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Peer Review Report for "Clinical Characteristics and Major Complications of *Echis carinatus* Envenomation: A Retrospective Cohort Study"

Author(s): Soroush Khorramdel, Nima Farhadi, Ali Hasan Rahmani

Review Timeline:

Submit Date:	10 Dec 2025
Revised Date:	4 Jan 2026
Accepted Date:	7 Jan 2026

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFeree : Reviewer

13 Dec 2025

The manuscript addresses an important public health problem, namely the clinical complications of *Echis carinatus* envenomation in southwest Iran. Snakebite envenoming remains a neglected tropical condition, and region-specific clinical data are valuable for improving management strategies. The relatively large sample size and the focus on major complications such as venom-induced consumption coagulopathy (VICC), acute kidney injury (AKI), and cellulitis are strengths of the study.

However, several important issues should be addressed to improve the scientific rigor and clarity of the manuscript. First, the study design is described as a "retrospective cross-sectional study," which is conceptually inaccurate. Based on the

methods, this work is better classified as a retrospective cohort or retrospective chart-review study, and the terminology should be corrected throughout the manuscript, including the title and abstract. Clear justification for the extensive exclusion of screened patients (1,699 out of 1,949) is also needed, with exact numbers provided for each exclusion criterion to allow readers to assess potential selection bias.

Second, some inconsistencies and probable data errors are present in the Results and tables. In particular, several laboratory variables show implausibly large standard deviations (e.g., potassium, hemoglobin, and red blood cell counts), suggesting possible data entry or unit errors. These values should be carefully rechecked against the raw data before publication. In addition, the length of hospital stay is reported inconsistently (mean vs. median) between the abstract and main text and should be standardized.

Third, while the statistical analyses are generally appropriate for a descriptive observational study, the manuscript should clarify whether odds ratios were derived from univariate analyses or regression models, and confidence intervals should be reported consistently. Causal language such as “predictor” or “predisposing” should be replaced with more cautious terms (e.g., “associated with”), given the retrospective design and limited power for subgroup analyses.

Finally, the Discussion would benefit from a clearer and more explicit limitations section, addressing retrospective design, referral bias, possible misclassification of snake species, lack of sample size calculation, and limited follow-up. With these revisions, the manuscript would be substantially strengthened and more suitable for publication.

REFeree : Reviewer

21 Dec 2025

This retrospective cross-sectional study investigates demographic and clinical complications of *Echis carinatus* envenomation in a regional referral center in Iran. The topic is clinically relevant, particularly in regions where snakebites pose a significant public health burden. The manuscript is generally well-structured, and the statistical methods are appropriately applied. However, several methodological and analytical limitations significantly affect the validity and interpretability of the findings. Addressing these concerns through substantial revisions is necessary before the manuscript can be considered for publication.

Major Concerns and Required Revisions:

1. This methodological limitation significantly impacts the interpretation of the AKI-related findings. The core issue is that defining baseline serum creatinine solely as the value measured at hospital admission is clinically and methodologically insufficient. This approach introduces a substantial risk of misclassification bias. Patients presenting with envenomation often exhibit hypovolemia, dehydration, or hypotension, which can cause an acute, transient elevation in serum creatinine due to reduced renal perfusion rather than true parenchymal injury. Consequently, using

this potentially elevated admission value as the baseline leads to two critical errors: first, it can falsely inflate the observed incidence of AKI by misclassifying patients with pre-renal azotemia as having intrinsic AKI; and second, it may paradoxically miss true AKI in patients with chronic kidney disease whose admission creatinine is chronically elevated but stable. This bias directly affects the validity of key results, including the reported 7.6% AKI incidence rate and the strong association between cellulitis and AKI (OR=10.64), as some diagnosed cases might represent hemodynamic artifacts rather than venom-induced nephrotoxicity. To rectify this, the authors should prioritize using pre-morbid creatinine values from medical records within the past 6-12 months as the true baseline. If such data are unavailable, applying the KDIGO criterion of a ≥ 0.3 mg/dL creatinine increase within 48 hours would be a more dynamic and reliable alternative, as it captures acute changes relative to the admission value. At a minimum, this significant limitation must be explicitly and thoroughly acknowledged in the Discussion section, detailing how it might have influenced the study's conclusions and suggesting caution when interpreting the AKI prevalence and associated risk factors.

