ORIGINAL ARTICLE

The prognostic value of the neutrophil/lymphocyte ratio in patients with snake bites for clinical outcomes and complications

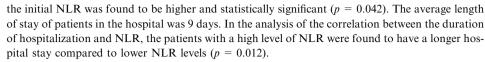**Bilal Elbey** [a,*]**, Burhan Baykal** [b]**, Ümit Can Yazgan** [c]**, Yılmaz Zengin** [d]

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Abstract *Introduction:* Snake bites have cardiotoxicity, neurotoxic, myotoxic, nephrotoxic, and hemotoxic features. The neutrophil/lymphocyte ratio (NLR) provides valuable information for the determination of the diagnosis and prognosis of various diseases. In this study, we aimed to investigate the relationship between NLR with the development of complications and duration of hospital stay in snakebite cases.

Method: In this study, 107 patients with snakebite complaints that applied to a tertiary care university hospital between 2011 and 2014 were retrospectively reviewed. The control group compromised of 107 age-and gender-matched healthy subjects. These patients were examined using their previous laboratory results, bite areas pictures, geographic location, and analysis of complications that developed during the hospitalization.

Results: Patients in our snake bites group ($n = 107$), included males (64%) and females (36%). When NLR1–NLR2, NLR1–NLR3, and NLR2–NLR3 were compared, a statistically significant difference was found ($p < 0.001$). No mortality was observed in our patients. In cases of snakebites, 4.67% of the patients underwent finger amputation. Compartment syndrome occurred in 3.73% of patients. In one case that developed compartment syndrome, a finger amputation was made. When 8 patients with a complication were compared with patients having snakebite but no complication,

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the initial NLR was found to be higher and statistically significant ($p = 0.042$). The average length of stay of patients in the hospital was 9 days. In the analysis of the correlation between the duration of hospitalization and NLR, the patients with a high level of NLR were found to have a longer hospital stay compared to lower NLR levels ($p = 0.012$).

Conclusion: NLR was significantly increased in patients that developed complications and needed a longer stay in the hospital.

1. Introduction

Approximately one-third of the 2500 snake species in the world are poisonous. However, of the venomous snakes, only 200 are dangerous for people (Okur et al., 2001b). Snake bites are common especially in the south and southeastern region of Turkey, which is where the majority of the snakes are located due to the climate and geographical features. Snake species *Vipera lebetina obtusa* are often seen in Southeastern Turkey and have venom that causes serious systemic and tissue damage (Kuru, 1999). The snake poison has cardiotoxicity, neurotoxic, myotoxic, nephrotoxic, and hemotoxic effects. The chemical makeup of snake venom is complex and consists of a combination of many toxic proteins and enzymes (Demir et al., 2005). Local and systemic symptoms are observed according to the severity of toxin. Regional findings such as pain, temperature increase, hemorrhagic edema, ecchymosis, and tissue necrosis may occur in the bite area. Generally, most snake bite cases heal without sequelae. However, severe necrosis requiring amputation of the finger and compartment syndrome may be observed with some serious complications in some cases (Bentur and Cahana, 2003; Michelarakis and Varouhaki, 2009). Though the number of poisonous snake bite cases in Turkey is largely unknown, the annual death rate is estimated to be low.

Snake venom has widespread effects throughout the bite area as well as the whole body (Baran, 1976; Warrell, 1992). This is mostly due to the levels of toxic proteins and enzymes (such as neurotoxin, hemolysis, cardiotoxin, nucleotidase) within the venom, which can cause tissue damage (Basoglu and Baran, 1980). Neutrophils are the first inflammatory cells to respond to site of inflammation in this process. Even though they are short-lived, their production is increased in the bone marrow and they move into the region of the inflammation joining the circulation in about 1 h (Altenburg et al., 1995). In addition to this response, progressive anemia, leukocytosis, thrombocytopenia, hypofibrinogenemia, disturbances in coagulation tests, and proteinuria, azotemia occur in snake bites (Currie et al., 1991; Gökel et al., 1997; Okur et al., 2001a; Spiller and Bosse, 2003).

