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Diagnostic Performance of a Gold Nanoparticle - Based Lateral Flow Assay Snake Venom Detection Kit: A Single-Centre Observational Pilot Study in Snakebite Victims

Dhirubhai Chhaganbhai Patel[1], Shama Bhat[2], Vivek Wayse[3], Prabhakar B.[4], Anil Yadav[5], Hardik Nagar[6], Nibedita Das[7], Ziniya B. Mallick[8], Ankit Pathak[9]

[1]Shree Sainath Surgical and Maternity Hospital, Valsad, Gujarat, India
[2]Bhat Biotech Pvt. Ltd., Bengaluru, Karnataka, India
[3]Biorad Medisys Pvt. Ltd., Pune, Maharashtra, India
[4]Sarsuag Enterprise, Bangalore, Karnataka, India
[5]Raut Serums India Pvt. Ltd., Pune, Maharashtra, India
[6]NAMO Medical Education and Research Institute, Silvassa, Dadra and Nagar Haveli, India
[7]Shree Sainath Surgical and Maternity Hospital, Valsad, Gujarat, India
[8]University of Calcutta, Kolkata, West Bengal, India
[9]WorkSure® India, New Delhi, Delhi, India

Corresponding Author: Vivek Wayse

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ABSTRACT

Background: A gold nanoparticle–based lateral flow assay snake venom detection kit (SVDK) may provide a simple, rapid, point-of-care method for venom detection and timely clinical decision-making in resource-limited facilities. This study evaluated diagnostic performance of a SVDK in serum samples from snakebite victims by comparing it to physician-based primary reference standard (PRS).
Methodology: This single-centre, prospective, observational pilot study enrolled venomous and nonvenomous snakebite cases, as confirmed through clinical presentation, evidence of snakebite and laboratory investigations (on physician's discretion) that served as the PRS. The SVDK testing was performed by an independent pathology service, blinded to PRS. Analysis of diagnostic performance of the SVDK was assessed by overall agreement (%) between SVDK and PRS for identification of venomous/nonvenomous cases.
Results: Forty snakebite cases were prospectively enrolled in this study, of whom 38 were included in the final analysis. Eighteen were venomous snakebite cases and 20 were nonvenomous snakebite cases, as per PRS. In analytical validation studies, the SVDK demonstrated a limit of detection of 10 ng/mL for Indian 'big four' snake venoms. Evaluation of the SVDK using serum samples from the enrolled patients showed complete concordance with the physician-based PRS (100% true positivity and true negativity).
Conclusion: The SVDK has shown high overall agreement vis-à-vis PRS in identifying venomous snakebite cases. Large population studies are required to determine its clinical utility as point-of-care diagnostic.

Keywords: Anti-snake venom; Gold nanoparticles; Lateral flow assay; Snake envenomation; Snake venom detection kit; Rapid detection

INTRODUCTION

Diagnosis of snakebite envenomation diagnosis is primarily based on combination of detailed patient history, syndromic clinical assessment, physical examination, and appropriate laboratory investigations. Information regarding the offending snake, when available through photographs, or specimens live/carcass brought to the hospital, may support diagnosis but are not conclusive as misidentification is common.[1] The simple, bedside 20 min whole blood clotting test (20WBCT) though widely used in resource-limited setting,[2] is time-dependent and only shows physiological consequences of envenoming post-coagulopathy.[3] Novel diagnostics like enzyme-linked immunosorbent assay (ELISA), infrared imaging, impedimetric immunoassay and polymerase chain reaction-based methods are difficult to perform in rural setting due to laboratory infrastructure requirements, technical expertise and longer processing times.[1, 4] An easy-to-use, stable, comprehensive, efficacious and affordable diagnostic tool at rural facilities may allow qualitative identification of envenoming and guide treatment decision, even by less experienced clinicians.[5]

A lateral flow assay (LFA)-based snake venom detection kit (SVDK) carrying gold-conjugated polyvalent equine anti-snake venom polyclonal antibodies that target the whole venom of big four[6] [Indian Cobra (*Naja naja*), Common Krait (*Bungarus caeruleus*), Russell's Viper (RV, *Daboia russelii*) and Saw-scaled Viper (SSV, *Echis carinatus carinatus*)] is developed. The LFA is an advanced, sensitive, specific, user-friendly, rapid (~5–20min) assay method.[7, 8] This study evaluated diagnostic performance of SVDK in qualitatively detecting the circulating snake venom antigens and differentiating venomous and nonvenomous snakebite cases. Additionally, the current standard and initiatives in diagnosis and management of snakebite in an Indian rural healthcare facility are summarized.