2. The study's analytical approach currently relies on univariate analyses to establish associations, such as between cellulitis and AKI or hypotension and VICC. While these initial findings are valuable, they present a significant methodological limitation because they do not account for potential confounding variables. This oversight substantially weakens the causal inference and clinical applicability of the results. Factors such as patient age, sex, the time delay between the bite and hospital presentation, the administered dose of antivenom, and pre-existing comorbidities (e.g., hypertension, diabetes, chronic kidney disease) are all clinically plausible variables that could independently influence both the risk factor (e.g., developing cellulitis) and the outcome (e.g., developing AKI). For instance, an older patient or one with delayed presentation might be more susceptible to both wound infection and renal complications, creating a spurious association in a univariate model. Without statistical adjustment for these confounders, we cannot determine whether the reported relationships are direct and independent or merely reflections of these underlying patient characteristics. To strengthen the validity and impact of the study, it is essential to perform multivariate logistic regression analyses. The authors should construct models that include their key exposures (cellulitis, VICC, hypotension) alongside a carefully selected set of potential confounders based on clinical relevance and prior literature. The results should then be presented clearly, showing both the unadjusted Odds Ratios (as in the current manuscript) and the adjusted Odds Ratios with 95% Confidence Intervals, ideally in a revised or supplementary table. Finally, the Discussion section must explicitly address how controlling for these variables affected the associations. For example, does the strong link between cellulitis and AKI remain significant after adjusting for age and time to presentation? This analysis is crucial for transforming observed correlations into more robust evidence that can reliably inform clinical risk stratification and management protocols.

3. The study's definition and analysis of cellulitis as a major complication present a significant gap that limits the interpretability of its key findings, particularly the

strong association reported between cellulitis and AKI. Relying solely on a clinical diagnosis (erythema, warmth, edema, necrosis, or infected bullae) without any supporting microbiological data is a major methodological shortcoming. In the context of snakebite, local tissue damage can produce signs indistinguishable from bacterial cellulitis, and the failure to confirm infection with wound cultures or Gram stains means that some cases classified as "cellulitis" might actually represent severe local envenomation reactions, venom-induced dermonecrosis, or non-infectious inflammation. This diagnostic uncertainty introduces substantial misclassification bias. Consequently, the reported association between cellulitis and AKI becomes difficult to interpret: is the observed renal injury driven by a true bacterial infection and subsequent systemic inflammatory response, or is it the result of severe local tissue damage and rhabdomyolysis from the venom itself, which also presents with similar local signs? Furthermore, the complete absence of information on infection management—such as the choice of empiric antibiotics, duration of therapy, or use of surgical debridement—leaves readers unable to assess the standard of care or consider how treatment might have modified the outcomes. To address this, the authors should, if data exists, provide a supplementary table summarizing available microbiological culture results and antibiotic regimens used. More critically, if this data is unavailable—as is often the case in retrospective studies—this must be explicitly and prominently stated as a key limitation in the Discussion. The authors must elaborate on how this diagnostic uncertainty could have biased their results, potentially overestimating the strength of the cellulitis-AKI link, and caution against inferring a direct causal role for bacterial infection without microbiological confirmation.