The neutrophil/lymphocyte ratio (NLR) measured in the blood has recently gained popularity since it is a practical method that is easy to measure. It provides valuable information for the determination of the diagnosis and prognosis of various diseases. NLR has been used in many cardiac and non-cardiac diseases as an indicator of inflammation (Azab et al., 2012). However, to the best of our literature search, no study has documented the NLR as a predictor of the complication and severity of patients with snake bites.

In this study, we aimed to investigate the relationship between NLR increase with the development of complications and duration of hospital stay in patients with snakebite.

2. Materials and methods

2.1. Methods

In this study, the medical records of the patients with snake bite complaints that applied to a tertiary care university hospital between 2011 and 2014 were retrospectively reviewed. The patients who were admitted for less than 6 h after bite were included in the study. The local ethics committee approved the study. The controls group compromised with age, and gender matched healthy subjects who were admitted to outpatient clinic. Patients who failed to fully conform to the information from the hospital records system; patients under 18 years of age; who had disease that may affect neutrophil and lymphocyte count such as diabetes mellitus, hypertension, had a history of chronic use of steroid or other medications, who develop an allergic reaction; with identified systemic infection; with active cancer, acute coronary syndrome, congestive heart failure, chronic obstructive pulmonary disease exacerbation period, and any chronic inflammatory disease were excluded. These patients were examined using their previous laboratory results, bite areas pictures, geographic location, and analysis of complications that developed during the hospitalization. The application of the patients; the third day and pre-discharge neutrophil, lymphocyte, and platelet counts, urea, creatinine, albumin, aspartate trans aminase (AST) ratio; the patient's NLR at admission (NLR1); NLR on the third day of the hospitalization (NLR2); and NLR on the day when the patient was discharged (NLR3) were recorded.

2.2. Statistical analysis

For statistical evaluations "Statistical Package for the Social Sciences 18" program (SPSS Inc., Chicago, IL, USA) was used. The Student's *t*-test and paired samples *t* test were used for the statistical analysis if the data were normally distributed, otherwise the non-parametric Mann Whitney *U*, and Wilcoxon's test were used. Chi-square test was performed for categorical comparisons. A *p* value less than 0.05 was accepted as statistically significant.

3. Results

Two hundred fourteen participants were included in the study. The demographics of the study population are given in Table 1.

Table 1 Comparison of the clinical and demographic parameters among the patient and control groups.

	Patients (n = 107)	Control group (n = 107)	p value
Age (year)	40.0 ± 18.1	39.9 ± 17.9	0.979
Hospitalization (day)	9 ± 8	–	
AST (U/L)	29 ± 19	19 ± 6	<0.001
Albumin (mg/dl)	3.6 ± 0.5	4.1 ± 0.3	<0.001
Urea (mg/dL)	35 ± 12	27 ± 8	<0.001
Creatinine (mg/dL)	0.77 ± 0.19	0.78 ± 0.16	0.621
Platelet (10^9/L)	203 ± 83	265 ± 67	<0.001
Neutrophil (at admission) (10^3/mm^3)	12.02 ± 5.90	4.69 ± 1.35	<0.001
Lymphocyte (at admission) (10^3/mm^3)	1.43 ± 0.78	2.53 ± 0.64	<0.001
Compartment syndrome (n, %)	4 (1.9)	0 (0)	
Amputation (n, %)	5 (2.3)	0 (0)	
Exitus (n, %)	0 (0)	0 (0)	
Neutrophil (on the third day of the hospitalization) (10^3/mm^3)	6.73 ± 3.47	–	
Neutrophil (pre-discharge) (10^3/mm^3)	5.02 ± 1.69	–	
Lymphocyte (on the third day of the hospitalization) (10^3/mm^3)	1.87 ± 0.73	–	
Lymphocyte (pre-discharge) (10^3/mm^3)	1.97 ± 0.59	–	

p: probability.
AST: aspartate transaminase.

There was no statistically difference between groups with respect to age ($p = 0.97$) and gender ($p = 1.0$).

