

The relationship between clinical findings and Plasma Phospholipase A2 levels in snakebite patients

PLA2 and clinical presentation in snake envenomations

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Abstract

Aim: Snake envenomation is a major cause of death in tropical and subtropical regions. Envenomed patients are treated according to snakebite severity. Snake venom contains phospholipase A2 (PLA2) enzyme, thus increasing levels may indicate envenomation. We investigated the correlation between clinical findings and PLA2 levels in snakebite patients.

Material and Methods: From May 2017 to October 2017, we recruited 22 snakebite patients and 28 healthy controls without a history of snakebites. Blood samples were collected and stored at -80°C after centrifugation and separation. Clinical and sociodemographic variables and PLA2 levels were analysed.

Results: Seven of 22 patients were female. Patients had a mean age of 48.8 ± 10.6 years, the mean height of 170.6 ± 10.5 cm and the mean weight of 75.9 ± 12.3 kg. The required antivenoms were significantly higher in patients with Grade 2 ($n = 10$) compared to initial phase envenomation ($n = 5$) ($p = 0.015$). The mean PLA2 levels were significantly lower in envenomed patients ($p = 0.017$), and the mean lactate levels in envenomed patients were significantly higher ($p < 0.001$).

Discussion: Snake envenomed patients had significantly decreased PLA2 levels, and there was no relationship between PLA2 levels and severity of snake envenomation in this preliminary study.

Keywords

Snake envenomation; Phospholipase A2; Lactate

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Introduction

Snake envenomation is a major cause of death and disability in tropical and subtropical countries. Approximately 1.8 million cases of snake envenomation and 100,000 deaths occur annually worldwide [1]. Snakebite cases are common in the Southeastern region of Turkey due to its climate and geography, leading to a high number of primary care and emergency department admissions due to snakebite and envenomation. The admission rate for snake envenomation is highest during the summer months [2].

Snake envenomation should be treated as a medical emergency, hence acute management is crucial. Upon presentation, it is treated according to the grade of severity. Wound incision, venom suctioning and tourniquets are typically ineffective and thus should be discouraged. Treatment with antivenom derived from polyvalent snake venom is considered standard of care for treating moderate or severe snake envenomation [3].

Phospholipase A2 (PLA2) is the primary component in snake venom [4-6]. Snake venom PLA2 plays a critical role in early morbidity and mortality in snake envenomation, resulting in death by paralysis as well as tissue damage and homeostatic imbalance [6].

Because venom toxicity is abundant in PLA2 activity, it is assumed that envenomed patients are inflicted with the PLA2 enzyme and have high PLA2 levels in their blood. Many studies have assessed PLA2 activity in snake venom, and testing for PLA2 levels may be considered standard for investigating venom activity [1].

Conversely, few data on PLA2 activity in patients with snake envenomation are available. There is also no data on the relationship between PLA2 activity and envenomation severity. Thus, the present study attempted to investigate the relationship between PLA2 blood levels and clinical features of envenomed patients at our hospital.

Material and Methods

In the summer of 2017 (May–October), a total of 22 snake envenomed patients were identified among approximately 150,000 emergency admissions. No patient was excluded from the study due to the low number of patients. The patients were categorised into 3 groups based on severity: Initial ($n = 5$), Grade 1 ($n = 7$), and Grade 2 ($n = 10$). Patient categorisation was performed according to previous studies [2]. The control group comprised 28 age- and sex-matched healthy individuals without a history of snakebites.

All patients admitted to the emergency department were treated according to a standard protocol. The local ethics committee approved the study protocol, and all participants gave their written informed consent for inclusion in the study.

Laboratory Analysis

Blood samples were obtained by venipuncture. Blood samples were collected from both groups and stored at -80°C after centrifugation and separation. The samples were centrifuged at 1000 g for 20 minutes. Serum levels of PLA2 protein were analysed using ELISA kits from Shanghai Sunred Biological Technology Co, Ltd., (Shanghai, China) according to the manufacturer's recommended protocol. To measure the corresponding protein concentrations, a standard curve was

plotted using a 4-parameter logistic regression.

