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ORIGINAL ARTICLE

Interspecies Variations in Clinical Envenoming Effects of Viper Snakes Evolutionized in a Common Habitat: A Comparative Study on *Echis carinatus sochureki* and *Macrovipera lebetina obtusa* Victims in Iran

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Abstract

Background: Despite sharing common evolutionary features, Viperidae species including *Echis carinatus* and *Macrovipera lebetina* possess venoms with different proportions of toxic agents, thereby causing clinical effects with potentially variable severity. This study was an effort to differentiate the clinical effects and outcomes of *E. c. sochureki* and *M. l. obtusa* victims.
Method: In this prospective cross-sectional study, snakebite patients treated at a reference poisoning center in northeast of Iran in 2012 were enrolled. The features of snakebite event, demographic and clinical data of patients were recorded in checklists.
Results: Twenty-seven patients (63% male) with mean age of 34.8 ± 18.1 years were included. The offending snakes were recorded as "*E. c. sochureki*" in 63%, "*M. l. obtusa*" in 25.9% and "unknown" in 11.1% of cases. The most common clinical findings were fang mark in 100%, local pain in 81.5% and local edema in 74% of patients. Although the victims of both species showed classic features of viper envenoming syndrome including marked local effect and hemostatic disturbances, the victims of *M. l. obtusa* had significantly higher creatine kinase levels (P = 0.031) and lower platelet counts (P = 0.043), whereas marked edema (> 15cm) was significantly more common in *E. c. sochureki* victims (P = 0.028). Envenomation severity, other clinical effects and outcomes did not differ between the two species. Patients with delayed presentation to hospital had greater envenomation severity and edema extent and higher rate of coagulopathy.
Conclusion: Species-specific description of clinical effects following snakebite envenoming is useful for syndromic approach to human victims. The clinical envenoming syndromes by *E. c. sochureki* and *M. l. obtusa* show many common similarities despite the difference in severity of some effects. The delay in hospital admission and antivenom therapy is a risk for increased severity of envenomation and development of poorer clinical outcomes.

Keywords: Antivenins; Envenomation Syndrome; Snake Bites; Species Specificity; Viperidae

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INTRODUCTION

Snakebite in Iran with temperate and subtropical climate characterized with long, hot and dry summers in most parts of the country is a toxicologic emergency. Estimated annual incidence of snakebite in Iran is 4.5 to 9.1 per 100,000 population (1-4). The two most common native venomous snakes in Iran are *Echis carinatus sochureki* and *Macrovipera lebetina obtusa*. Most viper bite envenoming cases admitted to tertiary care hospitals in the country are the victims of these two species (3, 4).

Mashhad Medical Toxicology Center (MTC) in Imam Reza Hospital is a referral poisoning treatment center in northeast Iran. Each year, 20 to 30 snakebite victims are treated at MTC with less than 1% mortality (3, 5, 6). Previous reports have shown that *E. c. sochureki* and *M. l. obstua* are the most common offending snakes responsible for envenoming cases admitted to the MTC (1, 3-6). These are vipers known to cause local damages and coagulopathy. However, available characterization studies on *E. c. sochureki* and *M. l. obtusa* venoms have shown that they possess variable proportions of toxic agents (7-9). Therefore,

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their victims may experience clinical effects and outcomes with variable severity.

In this report, the one-year data of snakebite patients managed at MTC is presented aiming at the comparison of clinical effects and outcomes of *E. c. sochureki* and *M. l. obtusa* victims.

METHODS

Study design and variables

In this prospective cross-sectional study, all snakebite patients treated at MTC in 2012 were enrolled. The catchment area of the study is shown in figure 1. Data collected to meet the study objectives comprised demographic features, circumstances of envenoming event, clinical findings and routine laboratory investigations of the patients prior to antivenom therapy as well as antivenom therapy details and ultimate outcomes. The routine laboratory investigations included complete blood count (CBC), coagulation studies and creatine kinase (CK) level. The extent (diameter) of local swelling/edema was measured considering the utmost rim of edema from the bite site. Envenomation severity of the patients was assessed using snakebite severity score (SSS) (10). Patients were treated according to a standardized snakebite envenomation management protocol for Iran (2, 6). Total antivenom dose given to the patients, elapsed time to achieve therapeutic response or initial control (as defined in the protocol (2, 6)) and duration of hospital stay were considered as outcome measures.

