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Investigation of the Usability of Serum Phospholipase A2, Neutrophil/Lymphocyte Ratio, Red Cell Distribution Width and Mean Platelet Volume Levels in the Grading of Scorpion Envenomation

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Aim: This study aimed to compare the serum phospholipase A2, neutrophil/lymphocyte ratio (NLR), red cell distribution width (RDW), and mean platelet volume (MPV) levels of patients that presented to our emergency department due to scorpion stings and to determine a laboratory parameter that could assist in clinical grading.
Materials and Methods: Sixty-three patients presenting to the emergency department due to scorpion stings and 33 volunteers presenting for other reasons between May and October 2018 were included in the study. The serum phospholipase A2, NRL, RDW and MPV levels of the patients were determined and compared with the control group.
Results: In the evaluation performed in the patient group, the mean serum leukocyte and serum lymphocytes were higher and the RDW mean was statistically significantly lower compared to the control group, ($p = 0.001$, 0.003, and 0.004, respectively). There was no significant difference between the patient and control groups in terms of the serum MPV, platelet and serum phospholipase levels ($p > 0.05$). When the patients' serum MPV values were compared according to their clinical grade, a statistically significant correlation was found (rho −0.432, $p < 0.001$).
Conclusion: In scorpion stings, as the clinical grade progresses, the MPV rate decreases. Therefore, the MPV level can be used as an auxiliary parameter to show the severity of scorpion stings.

Keywords: Scorpion sting, phospholipase A2, mean platelet volumes
Short Title in English: Serum Phospholipase, MPV of Scorpion Envenomation

Introduction

There are many types of scorpions worldwide, some of which can cause severe or deadly envenomation. Since ancient times, the existence and toxicity of scorpions have been known, but this species does not carry disease factors; however, in order to protect themselves, they can cause envenomation and death by stinging people and animals(1).

Scorpion stings can cause severe skin reactions, neurological and respiratory problems, and severe systemic manifestations that can lead to cardiovascular collapse. In terms of their geographical location in Turkey, scorpions are particularly common in Southeast Anatolia due to the climate and socio-economic structure. There are 1500 subspecies of scorpions worldwide and 50 subspecies are toxic to humans. In South America, North Africa, and Middle East, the subspecies of *Leiurusquinquestriatus*, *Androctonuscrassicauda* and *Buthusoccitonus* are dangerous. Turkey is estimated to be home to 13 subspecies of scorpions.

In East and Southeast Anatolia Regions of the country, *A. crassicauda* (Figures 1) and *L. quinquestriatus* (Figures 2) are relatively common and constitute the most causes of stings(2). Scorpion venom is a water-soluble, antigenic and heterogeneous mixture. The venom is formed by the release of neurotoxin, cardiotoxin, nephrotoxin, hemolytic toxin, phosphodiesterase, phospholipase, hyaluronidase, glycosaminoglycan, histamine, serotonin, tryptophan, and cytokine at different concentrations. However, the strongest toxin is neurotoxin(3).

In this study, the serum phospholipase A2, neutrophil/lymphocyte ratio (NRL), red cell distribution width (RDW), and mean platelet volume (MPV) levels of patients presenting to the emergency department after scorpion stings were compared to a control group. In addition, clinical grading was performed in the patient group, and by comparing the A2, NRL, RDW and MPV levels between the grades, the parameters that could determine the severity of envenomation was investigated.

Methods

This prospective case-control study was conducted in the emergency department of a tertiary hospital. Our emergency department serves approximately 25000 patients monthly with two attending physicians and three general practitioners. The study began after the approval of the local ethics committee (Number: 2017/9–26).

Sixty-three patients that presented to the emergency department due to scorpion stings and 33 volunteers presenting with other complaints between May and October 2018 were included in the study. The patients included in the study were graded according to their clinical features as follows: Grade 1, local pain and paresthesia; Grade 2, spread of local pain and paresthesia toward the proximal; Grade 3, central pain and paresthesia findings in addition to central nervous system findings (increased oral secretion, nystagmus, blurred vision, and rapid

tongue movements); and Grade 4, in addition to central nervous system findings, other system findings (rhabdomyolysis, multiple organ failure, etc.) (4).The blood samples obtained from the patients were analyzed in terms of the serum white blood cell count (WBC,normal range 4.5–10 $\times 10^3$/uL), phospholipase A2 (normal range 86.4–167.6 nmol/min/ml), NRL (normal range 1.65), RDW (normal range, 11.6–15.8%), and MPV normal range, 6.8–10.8 fL) and compared to the control group. All cases presenting to the emergency room were treated according to the standard protocol. Patients under the age of 18 years, pregnant women, and individuals who did not agree to participate in the study were excluded.