4. The description of the antivenom treatment protocol lacks the necessary detail to fully interpret the study's outcomes and limits the generalizability of its findings. While the manuscript states that treatment followed a national protocol and specifies a dose range of 3-12 vials, the critical, clinically relevant decision-making process is opaque. The reader is not informed of the precise, actionable criteria used for dose initiation and escalation. For example, what specific INR threshold (beyond " ≥ 2 ") or combination of systemic signs triggered the initial administration? What clinical or laboratory parameters determined the escalation from the standard 3-5 vials to 6-10 or up to 12 vials? Furthermore, the timing of antivenom administration relative to the bite and the onset of symptoms is not reported, which is a crucial prognostic factor. This lack of granularity makes it impossible to assess the appropriateness and consistency of the treatment provided or to replicate the protocol in other settings. Additionally, the study's exclusive focus on the Razi polyvalent antivenom, without any mention or comparison with the other licensed Iranian antivenom (Snafb by Padra Serum), represents a significant contextual gap. It is unclear if this was due to hospital policy, availability, or a deliberate exclusion. The efficacy, adverse effect profile, or patient outcomes might differ between these two products. To address these issues, the authors should provide a clear, detailed treatment algorithm—preferably as a table or flowchart—outlining the step-by-step clinical decision logic for antivenom use based on coagulation results and systemic involvement. If any patients received the Snafb antivenom during the study period, even a small comparative subgroup analysis would be highly valuable. If not, this must be

explicitly stated as a study limitation, acknowledging that the findings regarding treatment response and complication rates are specific to the Razi antivenom and may not be generalizable to all treatment settings in Iran. This clarity is essential for translating the study's results into actionable clinical practice.

5. The Discussion section, while competently summarizing the findings, remains largely descriptive and misses a critical opportunity to delve into the underlying pathophysiological pathways that could explain the observed associations. The manuscript reports strong statistical links—such as between cellulitis and AKI, and VICC and AKI—but offers only a superficial exploration of the plausible biological mechanisms connecting these complications. For instance, the connection between local cellulitis and systemic renal injury could be mediated by several interlinked pathways: the systemic release of pro-inflammatory cytokines (e.g., IL-6, TNF- α) from an infected wound, venom-induced or infection-exacerbated rhabdomyolysis releasing myoglobin, or secondary sepsis leading to hemodynamic instability and acute tubular necrosis. Similarly, the relationship between VICC and AKI warrants a deeper mechanistic discussion beyond "microthrombotic renal injury." This could involve venom-induced endothelial glycocalyx damage, fibrin deposition in the glomeruli leading to thrombosis and ischemia, or the role of platelet activation and neutrophil extracellular traps (NETs) in venom-induced thrombotic microangiopathy. By not engaging with these potential mechanisms, the discussion reads as a restatement of results rather than an interpretive synthesis that places the findings within the contemporary scientific understanding of envenomation pathology. Furthermore, the reliance on older references (some from the 1970s-1980s) in key areas weakens the context. The field of venom research has advanced significantly, particularly in understanding inflammation, coagulopathy, and endothelial biology. To strengthen the manuscript significantly, the authors must expand the Discussion to include a dedicated paragraph (or integrate throughout) that hypothesizes the mechanistic bridges between their clinical observations. They should cite recent reviews and primary studies (2023-2024) on snake venom-induced inflammation, immunothrombosis, and organ crosstalk. This will transform the section from a simple summary into a insightful narrative that explains how and why these complications might be linked, thereby enhancing the scientific contribution and clinical relevance of the work.

6. The absence of a reported power analysis, combined with the notably small sample size in key subgroups such as the AKI cohort ($n=19$), introduces significant uncertainty regarding the reliability of the study's negative findings and the robustness of its positive associations. Statistical power—the probability of correctly detecting a true effect when it exists—is intrinsically linked to sample size. When subgroup sizes are small, the study may be underpowered, meaning it lacks the sensitivity to identify clinically important differences even if they are present in the population. This elevates the risk of a Type II error (failing to reject a false null hypothesis). For instance, while the study found no statistically significant association between hypotension and AKI ($p=0.51$), this "negative" result cannot be confidently interpreted as evidence of no relationship; it may simply reflect an inability to detect it due to insufficient power, as only 1 patient with AKI presented with hypotension. Similarly, the lack of a significant association between VICC and cellulitis after

Bonferroni correction might be influenced by limited power in the cellulitis subgroup (n=19). To address this critical methodological gap, the authors should perform and report a post hoc power analysis for their primary comparisons (e.g., AKI vs. non-AKI groups for key predictors). This analysis would quantify the study's actual power to detect effect sizes of clinical relevance (e.g., an OR of 2.0 or greater) given the observed sample sizes and event rates. Reporting these power estimates would allow readers to gauge the confidence they can place in both the significant and non-significant results. If a formal post hoc analysis is not feasible, it is imperative that the authors explicitly and prominently acknowledge this limitation in the Discussion section. They must state that, due to the small number of events in some complication groups, the study may have been underpowered to detect certain associations, and therefore negative findings should be interpreted with caution. This transparency is essential for an accurate and balanced interpretation of the study's conclusions.