Neutrophil, lymphocyte, and platelet count, AST, albumin, NLR1 values were significant higher in the patient group.

Patients were from city center (9.3%) and 90.7% were from the country and urban rural. Most of the bites occurred on the right hand (Table 2). No difference was observed in terms of the relationship between the bite area and NLR ($p = 0.35$).

The patients were classified with respect to bite site as upper and lower extremity (Table 3).

All NLR parameters were significantly different between upper and lower groups compared to control subjects ($p < 0.001$). However there was no statistically difference between lower and upper groups with respect to NLR1 NLR2 and NLR3 ($p = 0.769$, $p = 0.151$ and $p = 0.435$, respectively).

When NLR1–NLR2, NLR1–NLR3, and NLR2–NLR3 were compared, a statistically significant difference was found ($p < 0.001$) (Tables 3 and 4).

No mortality was observed in our patients. In cases of snakebites, five patients (4.67%) underwent finger amputation. Compartment syndrome occurred in four patients (3.73%). In one case that developed compartment syndrome, a finger amputation was performed.

Table 2 Distribution of snake bites according to bite body site in Southeast of Turkey over the period (2011–2014).

	Bitten limb (n = 107)	Percent (%)
Both hands	1	0.5
Right foot	24	11.2
Right leg	5	2.3
Right hand	30	14
Right arm	1	0.5
Left foot	16	7.5
Left leg	8	3.7
Left hand	15	7
Left hip	1	0.5
Left arm	1	0.5

Table 3 NLR value of snake bites in lower and upper extremities.

	Bitten limb (n = 107)	Percent (%)	NLR1 (mean)	NLR2 (mean)	NLR3 (mean)
Upper extremity	51	47.6	11.63	4.32	2.54
Lower extremity	56	52.4	11.74	3.87	2.79

The initial NLR was found significantly higher ($p = 0.042$) in eight patients with a complication compared to the patients without complication. However, the NLR measured during the follow-up and pre-discharge was not statistically significant ($p = 0.001$).

The mean duration of hospitalization of the patients was 8.92 ± 8.52 days. In the analysis of the correlation between the duration of hospitalization and NLR, the patients with a high level of NLR were found to have a longer hospital stay compared lower NLR levels ($p = 0.012$, $r = 0.242$) (see Table 5).

4. Discussion

Snake venom has widespread effects throughout the body and at the bite region (Baran, 1976; Warrell, 1992). Snake venom

Table 4 Average of neutrophil/lenfosit ratio of patients.

	Average ± standard deviation
NLR1	11.69 ± 9.16
NLR2	4.07 ± 2.99
NLR3	2.67 ± 0.98

NLR1: neutrophil/lenfosit ratio at admission.
NLR2: neutrophil/lenfosit ratio on the third day of the hospitalization.
NLR3: neutrophil/lenfosit ratio of pre-discharge of the patients.

Table 5 Comparision of the NLR1, NLR2 and NLR3.

	Average ± standard deviation	95% the lower and upper limits of the confidence interval		P value
NLR1–NLR2	9.1 ± 1.0	7.03	11.18	< 0.001
NLR1–NLR3	10.8 ± 1.1	8.58	12.99	< 0.001
NLR2–NLR3	1.3 ± 0.3	0.64	2.00	< 0.001

NLR1: neutrophil/lenfosit ratio at admission.
NLR2: neutrophil/lenfosit ratio on the third day of the hospitalization.
NLR3: neutrophil/lenfosit ratio of pre-discharge of the patients.

comprises a combination of many toxic proteins and enzymes. These enzymes can facilitate an inflammatory response and create tissue and cell damage. In our study, we investigated the prognostic and predictive value of NLR at admission, complications in snake bites, and time of hospitalization. Collectively, we concluded that the increasing NLR would be effective in defining the length of stay and risk of complications in patients. To the best of our knowledge, our study is the first to investigate the role of NLR to predict the prognosis, development of the complications, and hospitalization time in patients with snakebites.