Laboratory Analysis

Blood samples were obtained by venipuncture from both groups and stored at –80 °C after centrifugation at 1000 g for 20 minutes and separation. The serum levels of the PLA2 protein were analyzed using ELISA kits supplied by Shanghai Sunred Biological Technology Co. Ltd.(Shanghai,China) according to the manufacturer's recommended protocol. Along with the serum PLA2 levels, selected clinical and sociodemographic variables, namely affected body region, number of antivenoms, electrocardiogram (ECG) tracing analysis, WBC, NRL, RDW, and MPV were recorded and analyzed.

Statistical Analysis

SPSS v.15.0 for Windows was used for the statistical analysis. Descriptive statistics were expressed as number and percentages for categorical variables, and mean, standard deviation, minimum, maximum and median values for numerical variables. The comparison of two independent groups of numerical variables was performed using the Mann-Whitney U test since the data did not meet the normal distribution condition. The ratios in the groups were determined by the chi-square analysis. The relationship of the ranks in the groups were

examined by linear-by-linear association, and the relationship between ordinal and numerical variables was examined by the Spearman correlation analysis. The alpha significance level was accepted as $p<0.05$.

Results

The study included 63 patients, 27 males and 36 females, with a mean age of 43.71 ± 18.63 years, and 33 controls, 12 males and 21 females, with a mean age of 41.06± 16.07 years. There was no statistically significant difference between the patient and control groups in terms of age and gender (Table 1, $p = 0.560$ and $p = 0.538$, respectively).

When the patient group was classified according to the clinical grade of envenomation, it was determined that most of them were in Grade 1 and least were in Grade 4. Concerning antivenom use, only nine (14.3%) patients received an antivenom and most were advanced-grade. The right upper extremity was the most affected body area (Table 2).

The mean serum leukocyte and serum lymphocytes were statistically significantly higher and the RDW mean was statistically significantly lower in the patient group compared to the control group ($p = 0.001$, $p = 0.003$, and $p =0.004$, respectively). A statistically significant difference was also observed in the RDW levels of the patient and control groups ($p =0.045$). However, no significant difference was found between the two groups in terms of the serum MPV, platelet and serum phospholipase levels (Table 3, $p > 0.05$).

In the patient group, the leukocyte, neutrophil, lymphocyte, NLR, phospholipase, D-dimer and RDW levels did not significantly differ according to the clinical grade ($p >0.05$). However, it was determined that the MPV level was significantly decreased as the grade progressed (rho –0.432, $p < 0.001$) (Table 4, Figures 3).

Discussion

Scorpion envenomation, widely seen especially in southeastern Turkey, is one of the frequent reasons for emergency presentations (5).Following a scorpion sting, certain anti-inflammatory and pro-inflammatory cytokines and mediators are released by the host depending on the scorpion subspecies. These released mediators and cytokines determine the degree of inflammation that can lead to major clinical effects, such as cardiac dysfunction, pulmonary edema, and shock(6).The mean platelet volume (MPV) reveals the presence of inflammatory load and disease activity in many diseases (7).In a study conducted with 76 cases of scorpion envenomation in 2014, Capan et al reported that the patient group had significantly higher WBC and platelet distribution width values and were significantly lower mean MPV values compared to the healthy control group (8). In another study, Gökay et al evaluated 189 cases of scorpion stings and found that the serum MPV value was within normal limits and there was no difference between the patient groups according to clinical grades (9).

Song et al examined the impact of Buthusmartensii Karsch-type scorpion venom on rabbits and showed that it significantly inhibited platelet aggregation. They attributed this to the increase in the ratio of PGI2/TXA2, indicating an association with increased PGI2 concentration in plasma (10).In a study on mice with and without scorpion venom, Nasr et al found that the platelet parameters were generally unaffected in both groups, but the MPV levels were decreased over time in the mice that had been given the venom. Such a big decline in the MPV levels within four hours of the administration of the venom cannot be explained by the production of new and small platelets. However, scorpion venom is likely to have an anticoagulant effect on existing platelets (11). In our study, there was no significant difference between the serum MPV levels of the patient and control groups but a significant decrease was observed in the MPV level of the patients as their clinical grade increased. Therefore, further studies are needed to elucidate this mechanism.