Minor Revisions:

1. Figure 1: Correct spelling errors ("eligibility" → "eligibility," "apllication" → "application").
2. Tables: Ensure column headings clearly indicate whether values are n (%) or mean ± SD. Consistently format confidence intervals.
3. Language Editing: Some sentences in the Results and Discussion are awkwardly phrased and would benefit from professional English editing

Rezvan Rajabzadeh : Reviewer

22 Dec 2025

I have reviewed the manuscript in my capacity as a Methodology and Statistics reviewer. Please carefully address the issues and points raised in the following comments, as they are essential for improving the methodological clarity and statistical transparency of the study.

- The use of the word "Retrospective" to describe a cross-sectional study is not appropriate. It is recommended to remove "Retrospective" from the article title and accurately describe the design as a cross-sectional study if appropriate.
- Please define all abbreviations at their first occurrence in the text; write the term in full followed by the abbreviation in parentheses.
- The concluding statement:

"Early detection of infections, stabilizing of blood pressure, along with close monitoring for coagulopathy and renal injury, can decrease the risk of long-term complications of *Echis carinatus* envenomation. Our research highlights the importance of early referral systems, increasing antivenom availability, and enhancing supportive care, particularly in rural areas."

While these recommendations are clinically reasonable, they extend beyond the direct scope of the study design and the evidence presented. Given the nature of your study (observational/case-series/retrospective — specify), causality cannot be established, and the data may not support such definitive claims regarding risk reduction or system-level interventions.

It is suggested to:

1. Re-frame the conclusion to align strictly with the strength and type of evidence obtained.
2. Present such points as suggestions or hypotheses for future research rather than confirmed outcomes.
3. Use cautious language (e.g., "may help reduce risk", "findings suggest the potential importance of...") to ensure the conclusions remain methodologically sound and supported by the data.

• I have carefully reviewed the manuscript, but I could not locate the results or outputs corresponding to the Kolmogorov–Smirnov test, Shapiro–Wilk test, Mann–Whitney U test, or Spearman correlation analysis in the Results section or tables. Please specify exactly where each of these statistical tests was used and present their results clearly. This will improve the transparency and reproducibility of your study.

REFEREE : Associate Editor

23 Dec 2025

The Ms needs some major revisions before suitable for publication in JJNPP.

REFEREE : EIC

23 Dec 2025

The manuscript has been examined by expert reviewers who have concluded that the work is of potential interest to the readership of JJNPP.

However, from their comments it is clear that major revision of this manuscript will be required before a decision can be made on its publication. I would be pleased to consider a revised manuscript that addresses the reviewers' concerns. Your revised manuscript will be returned to one or more of the original reviewers for further consideration.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reply to the Associate Editor and Editor-in-Chief

We sincerely thank the Associate Editor and the Editor-in-Chief for their careful assessment of our manuscript and for recognizing its potential relevance to the readership of *JJNPP*. We appreciate the opportunity to revise the manuscript and to address the reviewers' questions in detail.

In response to the decision for major revision, we undertook substantial revisions to enhance the scientific rigor, methodological transparency, and clarity of the manuscript. Specifically, we corrected and standardized the study design terminology to reflect a **retrospective cohort study**, rechecked and revised the Results and tables to resolve data inconsistencies, clarified the statistical methodology with explicit emphasis on univariable analyses, expanded the **Limitations** section, and revised the Discussion and Conclusion to ensure cautious interpretation consistent with the observational nature of the study.

A detailed, point-by-point response to each reviewer question is provided below. We believe that these revisions have substantially strengthened the manuscript and addressed all concerns raised.