MATERIALS AND METHODS

Study design

This is a single-centre, prospective, observational pilot study, carried out at Shree Sainath Surgical and Maternity Hospital in Gujarat, India. The rural secondary healthcare facility was selected as it receives a substantial number of snakebite patients from surrounding tribal and rural communities. The SVDK strips were provided to an independent pathology service (Spinos Lifesciences and Research Pvt. Ltd.) for SVDK testing. Study objective was to evaluate diagnostic performance of SVDK in serum samples from snakebite victims by comparing it to physician-based primary reference standard (PRS). The PRS was clinical decision by treating physician towards conclusive differentiation between venomous and nonvenomous snakebite cases. This was established using all available clinical information including history of snakebite, syndromic manifestations of envenoming, evidence supporting snakebite (viz. eyewitness accounts, identification of the snake by direct observation, specimen/carcass, or photograph, whichever available), and routine laboratory investigations (as per case requirement).

Consecutive patients presenting with snakebite, irrespective of age or gender, who had not received anti-snake venom serum (ASVS) before blood sample collection, were eligible for enrolment. Patients who used blood products, anticoagulant medications or had moribund condition, pre-existing coagulopathy, bite injury clearly attributable to animals other than snakes, were not eligible.

The study design flowchart is presented in Figure 1. Upon arrival, all participants were subjected to detailed clinical evaluation as

per the institute's standard protocol for snakebite screening. Subject demographics, symptoms/signs at presentation and venous blood samples were collected. Snakebite management decisions based on PRS were made solely by the treating physicians, who were blinded to the SVDK results. Similarly, pathology service personnel performing SVDK were blinded for PRS result. Diagnostic performance of SVDK was evaluated by comparing its results (identification of venomous/nonvenomous snakebite cases) with the PRS.

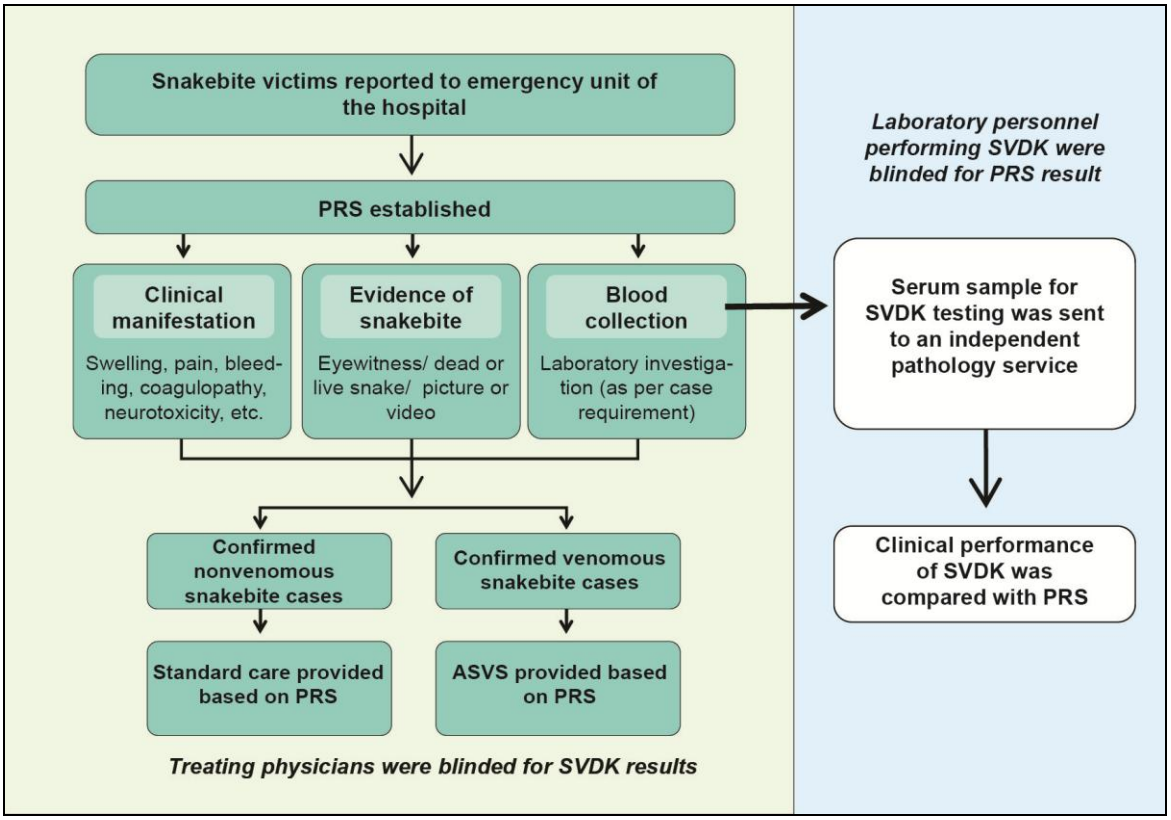

Figure 1: Study design flowchart. ASVS: Anti-snake venom serum; PRS: Primary reference standard; SVDK: Snake venom detection kit