Scorpion venom is a water-soluble, antigenic and heterogeneous mixture containing a large number of toxins and additional compounds, including various amounts of neurotoxin, cardiotoxin, nephrotoxin, hemolytic toxin, phosphodiesterase, phospholipase, hyaluronidase, glycosaminoglycan, histamine, serotonin, tryptophan, substances that increase cytokine release, and agglutinins. Any of these compounds can be dominant in the venom of scorpion species. Neuromuscular, neuroanatomic and local tissue effects are the most important clinical effects (12).

In our study, no significant difference was found in the serum phospholipase A2 level ofthe patients after a scorpion sting compared to that of the control group. In their2019 study on *Centruroidesedwardsii*, which is the most common scorpion species in Costa Rica. Diaz et al found that the *C. edwardsii*venom was rich in peptides, proteolytic and hyaluronidase enzymes but it did not haveany phospholipase A2 and fibrinogenolytic activity (13). Similarly, in our study, this enzyme may not have been present in scorpion venom, or a sufficient amount of poison may not have been transferred to the host. To clarify this issue, there is a need for further studies to determine the venom content of scorpions in our region.

RDW is a simple and inexpensive parameter that reflects the degree of heterogeneity (anisocytosis) of erythrocyte volume and is routinely used for the differential diagnosis of anemia. Increased RDW has been reported in various disease and disorders, such as cardiovascular disease, venous thromboembolism, cancer, diabetes, community-acquired pneumonia, chronic obstructive pulmonary disease, liver and kidney failure, and other acute or chronic conditions (14). In our study, the amount of RDW was determined to be lower in the patient group compared to the control group.

Limitations

This wasa single-center study with a low number of patients. In addition, only scorpion stings in our region were included in the study, and the species of scorpions that had stung the patients were not known. Therefore, the venom content of the scorpion species and how much venom the patients had been exposed to could not be determined. The blood samples were obtained from the patients only at the time of presentation, with no additional blood sample being taken during the follow-up. A study conducted in multiple centers to examine envenomation cases caused by different scorpion species can provide more comprehensive results.

Conclusion

We found that the serum leukocyte and lymphocyte levels were higher and the serum RDW level was lower in the patient group compared to the control group. In addition, a significant relationship was observed between clinical grading and the serum MPV level. Therefore, it is concluded that the serum MPV level can be used as an auxiliary parameter in determining the severity of envenomation in patients presenting with a scorpion sting.

References

1. Ozkan O.Scorpion Antivenom Production.*Turk Hij Den Biyol Derg*.2008; 65(2): 97−108.
2. Yılmaz F, Arslan ED, Demir A, Kavalci C, Durdu T,Yılmaz MS, et al. Epidemiologic and Clinical Characteristics and Outcomes of Scorpion Sting In The Southeastern Region of Turkey.*Ulus TravmaAcilCerrahiDerg*.2013;19(5):417−422.
3. Boju SL, Mogili HKR, Ram R, Vishnubotla SK. Nephrotic Syndrome after Scorpion Sting. *CEN Case Rep*.2016;5(1): 83−86.

4. Bawaskar HS, Bawaskar PH. Efficacy and Safety of Scorpion Antivenom Plus Prazosin Compared with Prazosin Alone for Venomous Scorpion (MesobuthusTamulus) Sting: Randomised Open Label Clinical Trial. *BMJ*. 2010;341:c7136.
5. Caglar A, Kose H, Babayigit A, Oner T,Duman M. Predictive Factors for Determining The Clinical Severity of Pediatric Scorpion Envenomation Cases in Southeastern Turkey. *Wilderness and environmental medicine*.2015;*26*(4): 451−458.
6. Petricevich VL. Scorpion Venom and The Inflammatory Response. *Mediators of Inflammation*. 2010;903295:1−16.
7. Shalaby MM, Sobeih AA, Abdulghany WE, Behiry EG, Ismail YM, Abd-El-Aziz MA. Mean Platelet Volume and Serum Uric Acid in Neonatal Sepsis: A Case-Control Study. *Annals of medicine and surgery*.2017; 20:97−102.
8. Capan K, Tekin M,ColakP,Uckardes F, Turgut M. An Overview of Platelet Indices for Evaluating Platelet Function inChildren with Scorpion Envenomation.*EXCLI Journal*.2014;13:801−808.
9. Gokay SS, Yilmaz HL, Yildizdas RD, Celik T, Ekinci F, Kendir OT. A Relationship Between Clinical and Laboratory Characteristics in Children with Severe Scorpion Envenomation in Cukurova, Turkey.*PediatrEmerg Care*. Epubahead of print 24 April 2018. DOI: 10.1097/PEC.0000000000001483.
10. Song YM, Tang XX, Chen XG, Gao BB, Gao E, Bai LLv XR. Effects of Scorpion Venom Bioactive Polypeptides on Platelet Aggregation and Thrombosis and Plasma 6-keto-PGF1a and TBX2 in Rabbits and Rats. *Toxicon*.2005;46:230–235.
11. Nasr HB, Bolon B, Hammami ST, Sahnoun Z, Jamoussi K, Lahyani A, et al. Clinical Pathology Alterations in Pregnant and Non-Pregnant Rats Following Scorpion Envenomation. *Basic and clinical pharmacology and toxicology*.2009;*105*(4):228–235.