Reviewer 0 – Point-by-Point Response

We thank Reviewer 0 for the thorough and constructive evaluation of the manuscript.

Question 1: Study design terminology and exclusion criteria

Reply:

The study design has been corrected throughout the manuscript (title, abstract, and Methods) to **retrospective cohort study**, which more accurately reflects the longitudinal assessment of patients during hospitalization.

Regarding exclusions, we clarified that although 1,949 patient records were initially screened, many were excluded due to predefined eligibility criteria (e.g., incomplete records, non-*Echis carinatus* envenomation, duplicate admissions, or referral after initial management elsewhere). Due to the retrospective design and incomplete archival screening logs, exact counts for each exclusion category could not be reliably retrieved. This limitation and its potential impact on selection bias are now explicitly acknowledged in the **Limitations** section.

Question 2: Implausible standard deviations and length of hospital stay

Reply:

All laboratory variables were rechecked against the original dataset. Variables with skewed distributions are now consistently reported as **median (interquartile range)** rather than mean $\pm$ standard deviation.

Length of hospital stay (LOS) has been standardized and is no longer reported as an overall cohort summary. Instead, LOS is described only in relation to major complications, with consistent reporting explained in the Methods and Limitations sections.

Question 3: Odds ratios and causal language

Reply:

The Statistical Analysis section now clearly states that all odds ratios were derived exclusively from **univariable analyses**. No multivariable regression was performed due to the limited number of outcome events, particularly AKI.

Confidence intervals are reported consistently, and causal terminology has been replaced with association-based language throughout the manuscript.

Question 4: Limitations section**Reply:**

A dedicated **Limitations** section has been added, explicitly addressing the retrospective design, referral and selection bias, possible snake species misclassification, absence of formal sample size calculation, limited statistical power for subgroup analyses, and lack of long-term follow-up.

Final remark to Reviewer 0:

Addressing these points has substantially improved the methodological clarity and interpretive rigor of the manuscript. We thank the reviewer for the insightful feedback.

Reviewer 1 – Point-by-Point Response

We sincerely thank Reviewer 1 for the detailed and clinically insightful critique.

Major Question 1: Baseline creatinine and AKI misclassification**Reply:**

We acknowledge that using admission creatinine as baseline is a significant limitation. Reliable pre-morbid creatinine values were unavailable for most patients. Although AKI was defined using KDIGO criteria, admission creatinine may have been transiently elevated due to hemodynamic factors. This limitation and its potential impact on AKI prevalence and associations are now explicitly discussed in the Discussion and Limitations sections.

Major Question 2: Lack of multivariable analysis**Reply:**

Multivariable analysis was considered but not performed due to the small number of AKI events ($n = 19$), which would risk overfitting. All reported odds ratios therefore represent **unadjusted associations**, as now clearly stated. Potential confounding by age, delay to presentation, antivenom dose, and comorbidities is explicitly acknowledged.

Major Question 3: Definition of cellulitis**Reply:**

Cellulitis was diagnosed clinically without routine microbiological confirmation. We now explicitly acknowledge that some cases may represent severe local envenomation or non-infectious inflammation, which may have biased the observed cellulitis–AKI association. This is discussed as a key limitation.

Major Question 4: Antivenom protocol**Reply:**

The Methods section was expanded to clarify antivenom initiation and escalation criteria. Only the **Razi polyvalent antivenom** was used due to institutional availability; no patients received SnaFab. Limited generalizability of treatment-related findings is now acknowledged.

Major Question 5: Mechanistic discussion**Reply:**

The Discussion has been expanded to address plausible mechanisms linking local tissue injury, inflammation, VICC, and AKI, including endothelial injury, immunothrombosis, microvascular thrombosis, cytokine release, and rhabdomyolysis, supported by recent literature.

Major Question 6: Power and interpretation of negative findings

Reply:

We explicitly acknowledge limited statistical power due to small subgroup sizes and emphasize that non-significant findings should be interpreted cautiously.