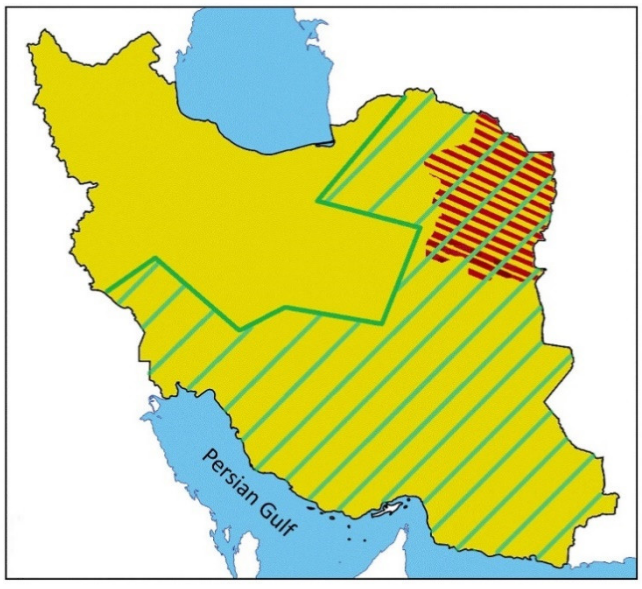

Figure 1. Geographical distribution of *Macrovipera lebetina* (yellow area) and *Echis carinatus* (striped green area) in Iran. *M. l. obtusa* lives in almost all parts of Iran and *E. sochureki* in two thirds of the country (data reproduced from the study by Dehghani et al (4)). Striped red area indicates the catchment area of the study.

Identification of the offending snakes

ELISA-based venom detection kits are the essential tools to detect the presence of venom in the blood and to identify the biting animal species. Nonetheless, no such kit for snake venoms has been developed in Iran, so far, despite being commercially available and routinely used in clinical practice in many countries (2, 11, 12). Hence, to identify the species of the offending snakes in this study, captured alive or dead snakes brought by the victims or their companions were evaluated according to the morphological details by an experienced zoologist. In addition, in MTC, a color atlas containing high quality photos of common regional snakes in different views including at least front and lateral views has been prepared to assist identifying the offending snake by the snakebite victims (if the offending snake has not been captured). This atlas was shown to each victim and if they could recognize the biting snake from the photos, then the name of the species was entered into the data checklist. Through either method, if the species was unidentifiable, the offending snake was recorded as "unknown".

Ethics

The study was approved by the institutional review board of the Mashhad University of Medical Sciences and AJA University of Medical Sciences. The patients cooperated with the study investigators and were informed about the study objectives giving their written informed consent.

Statistical analysis

The normality of quantitative data was analyzed using the Shapiro-Wilk test. The results are presented with mean ± standard deviation for normally distributed variables and with median and range for non-normal variables. For comparison of normally distributed data between two and three groups, independent T test and one-way ANOVA were used, respectively; while for non-normally distributed data, non-parametric alternatives, i.e. Mann-Whitney U test and Kruskal-Wallis H test, were used. The difference of qualitative data between groups was analyzed using chi-squared test. All analyses were done using SPSS for Windows (SPSS Inc., Chicago, USA). P values less than 0.05 were considered statistically significant.

RESULTS

Demographics

In 2012, twenty-seven snakebite patients (63% male) with mean age of 34.8 ± 18.1 years were admitted to MTC. Considering patient's occupation, the majority of victims were farmers (51.8%), followed by housewives (11.1%) and snake charmers (7.7%).

Snakebite circumstances

Except two snakebite cases that occurred in urban areas (one in the central fruit and vegetable market of Mashhad and the other one in a military base), the rest of events occurred in rural and desert areas. Snakebite events occurred during April to October with June having the highest rate of events (Figure 2).

Figure 3 shows the time of occurrence of snakebite on an hourly basis, which reveals that the majority of events happened during 8 am to 8 pm (85.1%), which by dividing this 12 hours into 4-hour sections, 12 to 4 pm (33.3%) had the highest rate of events closely followed by 4 to 8 pm (29.6%). No bites occurred during 8 pm to 4 am.