Standardization and validation of SVDK

Generation and characterization of polyvalent equine anti-snake venom polyclonal antibodies

Lyophilized venoms of big four were procured from the Irula Snake Catchers' Industrial Cooperative Society, Chennai, India. The venoms were reconstituted (in normal saline), mixed thoroughly, centrifuged and sterilized by filtration (0.22 µm membrane). Healthy adult equines (5–7 years old, ≥150 kg) received ~1 mg subcutaneous (multiple sites at neck and shoulder regions) injection of polyvalent venom emulsified with Incomplete Freund's Adjuvant. Booster immunizations with increasing venom doses were administered at scheduled intervals until adequate antibody response was achieved. Blood samples were tested for antibody development by indirect-ELISA. Highest plasma dilution producing greater optical density than the validated assay cut-off was the endpoint antibody titre. The production of polyvalent anti-snake venom hyperimmune equine plasma was in accordance with the World Health Organization (WHO) guidelines, Indian Pharmacopoeia and Central Drugs Standard Control Organisation regulatory requirements.

Hyperimmune plasma was collected from the immunized horses, stored (2–8 °C) and filtered (0.45 µm membrane) prior to

purification. Immunoglobulins (Ig) were purified using a downstream purification process and was concentrated by ultrafiltration, column chromatography, buffer exchanged into phosphate-buffered saline, sterile filtered, and stored (2–8 °C) until characterization (physicochemical and immunological properties). Protein concentration was determined by ultraviolet spectrophotometry (280 nm) and confirmed using BCA protein assay. Protein concentration and purity were determined by SDS-PAGE (under reducing and non-reducing conditions), antibody specificity against each of four venoms by indirect-ELISA, and cross-reactivity by ELISA and western blot. Functional inhibitory activity against venom enzymes including phospholipase A_2 and metalloproteinases was evaluated using validated in vitro enzyme inhibition assays. Immunoreactivity was further assessed by sandwich ELISA and immunoblotting for binding specificity and cross-reactivity. Functional neutralizing activity was determined using LD_{50} and ED_{50} assays according to the WHO-recommended procedures.

Gold-conjugation of antibodies and LFA prototyping

Gold nanoparticles (AuNPs) with an approximate diameter of 40 nm were synthesized using a modified citrate reduction method. Briefly, an aqueous solution of 0.02% $HAuCl_4$ was heated to boiling and reduced by the addition of 1% sodium citrate, resulting in the development of a characteristic cherry-red color. The reaction mixture was maintained under boiling conditions for 10 min, followed by continuous stirring for an additional 15 min. The resulting colloidal gold solution was subsequently cooled to room temperature and stored until further use. Antibodies were buffer-exchanged into ultrapure water and concentrated as required. The minimum antibody concentration necessary to achieve optimal stabilization of the AuNPs was determined, after which the antibodies were conjugated with the colloidal gold nanoparticles. Briefly, affinity purified polyvalent anti-snake venom antibodies were diluted to 1.0 mg/mL and gold solution pH was adjusted to 7.0 with carbonate buffer (pH 9.6). The predetermined amount of antibody required for stabilization of the gold nanoparticles was added to the colloidal gold suspension under gentle continuous stirring for 1 h. Subsequently, the remaining unbound sites on the nanoparticle surface were blocked by the addition of bovine serum albumin (BSA) to a final concentration of 0.1%. The antibody-conjugated gold nanoparticles were then centrifuged to remove unbound components and recover the conjugated nanoparticle fraction.

Ten different pHs were used for gold-conjugation, and the absorption spectra for each conjugated antibody were recorded. Blank Test line signals were used to identify optimal conjugation conditions. Once the conjugation completed, LFA prototypes were developed for each of the four sandwich pairs. The prototypes were used to detect a 10 µg/mL solution of whole venom. The intensities of 'Test line' and 'Control line' were quantified. To evaluate the ability of the prototype lateral flow assay (LFA) to detect the target antigen over a broad dynamic working range, the assay was tested using serially decreasing concentrations of the Big Four snake venom, ranging from 10 µg/mL to 0 ng/mL. Each venom concentration was spiked into pooled human serum, pooled human plasma, and freshly collected whole blood. Following completion of the assay, the intensities of the Test (T) and Control (C) lines were quantified, and the Test-to-Control (T/C) signal intensity ratio was calculated. The resulting T/C ratios were plotted against the corresponding venom concentrations to generate calibration curves and characterize the assay response across the tested concentration range. Visual (by naked eye) limit of detection (LoD) of the prototypes was assessed.