Final remark to Reviewer 1:

These revisions have strengthened the manuscript's transparency, interpretive caution, and clinical relevance.

Reviewer 2 – Point-by-Point Response

We thank Reviewer 2 for the careful methodological and statistical review.

Question 1: Study design terminology**Reply:**

The study design was clarified and standardized as a **retrospective cohort study** throughout the manuscript, reflecting longitudinal in-hospital follow-up.

Question 2: Abbreviations**Reply:**

All abbreviations are now defined at first occurrence in the abstract, text, tables, and figures.

Question 3: Conclusions and causal language**Reply:**

The Conclusion was revised to remove causal claims and to frame implications cautiously as hypotheses or suggestions for future research.

Question 4: Statistical test reporting**Reply:**

Normality tests (Kolmogorov–Smirnov and Shapiro–Wilk) were used solely to guide test selection and are described as such. Mann–Whitney U test results are now clearly linked to reported comparisons. Spearman correlation analysis was not performed and has been removed. These changes improve transparency and reproducibility.

Final remark to Reviewer 2:

We thank the reviewer for the valuable feedback, which has enhanced the methodological clarity of the study.

OPEN PEER REVIEW

Revision (1)

REFeree : Reviewer

6 Jan 2026

The authors have provided a comprehensive, well-structured, and respectful response to all reviewers' comments. The revisions implemented have significantly improved the manuscript, including:

Correction of the study design to "retrospective cohort" throughout.

Clear clarification that all analyses were univariable, with multivariable analysis not performed due to limited outcome events.

Addition of a dedicated "Limitations" section explicitly acknowledging key methodological constraints.

Revision of the Discussion and Conclusion to remove causal language and adopt a cautious, association-based interpretation.

Expansion of the Discussion to include plausible pathophysiological mechanisms.

Considering the relevance of the topic, the appropriateness of the methodology for the study aims, and most importantly, the authors' excellent and thorough response to constructive criticism, the manuscript now meets acceptable standards for publication. The inherent methodological limitations are clearly stated in the Limitations section, which guards against overinterpretation.

It is advised that accept the manuscript for publication in the journal.

REFeree : Reviewer

6 Jan 2026

The manuscript addresses an important and relevant public health problem, namely *Echis carinatus* envenomation and its associated complications in an endemic region of southwestern Iran. The topic is within the scope of the journal, and a brief search did not identify substantial overlap with previously published studies. The study provides updated, region-specific clinical data that add value to the existing literature.

The title accurately reflects the content of the manuscript and contains no unnecessary abbreviations. The abstract is structured and clearly summarizes the objectives, methods, results, and conclusions. The introduction clearly outlines current knowledge, existing gaps, and the aims of the study.

The materials and methods section is adequately described. Patient selection, inclusion and exclusion criteria, outcome definitions, and statistical analyses are appropriate for a retrospective cohort study. The use of accepted definitions for venom-induced consumption coagulopathy and acute kidney injury, as well as the conservative statistical approach, strengthens the methodological quality of the

study.

Results are presented clearly and logically, supported by appropriate tables and figures without unnecessary duplication. The discussion is balanced, appropriately interprets the findings, and acknowledges the limitations of the retrospective design and the limited number of outcome events.

Overall, the manuscript is well prepared, methodologically sound, and meets the scientific and reporting standards of the journal. I recommend acceptance (or acceptance with minor editorial revisions, if required).

REFEREE : Associate Editor

7 Jan 2026

The Ms is now suitable for publication in JJNPP

REFEREE : EIC

7 Jan 2026

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.

Your work underwent a rigorous peer-review process, and the reviewers and editorial board were impressed by the originality, scientific rigor, and relevance of your findings. We believe your contribution will be of significant interest to our readership and will advance the field of natural pharmaceutical products.

Before publication, our production team will contact you regarding final formatting, proofs, and any necessary administrative steps. Please ensure that all authors have approved the final version of the manuscript and that any required ethical or copyright documentation is in order.

On behalf of the editorial board, I congratulate you and your co-authors on this excellent work and thank you for choosing to publish in JJNPP.