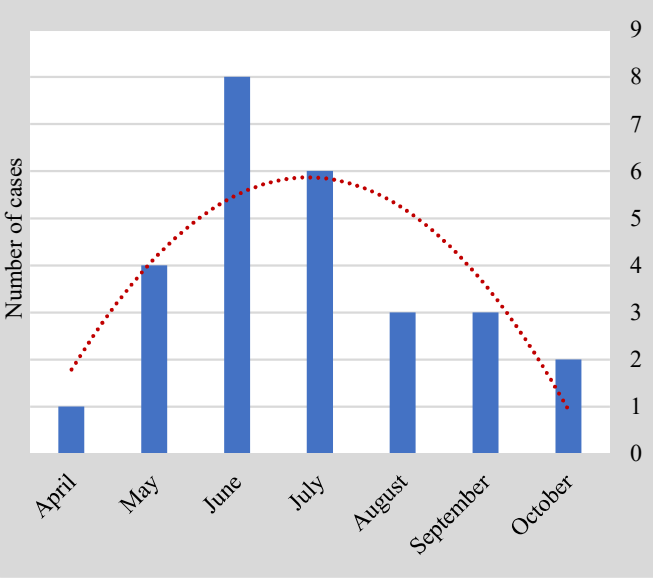

Figure 2. Monthly distribution of snakebite events (n = 27)

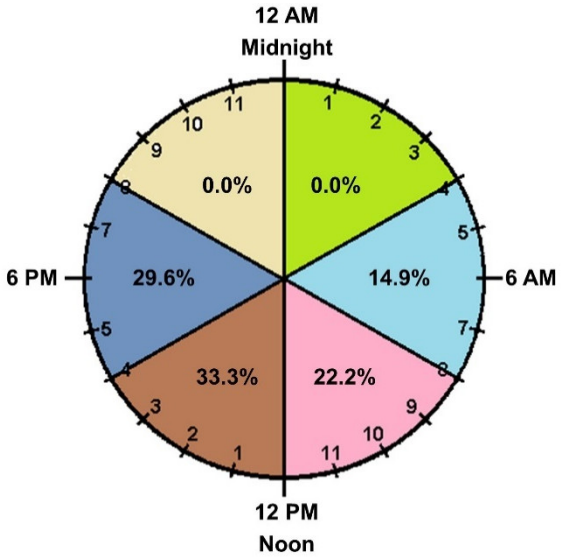

Figure 3. Distribution of daily time-points of snakebite events (n = 27)

The offending snakes were recorded as *E. c. sochureki* in 63% and *M. l. obtusa* in 25.9% of cases, while in 11.1% of cases the offending snake could not be identified. The median of elapsed time from the bite to hospital (tertiary) admission or the lag period was 5.5 (range, 1-72) hours. In over half of the events (55.6%), the patients were referred to the hospital in less than 6 hours.

Clinical findings of patients

At presentation to the MTC (on-admission or baseline visit), the most common clinical findings were fang mark in 100%, local pain in 81.5% and local edema in 74% of patients. Upper extremities were the most common location of bites (59.3%). Median SSS of all patients was 3.0 (0.0-10.0). In all patients, median of edema extent was measured to be 15.0 (0.0-70.0) centimeters.

Serious clinical findings including marked edema (> 15 cm), coagulopathy, thrombocytopenia (Platelet count < 150,000 cells/mm^3) and dermal necrosis were found in 15 (55.6%), 11 (40.7%), 9 (33.3%) and 7 (25.9%) patients, respectively. Only two patients had overt bleeding (one patient having epistaxis and the other one having epistaxis and gingival bleeding simultaneously), who both presented to the hospital with a great delay from the snakebite event, 29 hours and 55 hours, respectively. No patient developed internal hemorrhage. Rhabdomyolysis occurred in right leg of a 7-year-old boy, whom his parents had tied a tight tourniquet above the bite site for 7 hours prior to the hospital admission.