Preparation of SVDK strip

The test strips were manufactured by Bhat Biotech India (P) Ltd., Karnataka, India. The test strip consists of four major components: a sample port where the test sample (serum/ plasma/ fresh whole blood) is applied, a conjugate pad containing mobile antibodies labelled with gold nanoparticles (detection antibody), a ~8 µm nitrocellulose (NC) membrane containing immobilized polyvalent anti-snake venom equine polyclonal antibodies (capture antibody) forming the Test line, and anti-mouse IgG forming the Control line (Figure 2a).

The conjugate pads were prepared by spraying diluted gold conjugate solution on conjugate release pad followed by drying for 90 min in an incubator (37 ºC) and for 15 min in a vacuum oven (37 ºC). The anti-mouse antibodies were immobilized on the plastic backed NC membrane using IsoFlow dispenser. These membranes were dried (37 ºC) for 1 hr. The LFA strip assembly involved positioning of the absorbent pad on Control line end and the conjugate pad on Test line end, both overlapping the NC membrane. The distance between each line was 6 mm. The sample port was placed overlapping the conjugate pad opposite to the NC membrane.

The 3.5 mm wide SVDK strips were housed in plastic cassettes and stored at room temperature away from sunlight and humidity.

SVDK principle

The SVDK is a gold nanoparticle–based LFA designed for rapid detection of circulating snake venom antigens (Figure 2b). During testing, venom antigens present in the sample bind to gold-conjugated antibodies and migrate along the membrane by capillary action. The antigen–antibody complexes are subsequently captured at the Test line by immobilized antibodies, producing a visible coloured band. Two visible bands at Test line and Control line indicate a positive result, whereas single band at the Control line signifies a negative result. The test is considered invalid when the Test line appears alone or when no line appears on the strip.