12. Al B,Yılmaz DA, Sogut S, Orak M,Ustundag M, Bokurt S.Epidemiological, Clinical Characteristics and Outcome of Scorpion Envenomation in Batman,Turkey: An Analysis of 12O Cases.*JAEM*. 2009;8(3):9–14.
13. Diaz C, Rivera J, LomonteB, Bonilla F, Diego-Garcia E, Camacho E, et al. Venom Characterization of The Bark Scorpion Centruroidesedwardsii (Gervais 1843): Composition, Biochemical Activities and In Vivo Toxicity for Potential Prey. *Toxicon*.2019;*171:*7–19.
14. Salvagno GL, Sanchis-Gomar F, PicanzaA,Lippi G. Red Blood Cell Distribution Width: ASimple Parameter with Multiple Clinical Applications. *Crit. Rev. Clin. Lab. Sci. Informa Healthcare USA, Inc* 2015;52:86–105.

Table 1. Distribution of age and gender in the patient and control groups

		Patient Group (n=63)	**ControlGroup (n=33)**	***P* value**
Age (Mean.±SD, Min−Max)		43.7±18.6 (15−92)	41.1±16.1 (20−74)	0.560
Gender n(%)	Male	27 (42.9)	12 (36.4)	0.538
	Female	36 (57.1)	21 (63.6)	

SD: standard deviation

Table 2. General characteristics of the patient group

Time to presentation (minutes) Mean±SD (Min−Max)		101.0±121.9 (12−600)
Grade n (%)	1	35 (55.5)
	2	23 (36.5)
	3	3 (4.8)
	4	2 (3.2)
Electrocardiogramn (%)	Normal Sinus Rhythm	63 (100)
Number of antivenoms used n (%)	0	54 (85.7)
	1	8 (12.7)
	2	1 (1.6)
Affected body area n (%)	Right upper extremity	23 (36.5)
	Left upper extremity	12 (19.0)

	Right lower extremity	12 (19.0)
	Left lower extremity	10 (15.9)
	Head-neck, trunk	6 (9.5)

Table 3. Comparison of the patient and control groups in terms of laboratory parameters

	PatientGroup		**ControlGroup**		
	Mean ±SD	Min–Max (Median)	Mean ±SD	Min–Max (Median)	*P* value
Leukocyte(10^3/uL)	9.76 ± 2.49	4.64-16.94 (9.41)	8.05 ± 1.56	4.16–11.27 (8.13)	0.001
Neutrophil(10^3/uL)	5.40 ± 1.92	1.97-11.37 (4.86)	4.72 ± 1.45	2.05–7.53 (4.66)	0.104
Lymphocyte(10^3/uL)	3.28 ± 1.20	1.46-7.45 (2.95)	2.54 ± 0.66	0.87–3.79 (2.67)	0.003
NLR	1.83 ± 0.91	0.65-5.71 (1.66)	2.12 ± 1.49	0.91–8.62 (1.72)	0.471
RDW (%)	12.21 ± 1.44	10.7-19.9 (11.83)	12.78 ± 1.25	11.14–16.3 (12.55)	0.004
<11.6	23 (36.5)		5 (15.2)		0.045
11.6-15.8	39 (61.9)		26 (78.8)		
>15.8	1 (1.6)		2 (6.1)		
MPV(fL)	7.99 ± 1.77	5.5–13.5 (7.487)	8.39 ± 2.34	5.83–16.01 (7.63)	0.622
<6.8	17 (27.0)		9 (27.3)		0.697
6.8–10.8	40 (63.5)		19 (57.6)		
>10.8	6 (9.5)		5 (15.2)		

Platelet(10^3/uL)	268.14 ± 80.57	135.8–486.6 (260.9)	235.68 ± 64.48	130.5–373.8 (226.6)	0.053
Phospholipase (nmol/min/ml)	112.31 ± 25.59	62.7–199.7 (111.2)	116.50 ± 22.40	86.4–167.6 (114.4)	0.497