Interspecies difference of clinical findings and outcome measures

Table 1 summarizes the major baseline (on-admission) clinical findings and laboratory results of snakebite patients as well as outcome measures plotted against the offending snake species. Comparing the clinical findings of the snakebite patients of all snake categories (*E. c. sochureki, M. l. obtusa and unknown* cases) with each other; extent of edema (P = 0.033), SSS (P = 0.048), CK level (0.013) and the rate of marked edema (P = 0.012) were found to be significantly different. However, if only *E. c. sochureki* and *M. l. obtusa* victims were taken into account, the differences of CK level (P = 0.031), platelet count (P = 0.043) and marked edema incidence (P = 0.028) were statistically significant. In this context, the extent of edema and SSS in *E. c. sochureki* and *M. l. obtusa* victims were significantly higher than patients bitten by unknown snakes, whereas they were not significantly different between *E. c. sochureki and M. l. obtusa* victims. In addition, CK levels in *M. l. obtusa* victims were significantly higher than *E. c. sochureki* victims and patients bitten by unknown snakes. On the other hand, marked edema was significantly more common in *E. c. sochureki* victims compared with the other two categories. Ecchymosis was also more commonly seen in *E. c. sochureki* victims, though the analysis of difference with the other two categories only showed approaching to the level of significance (P= 0.057). Platelet count, nonetheless, was significantly lower in *M. l. obtusa* compared with *E. c. sochureki* victims.

Treatments and outcomes

All patients were managed successfully with no significant morbidity. The median of total antivenom dose given to all patients was 10 (0-24) vials and the median of time to achieve therapeutic response (initial control) was 2 (1-9) hours. The total antivenom doses required for *E. c. sochureki* and *M. l. obtusa* victims were significantly higher than patients bitten by unknown snakes (P = 0.039), whereas this parameter was not significantly different between *E. c. sochureki and M. l. obtusa* victims (Table 1). Regarding the time to achieve initial control, no significant differences existed among the groups. All patients were discharged in good health conditions after a median of 2 (0-6) days and no mortality occurred (one patient bitten by *M. l. obtusa* left

Table 1. Baseline clinical findings of snakebite victims and outcome measures plotted against offending snake species

Parameters	Total	Based on offending snake			P value	
		Echis carinatus sochureki	*Macrovipera lebetina obtusa*	Unknown	Comparison among all categories	*E. c. socureki* vs. *M. l. obtusa*
No. of victims	27	17	7	3		
Extent of edema, cm, median (range)	15.0 (0.0-70.0)	20.0 (0.0-70)	15.0 (0.0-50.0)	0.0 (0.0-3.0)	0.033*	0.184**
SSS, score, median (range)	3.0 (0.0-10.0)	3.0 (0.0-10.0)	3.0 (0.0-8.0)	1.0 (0.0-1.0)	0.048*	0.383**
Creatine kinase, U/L, median (range)	147.0 (52.0-865.0)	178.0 (52.0-407.0)	291.5 (118.0-865.0)	132.0 (112.0-217.0)	0.013*	0.031**
PT, sec, median (range)	13.0 (10.8-60.0)	13.1 (10.8-60.0)	13.1 (12.5-60.0)	12.5 (12.0-13.4)	0.428*	~1.000**
PTT, sec, median (range)	26.5 (22.8-180.0)	25.0 (22.8-180.0)	25.0 (22.8-180.0)	25.0 (22.8-180.0)	0.421*	0.235**
INR, ratio, median (range)	1.1 (1.0-8.3)	1.1 (1.0-8.3)	1.1 (1.0-8.3)	1.1 (1.0-1.1)	0.501*	0.791**
WBC count, cells x $10^3/mm^3$, median (range)	9.3 ± 3.2	10.3 ± 3.1	7.9 ± 2.6	6.3 ± 2.7	0.072#	0.121##
Platelet count, cells x $10^3/mm^3$, median (range)	185.7 ± 90.8	196.5 ± 98.7	127.6 ± 48.2	244.6 ± 61.6	0.139#	0.043##
Local pain, n (%)	22 (81.5)	15 (88.2)	6 (85.7)	1 (33.3)	0.074¶	0.865¶
Local edema, n (%)	20 (74.1)	15 (88.2)	4 (57.1)	1 (33.3)	0.067¶	0.126¶
Marked edema, n (%)	15 (55.6)	13 (76.5)	2 (28.6)	0 (0.0)	0.012¶	0.028¶
Ecchymosis, n (%)	13 (48.1)	11 (64.7)	2 (28.6)	0 (0.0)	0.057¶	0.106¶
High CK, n (%)	12 (44.4)	6 (35.3)	5 (71.4)	1 (33.3)	0.248¶	0.106¶
Coagulopathy, n (%)	11 (40.7)	7 (41.2)	4 (57.1)	0 (0.0)	0.241¶	0.476¶
Thrombocytopenia, n (%)	9 (33.3)	5 (29.4)	4 (57.1)	0 (0.0)	0.182¶	0.202¶
Lymphadenitis, n (%)	8 (29.6)	6 (35.3)	2 (28.6)	0 (0.0)	0.466¶	0.751¶
Nausea/ vomiting, n (%)	7 (25.9)	5 (29.4)	1 (14.3)	1 (33.3)	0.709¶	0.437¶
Dermal necrosis, n (%)	7 (25.9)	5 (29.4)	2 (28.6)	0 (0.0)	0.553¶	0.967¶
Blister formation, n (%)	3 (11.1)	3 (17.6)	0 (0)	0 (0)	0.371¶	0.235¶
Total AV dose, vial, median (range)	10 (0-24)	10 (0-24)	8 (0-22)	0 (0-5)	0.039*	0.619**
Time to achieve initial control, hour, median (range)	2 (1-9)	2.5 (1-8)	2.5 (1-9)	1.5	0.524*	0.747**
Hospital stay, day, median (range)	2 (0-6)	2 (1-6)	2 (0-5)	1 (1-1)	0.189*	0.757**