According to the severity of the toxins in snakebites, local and systemic symptoms can occur in patients. Local findings are lesions as edema, hematoma, and gangrenous. Fever, nausea, vomiting, circulatory collapse, mild jaundice, delirium, convulsions, and coma may occur as systemic findings. Death can occur in as little as 6–48 h due to the secondary infections, disseminated intravascular coagulopathy, acute renal insufficiency, neurotoxicity, and intracranial hemorrhage. In addition, progressive anemia, leukocytosis, thrombocytopenia, hypofibrinogenemia, disturbances in coagulation tests, proteinuria, and azotemia can also be counted among the causes of death (Currie et al., 1991; Gökel et al., 1997; Okur et al., 2001a; Spiller and Bosse, 2003).

The number of leukocytes and their subtypes (granulocytes, lymphocytes and monocytes) rates has been recognized as a marker of inflammation in cardiovascular diseases (Acanfora et al., 2001; Horne et al., 2005). Neutrophil count is elevated beginning from the first hour due to the inflammation whereas, lymphopenia occurs in response to physiological stress (Acanfora et al., 2001). Data related to the role of inflammation in snake bites are limited. In a study by Açıkalın and Gökel, increased IL-6 and TNF-alpha values indicate that the inflammation occurs through snake bites (Açıkalın and Gökel, 2011). In the study by Al Durihim et al. (2010), leukocytosis is an encountered finding in the complete blood count of patients admitted to hospital with snakebite. The increase in the number of leukocytes of patients has been reported to be associated with the clinical situation (Horne et al., 2005). The increased NLR levels in our study support the role of inflammation in snakebites.

NLR as a marker of inflammation has recently gained popularity. Arachidonic acid metabolites, which trigger inflammation, lead to the release of factors activating thrombocytes. Accordingly, neutrophil levels improve. NLR elevation takes place as a result of increase in the number of neutrophils and the decrease in the number of lymphocytes (Sarraf et al., 2009).

NLR has been determined to change in diseases associated with inflammation like obesity, metabolic syndrome (Bahadır et al., 2014), Behcet's disease (Rifaioglu et al., 2014), and coronary artery disease (Gillum et al., 2005; Sönmez et al., 2013; Tamhane et al., 2008). Sönmez et al. determined that NLR level was higher in coronary artery disease (Sönmez et al., 2013), and that NLR increased with the increase in the prevalence of coronary artery disease. In inflammation-included tuberculosis pleurisy, evaluation of pleural fluid adenosine deaminase activity together with NLR was reported to be a valuable method for providing diagnosis (Yurdakul et al., 2000). In addition to this, another study reported that NLR value in psoriasis patients was higher than in control groups (Ataseven et al., 2014). In our study, the NLR was found to be higher in snakebites compared to control subjects. We suggest that the snake venom may increase NLR secondary to inflammation. In addition, higher levels of NLR at admission in the complication-contained group showed that NLR may be a useful method to predict the development snakebite complications. When examining the blood measured at different times, a statistical significance was found between NLR1, NLR2, and NLR3 when compared with each other in the pair.

Salciccioli et al. stated in their study that mortality was higher in patients with increased NLR (Salciccioli et al., 2015). Although complications occurred in some cases of snakebites, no mortality was observed in our study.

Albumin is a negative acute phase reactant that decreases in inflammation. This decrease results from inflammatory cytokines like IL-6 and TNF-alpha to suppress the albumin synthesis through inhibition of albumin gene transcription. In our study, the serum albumin level was significantly lower in patient group, that is consistent with previous reports.

There are some limitations in our study. First, it has a relatively small sample size and it is an observational and retrospective study. In addition, other inflammatory markers such as C-reactive protein and fibrinogen were not routinely examined in the study population.

5. Conclusion

In conclusion, NLR was significantly increased in patients that developed complications and needed a longer hospitalization. Our results show that the use of NLR at admission may be a useful parameter in predicting the development of complication and stay-period of the patients. Prospective studies are warranted to define the predictive value of NLR and its association with the development of snakebite complications and the hospitalization time.

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