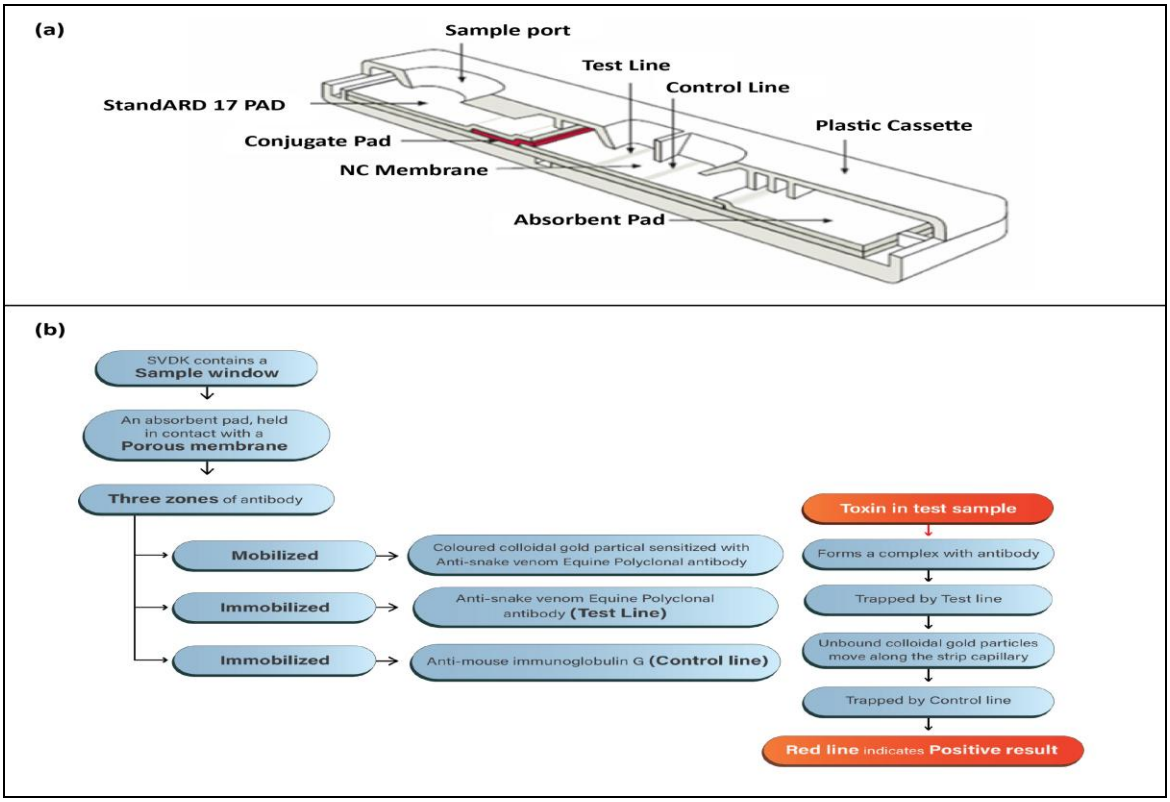

Figure 2: SVDK test strip (a) design and (b) step-by-step principle

Agreement between SVDK and sandwich ELISA

Twenty known positive and 30 known negative samples were tested by both SVDK and sandwich ELISA to assess the inter-method agreement for the diagnosis results.

Ethics statement

The study was approved by the Institutional Ethics Committee at Shree Sainath Hospital, Gujarat, India (Reference Number: ECR/1846/Inst/GJ/2023). Written informed consent was obtained from all conscious participants or from a legally authorized representative (if unconscious), after explaining the study purpose and procedures.

Laboratory investigations

Upon arrival, ~6 mL of venous blood was collected aseptically from each participant for blood parameter and SVDK testing. Serum samples (200 µL) were isolated, aliquoted and stored (−20 °C), and transported (under strict cold-chain conditions to preserve venom antigen integrity) to the pathology service for SVDK testing. Approximately 2 mL of venous blood was freshly drawn in a 10 mL glass test tube for 20WBCT testing.

Blood and urine test

Blood haematology, random blood glucose and serum creatinine along with urine routine microscopy were evaluated at physician's discretion.

Routine 20WBCT

This was performed in all snakebite cases according to the standard protocol. The test tube with ~2 mL freshly drawn venous blood left undisturbed in an upright position at room temperature. After 20 min, the tube was gently tilted to assess clot formation. Complete clot formation was interpreted as a negative result, whereas failure of clot formation as a positive result, suggestive of coagulopathy and haemotoxic envenomation.

SVDK testing procedure

The pathology service personnel used the provided test strips and serum samples for SVDK testing, and were blinded to the PRS. Briefly, 50 µL of serum was added to the sample pad, followed by addition of one drop assay buffer (test diluent). After 20 min of sample application, the test results (visible bands) were interpreted as positive or negative, and the snakebite cases were classified as venomous/nonvenomous accordingly. The SVDK results did not influence the institutional standard snakebite management protocol.

Percentage agreement of SVDK with PRS

Overall percentage (%) agreement of the index test (SVDK) with PRS was determined through true positivity, true negativity, positive predictive value (PPV) and negative predictive value (NPV).

Statistical consideration

Statistical analysis was done using SPSS version 25.0. Descriptive analysis of the data was performed by calculating mean, standard deviation (SD), median, range and percentage.

RESULTS

Assay performance of SVDK strip

The Test line signal intensity increased with venom antigen concentration, reaching a visual LoD at 10 ng/mL for all big four venoms (Table 1). At concentrations above 10 µg/mL (for all four venoms) the Test line intensity started to decrease, indicating the hook effect.

The qualitative results obtained using SVDK test strip showed complete agreement in detecting positive and negative samples when compared to sandwich ELISA.

Table 1: Limit of detection of snake venom detection kit

Antigen concentration	Signal intensity			
	Indian Cobra	Common Krait	RV	SSV
10 µg/mL	Positive (3+)	Positive (2+)	Positive (2+)	Positive (2+)
1 µg/mL	Positive (3+)	Positive (2+)	Positive (2+)	Positive (2+)
200 ng/mL	Positive (3+)	Positive (2+)	Positive (2+)	Positive (2+)
20 ng/mL	Positive (1+)	Positive (1+)	Positive (1+)	Positive (1+)
10 ng/mL	Positive (1+)	Positive (1+)	Positive (1+)	Positive (1+)

RV: Russell's Viper; SSV: Saw-scaled Viper

Classification of cases

Fifty consecutive snakebite cases, reported to the emergency facility of the hospital, were screened. Ten patients, who had already received antivenom at the peripheral facilities during referral, were excluded. Out of 40 enrolled cases, one was excluded because of grossly turbid blood sample and one due to extreme delayed presentation (7700 min post-snakebite). Amongst final 38 [mean age: 39.0±16.1 years] included cases, 60.5% were men. Median time to presentation was 95 min (range: 20-2260); 25 (65.8%) cases presented within 2 hr of the bite.