* Analyzed with Kruskal-Wallis H test
** Analyzed with Mann-Whitney U test
# Analyzed with one-way ANOVA
Analyzed with Student's T test
¶ Analyzed with chi-squared test

the hospital before completion of treatments and against medical advice). The duration of hospital stay was not significantly different among all groups, nor between *E. c. sochureki and M. l. obtusa* victims.

Risk analyses

Analysis of bivariate relationship revealed that the lag period (time from snakebite event to hospital admission) was significantly correlated with the extent of edema (r = 0.450, P = 0.018) and SSS (r = 0.404, P = 0.037) (Figure 4).

In univariate analysis, also, patients with marked edema had longer lag period and hospital stay, and received more antivenom vials. The association between lag period and

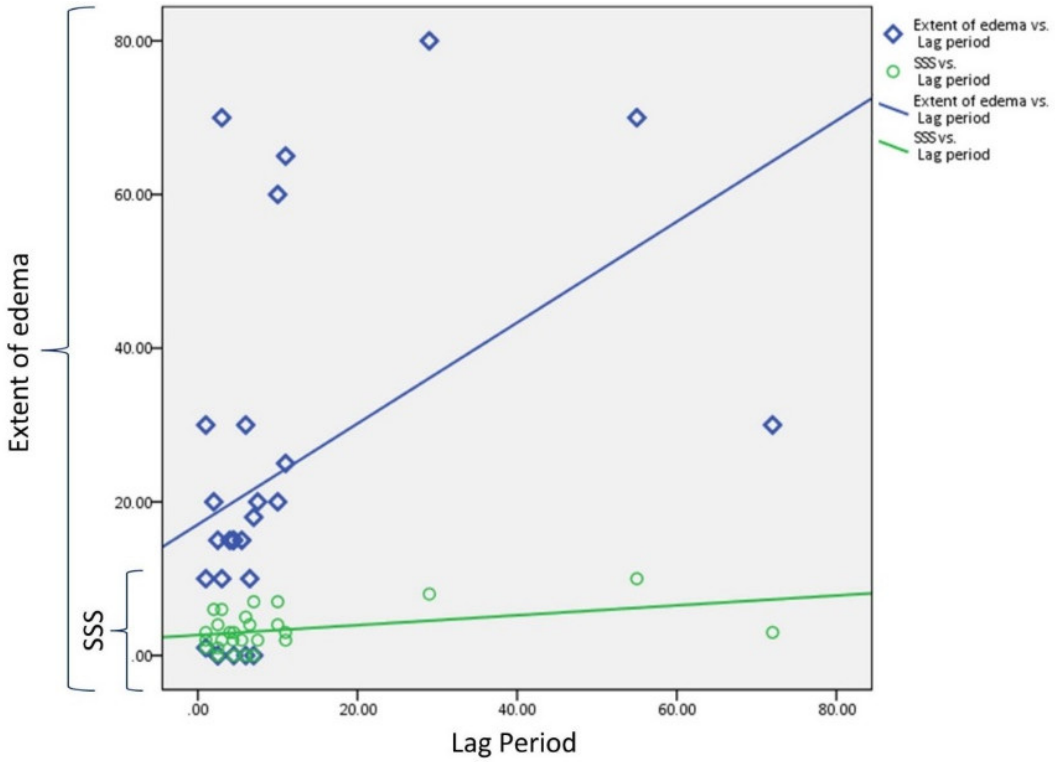

Figure 4. Relationship between lag period and extent of edema/SSS

presence of coagulopathy was close to the level of significance (P = 0.064). Presence of any serious clinical effect had no impact on the time to achieve initial control (Table 2).

DISCUSSION

Despite belonging to same family of snakes and sharing some common evolutionary features, different species evolved in a common habitat may have diverging venom compositions, and so cause diverse clinical venom effects in the prey/victim (13, 14). Hence, in modern clinical toxicology, scientists and clinicians seek out for species-oriented information of venom effects/envenoming syndrome. This type of information has not been available in Iran, so far, owing to a couple of reasons: First, no snake venom detection kit is available in the country. Second, the Iranian antivenom (Razi™ Antivenin) is poly-specific and has been effective in treating almost all snakebite cases in the country and even in the neighboring countries with a very limited mortality rate (3, 5, 6, 15, 16). Therefore, as almost always the antivenom has been clinically effective, Iranian physicians and researchers have never been motivated to develop a detection kit to distinguish the offending snake or to outline a species-specific description of envenoming syndrome. In fact, the detection kits are mandatory in regions where monovalent antivenoms are available and the range of native snakes is broad (11, 17).

