the PT/INR is an effective and simple means of monitoring recovery from snake bite coagulopathy; both whole blood clotting tests should be performed for 30 minutes.

## CONCLUSIONS

This study has attempted to see whether WBCT30 is better than WBCT20 in any way. In 93% of cases, both tests yield similar results. In our study, WBCT20 did not yield satisfactory results with a sensitivity of 84.72% and a specificity of 64.29% with INR as standard. The results of 30-minute WBCT are also not satisfactory, with a sensitivity of 80.28% and a specificity of 72.41%. However, combining WBCT20 and WBCT30 has marginally improved the specificity from 64.72% to 72.41%. However, given easy bedside testing that less experienced healthcare personnel can do in the peripheral healthcare centers, WBCT20 and WBCT30 should be done without good alternative bedside tests. Further testing is required using ordinary test tubes, which is recommended in the guidelines. WBCT should be done at 20 and 30 minutes to improve the specificity.

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