Along with serum PLA2 levels, selected clinical and sociodemographic variables were recorded and analysed including patient comorbidities, affected body region, bite-to-hospital time, number of antivenoms, electrocardiogram (ECG) tracing analysis, pO₂, lactate, D-dimer, neutrophil count (NEU), creatine kinase (CK) and length of hospital stay.

Statistical Analysis

Continuous variables were reported as mean and standard deviation, and categorical variables were reported as numbers and percentages. Normality of numerical variables was evaluated using the Kolmogorov–Smirnov test. Numerical data with normal distribution were analysed using independent samples t-test, while data with skewed distribution were analysed using the Mann–Whitney U test. Categorical variables were reported as percentages on the chi-squared analysis. One-way ANOVA and the Kruskal–Wallis test were used to compare multiple groups. The differences between parametric variables were analysed using the Tukey test. The Dwass–Steel–Critchlow–Fligner test was used to evaluate differences between non-parametric variables, and the Pearson Chi-Squared test was used to analyse differences between categorical variables. Correlation of variables was assessed using Spearman and Pearson correlation analyses. We evaluated the receiver operating characteristic (ROC) curve and the corresponding areas under the curve for accuracy and respective best cut-off values for PLA2 in snake envenomation patients.

All statistical analyses were performed using Jamovi project computer software (Version 0.9, retrieved from <https://www.jamovi.org>) and JASP computer software (Version 0.9). A p-value of < 0.05 was considered statistically significant.

Results

Overall, 7 (31.8%) of 22 patients recruited to the study were female. The mean age of the patients was 48.8 ± 10.6 years (range, 25–74), the mean height was 170.6 ± 10.5 cm and the mean weight was 75.9 ± 12.3 kg. Six patients reported a history of accompanying diseases, hence a detailed statistical analysis was not performed on this variable. Ten patients were bitten on the right hand ($n = 10$, 45.4%), 3 on the right foot ($n = 3$, 13.6%), 2 on the right arm ($n = 2$, 9.09%) and the remaining patients were bitten on other body parts.

The control group comprised 28 individuals, with 11 being (39.2%) female. Differences in clinical and demographic features were not statistically significant between the patient and control groups (Table 1).

ECG findings revealed right bundle branch block in 1 patient and sinus bradycardia in 1 patient, while the remaining patients ($n = 20$) had normal sinus rhythm without any abnormalities.

Differences in laboratory and clinical variables were not significant between the sexes (Table 2), nor were differences among envenomation severity groups. A significantly higher number of vials of antivenom was administered in patients with Grade 2 severity ($n = 10$) when compared to those in the initial phase ($n = 5$) ($p = 0.015$). Likewise, Grade 2 patients had a longer length of hospital stay compared with those in the Grade 1 or initial phase ($p = 0.007$) severity.

NEUs were positively correlated with the time-from-bite to

emergency department admission, D-dimer level and length of hospital stay ($r = 0.678, p < 0.001$; $r = 0.511, p = 0.015$ and $r = 0.429, p = 0.046$, respectively). In addition, the number of antivenom vials and D-dimer levels were also positively correlated with the length of stay ($r = 0.517, p = 0.014$ and $r = 0.477, p = 0.025$, respectively). Time-to-admission was not related to the number of antivenom vials, pO₂, or length of stay and no relationship existed between the number of antivenom vials and D-dimer levels or NEU. Furthermore, no significant relationship was demonstrated between severity of envenomations and PLA2 activity or other variables.

Table 1 summarises a comparison of patients and controls by sex, laboratory variables and clinical variables. Mean PLA2 levels were significantly lower in the envenomed patients than in controls ($p = 0.017$). Likewise, envenomed patients had a significantly lower mean pulse rate and diastolic blood pressure ($p < 0.001$ and $p = 0.008$, respectively). Lactate levels were significantly higher in envenomed patients than in controls ($p < 0.001$). ROC curve analysis failed to produce an optimal cut-off point for PLA2 level in snake envenomed patients ($p = 0.17$).