Primary reference standard

A total of 20 (52.6%) cases were bitten by nonvenomous snakes including common vine (*Ahaetulla oxyrhyncha*) and trinket (*Coelognathus helena*) (Figure 3a-3b). The cases neither exhibited symptoms on presentation nor throughout the 24 hr observation period.

Figure 3: Pictorial presentation of (a) Trinket snake photograph, (b) Common Vine snake carcass, (c) Cobra snake photograph, and (d) ptosis in the Cobra snakebite case

Out of 18 venomous snakebite cases, 11 (61.1%), 6 (33.3%) and 1 (5.6%) were bitten by RV, SSV and Cobra, respectively. Local symptoms of pain, swelling and tenderness were observed in all cases. One case with the cobra envenomation was presented with pain, swelling, tenderness and bleeding at the bite site, elevated blood pressure (BP), and neurotoxic manifestations including ptosis, slurred speech and blurred vision (Figure 3c-3d). The symptoms in this case progressed to respiratory distress, requiring

oxygen support. The recorded clinical symptoms and categories of evidence for venomous snakebite cases are presented in Figure 4a-4b.

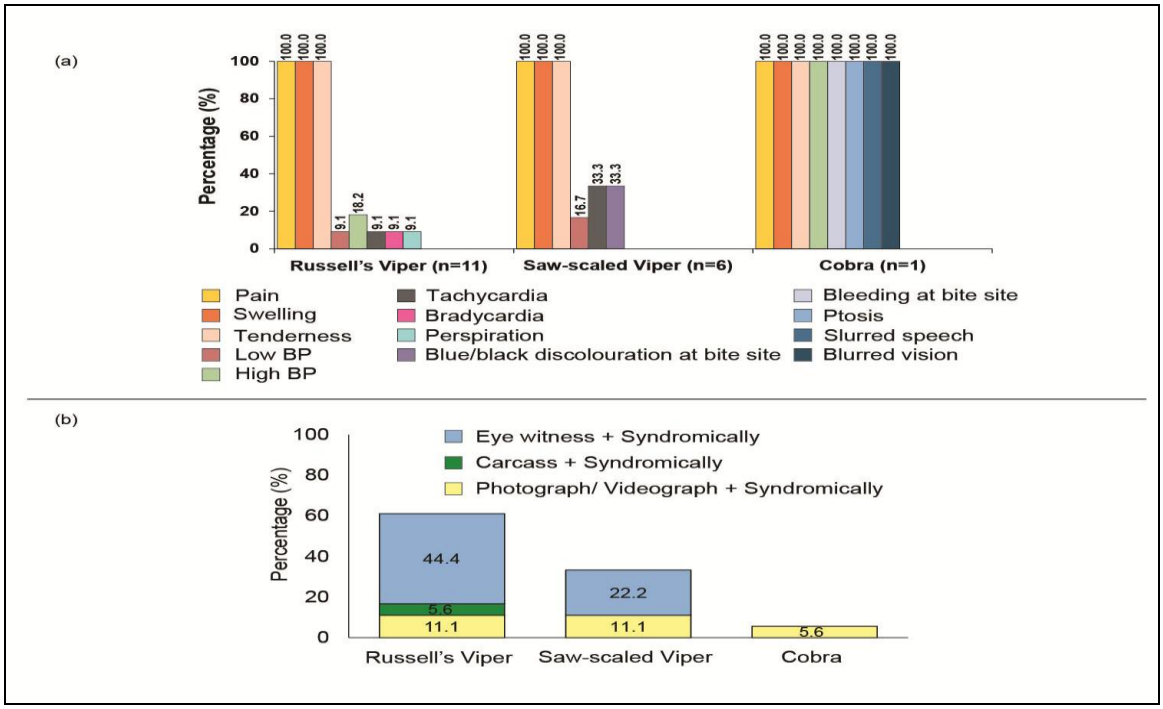

Figure 4: Graphical presentation of (a) clinical symptoms at presentation and (b) approach to identify species of venomous snakebite cases (n=18). BP: Blood pressure

Laboratory findings of all snakebite cases at baseline are presented in Table 2. Random blood glucose, creatinine and routine urine analysis were not performed in all 20 nonvenomous cases at physician discretion. Routine urine analysis revealed presence of urine albumin in 4/18 (22.2%) venomous snakebite cases; in rest of the envenomed cases, the findings appeared normal.