This study was an effort to describe species-oriented clinical envenoming syndrome of two common vipers of Iran. In this study, the victims of both *E. c. sochureki* and *M. l. obtusa* developed severe local and systemic effects including marked local edema and sometimes dermal necrosis as well as thrombocytopenia and coagulopathy, which are pathognomonic features in envenoming by snakes of Viperidae family (6, 8, 18-23). Nonetheless, we found that the extent of local effects was more severe in victims of *E. c. sochureki* compared with those of *M. l. obtusa*. On the other hand, victims of *M. l. obtusa* experienced considerable reductions in platelets and greater increase in serum CK levels. These variations can be attributed to the difference of venom compositions between these two species. Cumulative evidence from various characterization studies of viper venoms show that the concentrations of phospholipase A2, which is notorious for causing tissue degradation and edema formation , is higher in *E. carinatus* venom compared with *M. lebetina* venom (24-29). In addition, the presence of hyaluronidase, a relatively common component of *E. carinatus* venom responsible for causing extracellular matrix disruption (30), is very limited to none in *M. lebetina* subspecies (31). Moreover, there have been reports of *M. lebetina* venom containing VLH2, a metalloproteinase known for causing hemorrhagic and strong myotoxic activity (32), as well as serine proteases, recognized as a platelet aggregating agent (24, 31), which both might be responsible for marked thrombocytopenia and high CK levels in the victims. Serine proteases are also present in *E. carinatus* venom but in much lesser extent (*M. lebetina* venom contains 4-fold more serine proteases compared to *E. carinatus*

Table 2. Serious clinical venom effects plotted against lag period as a risk factor and duration of hospital stay, total antivenom dose and lag time to achieve initial control as outcome measures