Table 1. Comparison of sex, laboratory and clinical variables in patients and controls

	Group		p
	Case	Control	
Sex			
Male	15 (68.18)	17 (60.71)	0.585***
Female	7 (31.82)	11 (39.29)	
Age (years)	48.41 ± 15.98	49.14 ± 2.94	0.812*
Lactate	1.8 (1.3–2.6)	1.15 (1–1.45)	<0.001**
Phospholipase A2	1131.32 ± 151.43	1439.61 ± 567.92	0.017*
Pulse rate (bpm)	75.73 ± 9.57	86.07 ± 10.29	<0.001*
Systolic blood pressure (mm Hg)	120.41 ± 14.86	127.5 ± 11.67	0.065*
Diastolic blood pressure (mm Hg)	71.27 ± 9.7	78.04 ± 7.62	0.008*

* Independent samples t-test was performed. Descriptive statistics were reported as mean ± standard deviation.

** Mann–Whitney U test was performed. Descriptive statistics were reported as median (interquartile range, IQR).

*** Pearson Chi-Squared test was performed. Descriptive statistics were reported as N (%).

Discussion

The present study showed that mean PLA2 levels in blood samples of envenomed patients were significantly lower compared to those of controls. We also demonstrated that mean lactate levels were significantly higher in the envenomed patients. However, no significant differences in laboratory or clinical variables were observed between the groups with varying severity of envenomation.

Snake envenomation is an under-recognised global health problem resulting in substantial mortality, disability and physical and psychological morbidity, particularly in rural tropical and subtropical zones. Antivenom treatment is currently the standard practice for treating envenomed patients. PLA2 has a wide range of pharmacological effects and plays a pivotal role in damage due to envenomation. Local tissue damage is inflicted by PLA2 activation. PLA2 impairs muscular fibre membranes, leads to cytosolic calcium influx, increases muscle filament contractions, causes mitochondrial dysfunction and results in irreversible damage to muscle cells [7–9]. These mechanisms cause chronic musculoskeletal sequelae, which are long-term health complications of snake envenomation [10]. Hence, neutralising snake venom PLA2s could decrease or inhibit toxic damage and may also prevent inflammatory reactions [11].

Because PLA2 activity plays a key role in venom toxicity, it is assumed that envenomed patients have the PLA2 enzyme and high levels of the compound in blood serum. Many studies have investigated PLA2 activity in snake venom, and it is typically used as a standard test for assessing venom activity [1]. Neri-Castro et al. [12] reported that venom from 3 snake species revealed differences in biological activity. Snake venom potency was assessed by lethality in mice, and PLA2 activity was highest in the most lethal venom. Thus, increased PLA2 activity is associated with complications of snake envenomation [12]. Kalita et al. [13] also reported a higher PLA2 level in one of the deadliest snakes reported in their study. Moreover, they indicated that low neutralisation of PLA2 is a major concern for treating the most lethal snake envenomations [13]. However, few studies have analysed PLA2 activity in the blood of snake envenomed patients. Maduwage et al. showed higher PLA2 activity in serum samples of envenomed patients compared

Table 2. Binary comparisons of selected laboratory and clinical variables, sex and severity of envenomation