Table 2: Baseline laboratory investigation in nonvenomous and venomous snakebite cases

Laboratory parameters	Nonvenomous snakebite cases (n=20)	Venomous snakebite cases (n=18)
Random blood glucose (mg/dL)	NP	97.7±25.3
Haemoglobin (g/dL)	12.0±1.4	12.6±2.1
Total RBC (mill/cmm)	5.5±0.9	5.8±1.1
Total WBC (mill/cmm)	6358.0±2108.0	7487.8±3398.6
Platelet (per cmm)	261350.0±59815.5	240766.7±85157.8
Neutrophil (%)	63.8±8.6	67.7±11.9
Lymphocyte (%)	29.1±7.3	25.3±10.1
Eosinophil (%)	1.3±0.9	1.9±2.2
Monocyte (%)	4.7±1.5	4.5±1.7
Basophil (%)	1.0±1.6	0.7±0.4
PCV (%)	38.1±4.7	40.0±7.3
MCV (fL)	70.1±8.1	69.7±10.8
MCH (pg/cell)	22.2±3.0	22.0±3.8
MCHC (g/dL)	31.6±0.9	31.5±1.3
RDW (%)	14.4±2.2	16.2±2.1
Creatinine (mg/dL)	NP	1.2±0.2

Data is presented as Mean±SD; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; MCV: Mean corpuscular volume; NP: Not performed; PCV: Packed cell volume; RBC: Red blood cell; RDW: Red blood cell distribution width; WBC: White blood cell

Outcomes of 20WBCT

Clotted 20WBCT was found in all 20 (100.0%) nonvenomous snakebite cases. Among clinically venomous snakebite cases, 14/18 (77.8%) had a clotted 20WBCT, while 4/18 (22.2%) had non-clotted results. Of these, three were brought in within 2 hr and one after 6 hr of snakebite. Proportions of clotted and non-clotted 20WBCT according to the sampling times are presented in Table 3. Of note, among the 14 clotted results, one was the cobra bite case.

Table 3: 20WBCT outcomes at different sampling times in venomous snakebite cases

Bite to sample time (min)	Venomous snakebite cases (n=18)	Clotted blood n (%)	Non-clotted blood n (%)
<120	9	6 (66.7)	3 (33.3)
120–240	4	4 (100.0)	0
241–360#	0	0	0
>360	5	4 (80.0)	1 (20.0)
Total	18	14 (77.8)	4 (22.2)

Data is presented as frequency (percentage); # None of the subjects presented during 241–360 min; 20WBCT: 20 min whole blood clotting test

Management of snakebite

Clinical management was guided by the treating physician's assessment based on PRS. The 20 nonvenomous snakebite cases were kept under observation for 24 hr as per the Standard Treatment Guidelines. At the time of admission, mean pulse rate and oxygen saturation of the clinically envenomed cases were 88.1±16.6 beats/min and 98.6±0.8%, respectively. Their mean systolic BP and diastolic BP were 122.0±22.5 and 73.7±11.3 mmHg, respectively. All 18 venomous snakebite cases received ASVS, at a median time of 121.5 min (range: 50-2270 min). Five vials of ASVS were administered to 9/18 cases (50.0%), 10 to 7/18 (38.9%), 15 to 1/18 (5.6%) and 30 to 1/18 (5.6%) cases. Additional supportive care, including intensive care when clinically indicated, was provided according to the institutional management protocol. No deaths occurred during the study.

Percentage agreement of SVDK with PRS

In the result window of SVDK strips, both Test line and Control line appeared in 18 snakebite cases, indicating that the samples were reactive for snake venom, whereas, only Control line appeared in 20 cases, indicating negative (for snake venom) results (Table 4). After comparing this observation with the PRS, no false positive or false negative was observed. The SVDK demonstrated complete agreement with PRS in identifying 18 true positive (venomous) and 20 true negative (nonvenomous) cases.

Table 4: Outcomes of SVDK in nonvenomous and venomous snakebite cases

SVDK result	Total snakebite cases (n=38)	SVDK prediction	Agreement with PRS (%)	TP (%)	TN (%)	FP (%)	FN (%)	PPV (%)	NPV (%)
Negative (TL-/ CL+)	n=20	Nonvenomous snakebite cases	100.0	0	100.0	0	0	0	100.0
Positive (TL+/ CL+)	n=18	Venomous snakebite cases	100.0	100.0	0	0	0	100.0	0

CL: Control line; FN: False negative; FP: False positive; NPV: Negative predictive value; PPV: Positive predictive value; PRS: Primary reference standard; SVDK: Snake venom detection kit; TL: Test line; TN: True negative; TP: True positive

DISCUSSION

To our knowledge, this represents the first prospective, observational, pilot study, conducted in an Indian rural clinical setting where SVDK results demonstrated complete agreement, when independently matched with physician-based PRS, without affecting the snakebite management decision. The SVDK is designed to overcome the challenges of resource-limited setting by detecting direct venom antigen in blood samples of the suspected snakebite cases. This enables objective identification of envenomation independent of the development of downstream pathophysiological changes. The strength of this study is that analytical standardization of the SVDK followed clinical validation in snakebite victims.