Clinical effect	No. of patients	Lag period, hour, median (range)	Total AV dose, vial, median (range)	Time to initial control, hour, median (range)	Hospital stay, day, median (range)
Marked edema					
Yes	15	7.5 (1-72)	10 (5-24)	3 (1-8)	2 (1-6)
No	12	3.5 (1-7)	2.5 (0-15)	2 (1-9)	1 (0-3)
P value		0.010	0.005	0.157	0.006
Coagulopathy					
Yes	11	6.2 (1-72)	9.5 (5-22)	2.5 (1-9)	2 (1-6)
No	16	5.1 (1-11)	5 (0-24)	2 (1-8)	1 (0-5)
P value		0.064	0.182	0.706	0.574
High creatine kinase*					
Yes	12	7 (1-55)	5 (0-24)	2 (1-9)	1.5 (1-5)
No	15	5.5 (1-72)	10 (0-22)	2.5 (1-7)	2 (0-6)
P value		0.270	0.361	0.683	0.559
Thrombocytopenia					
Yes	9	6 (1-72)	8 (5-22)	3 (1-7)	3 (1-6)
No	18	5 (1-11)	9 (0-24)	2 (1-9)	2 (0-5)
P value		0.354	0.658	0.630	0.141
Dermal necrosis					
Yes	7	4.5 (3-11)	10 (0-24)	3 (1-8)	2 (1-5)
No	20	5.8 (1-72)	8 (0-22)	2 (1-9)	2 (0-6)
P value		0.618	0.341	0.278	0.708

P values are analyzed with Mann-Whitney U test
* > 190 U/L (male) or > 165 U/L (female)

venom) (24-26). In this study, cases bitten by unknown snakes developed very limited local effects and no systemic effects, which might be due to the fact they were either bitten by small/new born snakes or non-/mildly- venomous snakes (2, 6, 20, 33).

In this study, the elapsed time for achievement of therapeutic response to Iranian antivenom was similarly around 2 hours in victims of *E. c. sochureki* and *M. l. obtusa*, which is an expected time-span to observe therapeutic effects from $F(ab')_2$ antivenoms (3, 34). Moreover, the average required doses of antivenom and the length of hospital stay were comparable in the victims of both species. Altogether, the outcome measures were not significantly different in envenomation by *E. c. sochureki* and *M. l. obtusa*.

Human snake bites are a healthcare problem of warm months in the regions with subtropical and temperate climates, when these cold-blooded reptiles are more active (18, 20, 23, 33, 35). Therefore, it is not surprising that all events occurred between April and October in this study. In addition, the occurrence of these events is also related to when humans are active in outdoors (36), so at nights the incidence is much lower (36, 37). Further, snakes are usually less active during nights (38, 39). Hence, the majority of snakebite events expectedly occurred around mid- day; whereas no incident is recorded at night, midnight and early morning in the present study. Nonetheless, whenever such incident occurs, the victims and their relatives/companions should not lose vital time to reach healthcare centers to receive appropriate treatments. When this lag period extends to over 6-7 hours, the risk for development of grievous local effects increases and severer hemostatic disturbances significantly, as ascertained in the present study. Hafezi et al correspondingly found that delay in antivenom therapy is significantly associated with increased risk for coagulopathies (40). Some other studies, also, established delay in hospitalization and delayed antivenom administration as determinant factors of poorer outcomes following snake envenoming (41-43).

LIMITATIONS

Lack of a venom detection kit to identify the offending snake species was the major limitation of this study. This problem is not limited only to Iran and many other countries with high prevalence of snakebite events struggle with this drawback, as well. To overcome this limitation, a photo atlas of common regional snakes and the morphologic assessment by an expert zoologist were the possible solutions. Similarly, in Indian subcontinent, clinicians identify the type of the offending snake by evaluating photographs or preserved specimens of the bite (44). The other limitation of this study

might be the small sample size. Analysis with different statistical tests was an effort to circumvent this limitation.

CONCLUSIONS

Species-specific description of clinical effects following snakebite envenoming is essential and useful for syndromic approach to human victims. The envenoming syndrome guides the clinician to differentiate between the victims of vipers and elapids and clearly influences the medical decision making. It may also help identify the species of the offending animal in a snakebite event, though this requires further and large-scale evaluations. As ascertained in this study, the clinical envenoming syndromes by *E. c. sochureki* and *M. l. obtusa* show many common similarities. However, the local effects following *E. c. sochureki* envenomation is more severe, whereas some systemic effects are more intense in *M. l. obtusa* envenomation. The delay in hospital admission and antivenom therapy is a risk for increased severity of envenomation and development of poorer clinical outcomes.

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