	Sex		p	Grade of severity			p
	Male (n = 15)	Female (n = 7)		Initial (n = 5)	Grade 1 (n = 7)	Grade 2 (n = 10)	
Number of antivenoms used	1 (0–2)	1 (0–2)	0.911 ²	0 (0–0)	1 (0–1)	2 (1–3)	0.015 ⁴
pO ₂	58.43 ± 21.19	46.66 ± 11.03	0.185 ¹	61.32 ± 18.53	48 ± 10.76	56.04 ± 23.7	0.493 ³
Lactate	1.5 (1.1–2.3)	2.4 (1.5–3.1)	0.216 ²	1 (1–1.1)	2.2 (1.5–3.1)	2 (1.4–3.2)	0.056 ⁴
D-dimer	928 (624–6000)	1080 (591–3100)	0.832 ²	839 (698–928)	1080 (330–1680)	3375 (702–9000)	0.494 ⁴
Neutrophil count	69.15 ± 18.57	60.63 ± 11.04	0.277 ¹	55.91 ± 15.37	62.34 ± 11.71	74.58 ± 17.65	0.088 ³
Creatine kinase	144 (98–239)	183 (135–419)	0.397 ²	150 (135–154)	135 (122–419)	167.5 (98–333)	0.978 ⁴
Phospholipase A2	1151.33 ± 144.45	1088.43 ± 168.61	0.377 ¹	1081.8 ± 164.69	1181.57 ± 185.23	1120.9 ± 123.01	0.531 ³
Length of stay (days)	2 (1–4)	1 (1–2)	0.180 ²	1 (1–1)	2 (1–2)	4 (2–5)	0.007 ⁴

¹ Independent samples t-test was performed. Descriptive statistics were reported as median ± standard deviation.

² Mann–Whitney U test was performed. Descriptive statistics were reported as median (interquartile range, IQR).

³ One-way ANOVA test was performed. Descriptive statistics were reported as median ± standard deviation.

⁴ Kruskal–Wallis Test was performed. Descriptive statistics were reported as median (interquartile range, IQR).

with non-envenomed patients [1].

We identified lower PLA2 activity in snake envenomed patients than in controls. This result may be related to only our study group, which had relatively fewer patients compared to the previous study [1]. Lower PLA2 activity might also be associated with the type of snakes involved because PLA2 activity varies among snake species. For example, Maduwage et al. [1] reported that median PLA2 activity was 55.7 $\mu\text{mol/ml/min}$ for Russell's viper envenomation, 13.6 $\mu\text{mol/ml/min}$ for hump-nosed viper, 14.8 $\mu\text{mol/ml/min}$ for cobra, 17.2 $\mu\text{mol/ml/min}$ for krait and 98 $\mu\text{mol/ml/min}$ for black snake. Divergent PLA2 activities in patients and controls might be due to variations among snake species.

The laboratory signs of envenomation reveal the effects of the venom on the haematopoietic and inflammatory systems. Snake envenomation may increase systemic inflammatory markers. Previous studies have examined the relationship between the severity of snake envenomation and such biomarkers. Aktar et al. [14] concluded that leukocyte count, AST/ALT ratio, CK, hypoproteinemia, hypoalbuminemia and hypocalcaemia could be associated with severity of snake envenomation [14]. Aktar et al. [15] also investigated the relationships between diagnosis or outcome prediction in patients with snake envenomation and the mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), concluding that MPV, NLR and PLR might be useful inflammatory markers in snake envenomation. They also observed strong associations with leukocytosis, neutrophilia and thrombocytopenia and severe snake envenomations [15]. We did not detect any differences in D-dimer levels, neutrophil count and CK between the snake envenomed patients and controls. We also did not find a relationship between the haematological markers and severity of snake envenomation.

This study has some limitations. First, our sample size was relatively small. Second, this study was performed in one region at a single institution. We determined low level PLA2 in our envenomed patients. This result may originate from the scanty snake species in our region. Moreover, we could not evaluate the relationship between the severity of snake envenomation and PLA2 activity. Finally, we could not examine the relationship between the area of the body in which the snakebite occurred and severity of snake envenomation.

Conclusion

PLA2 levels were significantly decreased in the snake envenomed patients. No relationship was seen between PLA2 levels and severity of snake envenomation in this preliminary study. Future case-control studies are needed to clarify the relationship between PLA2 levels and severity of snake envenomation.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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