Shaikh et al. developed a dot ELISA-based rapid venom detection test, capable of identifying big four snake venoms within 20-25 min at LoD of 1 to 0.1 ng/mL; however, it was a process prototype and not clinically tested in snakebite victim samples.[9] Lin et al. reported 5 ng/mL LoD for identifying medically important cobra venoms using a rapid (20 min) lateral flow immunochromatographic device.[5] The LFA-based SVDK of the present study recorded a LoD of 10 ng/mL for the big four venoms within 20 min of runtime during its analytical validation. Moreover, the SVDK clinically detected venom in a range of presentation from 20-2260 min, corroborating its clinical use in rural healthcare facility, where late arrivals are common owing to cultural beliefs, lack of transport, poor awareness and poverty.[10] Snake venom in circulation follows biphasic kinetics of distribution within 60 min and a prolonged elimination in 12-24 hr.[4] This observation warrants further investigation into the ability of SVDK to detect circulating venom antigens in snakebite cases with delayed presentations.

Liu et al. (Taiwan) reported 100% specificity and sensitivity of a lateral flow strip assay that detected neurotoxic envenomation (within 5-20 min) in 5 suspected snakebite victims.[7] A single centre, prospective cohort study from Australia evaluated a SVDK with ability to distinguish five most medically significant snake venoms. The study recorded zero false positive each out of 61 for simulated bite site, 61 for unbroken skin swab and 59 for the urine sample.[11] The present study also recorded 100% true positivity and true negativity with SVDK, thereby reaching complete agreement with the PRS in identifying clinically venomous (both haemotoxic and neurotoxic) and nonvenomous snakebite cases. There were zero false positive or false negative reports. However, the small sample size and subjective reference standard (PRS), may raise ambiguity regarding the SVDK outcomes.

The 20WBCT is routinely performed in resource-limited setting (or in absence of clotting assays) for identifying systemic haemotoxic envenoming and post-bite severe coagulopathy.[12] The 20WBCT, performed in the present study only identified a minority of clinically venomous snakebite cases, highlighting the potential value of direct venom antigen detection as a complementary diagnostic approach. The SVDK can be a guide to snakebite cases presenting with minimal to no clinical evidence of systemic envenomation and/or when the offending snake species remains unidentified. The SVDK integration in peripheral healthcare settings can only be established through larger multicentre studies across different ecological and clinical settings in India.

Economically productive rural populations are mostly affected by snakebite envenomation.[13] Delay in antivenom administration due to lack of reliable early diagnostic tests may result in life-threatening acute as well as chronic effects and increase the medical costs.[10] In this study, males in their productive age-groups were mostly envenomed and required ICU support. The PRS assisted the treating physician in providing ASVS within a median time of 121.5 min. Further research

is required to investigate the utility of SVDK in accelerating ASVS administration and minimizing the economic burden associated with snakebite complications.

The study has several limitations. As a single-centre pilot study with a limited sample size, the findings require confirmation in larger cohorts with more precise estimates of diagnostic performance. A subjective reference standard, the physician-based PRS, has been used for evaluation of SVDK accuracy instead of a diagnostic reference standard. This may add to the ambiguity regarding SVDK outcomes. The study population predominantly comprised haemotoxic envenomation, with limited representation of neurotoxic bites; therefore, further evaluation across diverse clinical presentations and geographical regions is warranted. Although clinical classification by treating physicians reflects real-world diagnostic practice, additional studies incorporating laboratory-based venom quantification may provide further validation.

CONCLUSION

The SVDK tested in this pilot study has shown high overall agreement vis-à-vis PRS and has potential for guiding clinical decision in snakebite management, even by less experienced clinicians in rural settings. This could be a method of choice for verifying and confirming venomous/nonvenomous snakebite cases, while avoiding unnecessary use of antivenom. However, given the small sample size and subjective reference standard, uncertainty may exist regarding the SVDK outcomes. Future systematic large population study evaluating prospective point-of-care application of SVDK in emergency and peripheral healthcare settings will be important to determine its practical utility.

Declaration

Ethical Approval: The study was approved by the Institutional Ethics Committee at Shree Sainath Hospital, Gujarat, India (Reference Number: ECR/1846/Inst/GJ/2023).

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