SD: standard deviation; NLR: neutrophil/lymphocyte ratio; RDW: red cell distribution width; MPV: mean platelet volume

Table 4. Rho and p values of the laboratory parameters according to clinical grades in the patient groups

	Clinical Grade	
	rho	*P* value
Leukocyte(10^3/uL)	0.131	0.307
Neutrophil (10^3/uL)	−0.005	0.967
Lymphocyte(10^3/uL)	0.162	0.206
NLR	−0.195	0.126
RDW (%)	−0.183	0.152
MPV(fL)	−0.432	<0.001
Platelet(10^3/uL)	0.164	0.199
Phospholipase (nmol/min/ml)	0.081	0.530
D-Dimer (µg/L)	−0.001	0.996

NLR: neutrophil/lymphocyte ratio; RDW: red cell distribution width; MPV: mean platelet volume

Table 5. Comparison of the laboratory values of the patients according to clinical grades

	Grade 1	Grade 2	Grade 3*	Grade 4*	p#
Leukocyte(10^3/uL)	9.56 ± 2.50 (9.2)	9.73 ± 2.39 (9.4)	11.29 ± 3.09 (10.8)	11.22 ± 3.73 (11.2)	0.709
Neutrophil (10^3/uL)	5.51 ± 2.13 (4.9)	5.12 ± 1.30 (4.8)	6.91 ± 3.54 (5.4)	4.50 ± 1.03 (4.5)	0.874
Lymphocyte(10^3/uL)	3.02 ± 0.84 (2.9)	3.50 ± 1.43 (3)	3.22 ± 0.64 (3.4)	5.37 ± 2.58 (5.4)	0.465
NLR	1.97 ± 1.01 (1.7)	1.63 ± 0.55 (1.7)	2.35 ± 1.71 (1.4)	0.89 ± 0.24 (0.9)	0.276
RDW (%)	12.27 ± 1.19 (11.9)	12.32 ± 1.85 (11.9)	11.30 ± 0.14 (11.2)	11.32 ± 0.58 (11.3)	0.715
MPV(fL)	8.53 ± 1.76 (8.2)	7.54 ± 1.61 (7)	6.43 ± 0.89 (6.6)	6.11 ± 0.35 (6.1)	0.016
Platelet(10^3/uL)	259.02 ± 78.85 (242.4)	277.84 ± 81.35 (272.1)	253.20 ± 109.22 (272)	338.55 ± 77.15 (338.6)	0.348
Phospholipase (nmol/min/ml)	112.48 ± 20.23 (110)	110.04 ± 34.02 (112.6)	121.13 ± 17.75 (114.2)	122.15 ± 7.99 (122.2)	0.805
D-dimer (µg/L)	737.66 ± 1747.44 (304)	1606.39 ± 5188.88 (354)	282.00 ± 50.32 (256)	192.50 ± 89.80 (192.5)	0.320

Values given in mean ± standard deviation (median)
*not included in analysis
#Mann–Whitney U test
NLR: neutrophil/lymphocyte ratio; RDW: red cell distribution width; MPV: mean platelet volume

Figures

Figure 1. Androctonus crassicauda

Figure 2. Leiurus quinquestriatus

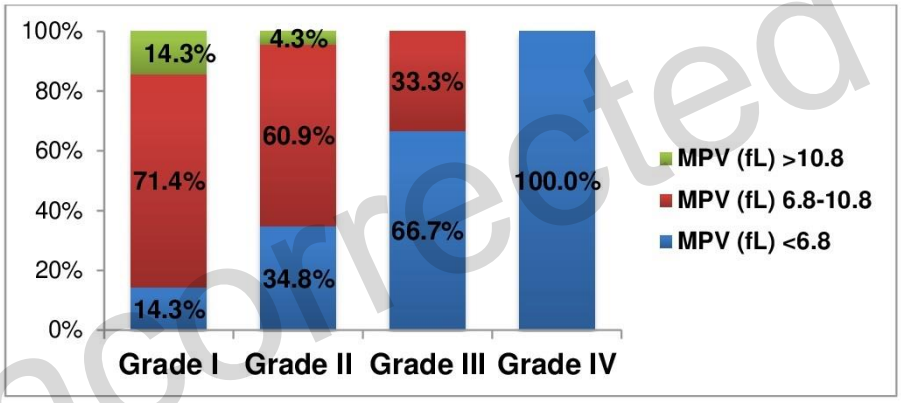

Figure 3. Mean platelet volume levels of the patient group according to clinical grade