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ARAŞTIRMA / RESEARCH

The role of neutrophil-lymphocyte ratio as a predictor of acute hepatic failure in mushroom poisoning

Mantar zehirlenmesinde akut karaciğer yetmezliğinin yordayıcısı olarak nötrofil-lenfosit oranının rolü

Bengü Gülhan Aydin[1], Gamze Küçükosman[1], Özcan Pişkin[1], Bilgehan Açikgöz[2], Rahşan D. Okyay[1], Hilal Ayoğlu[1]

[1]Bülent Ecevit University Faculty of Medicine, Department of Anesthesiology and Reanimation, Zonguldak, Turkey
[2]Bülent Ecevit University Faculty of Medicine, Department of Public Health, Zonguldak, Turkey

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Abstract

Purpose: This study aimed to evaluate the value of neutrophil/lymphocyte ratio (NLR) together with the demographic characteristics in predicting liver insufficiency or transplantation need following mushroom poisoning.

Materials and Methods: The patients were evaluated in terms of various parameters including gender, age, the month of admission, symptoms on emergency service admission, vital findings, laboratory findings, poisoning severity score (PSS) according to initial symptoms, and onset time of symptoms. The patients were divided into two groups according to whether the liver function tests were deteriorated or not. NLR was assessed according to PSS in patients with and without acute liver failure.

Results: A total of 84 patients, aged 16-85 years, were included in the study. Neutrophil/lymphocyte ratio was 10.82±8.7 in patients with impaired liver function tests and statistically significant compared to the patients with normal liver function tests (5.66±4.71). NLR was statistically significantly higher in patients who developed acute liver failure and in those with high poisoning severity score. The cut-off value was founded as 9.65.

Conclusion: NLR may be a useful predictor of liver failure or transplantation need, which develops as a result of mushroom poisoning.

Keywords: Acute hepatic failure, mushroom poisoning, neutrophil lymphocyte ratio

Öz

Amaç: Çalışmamızda mantar zehirlenmeleri sonucunda karaciğer yetmezliği veya transplantasyon ihtiyacını önceden tahmin edilmesinde nötrofil/lenfosit oranı (NLO) ve demografik özelliklerin birlikte değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Hastalar cinsiyet, yaş, hastaneye başvuru ayı, acil servise başvuru semptomları, vital bulgular, laboratuvar değerleri, ilk semptomlarına göre zehirlenme ciddiyet skoru (ZCS), şikayetlerinin başlama zamanı açısından değerlendirildi. Hastalar karaciğer fonksiyon testlerinin bozulup bozulmamasına göre iki gruba ayrıldı. ZCS'ye göre ve akut karaciğer yetmezliğinin olup olmamasına göre NLO değerlendirildi.

Bulgular: Yaşları 16-85 arasında olmak üzere 84 hasta çalışmaya dahil edildi. NLO karaciğer fonksiyon testleri bozulmuş hastalarda (10.82±8.7), karaciğer fonksiyon testleri normal olanlara (5.66±4.71) göre istatistiksel olarak anlamlıydı (p= 0.042). NLO'nun akut karaciğer yetmezliği gelişen hastalarda (17.77±9.44) şifa ile taburcu edilen hastalara göre (5.72±4.64) anlamlı derecede yüksek olduğu gözlendi. Cut-off değerinin 9.65 olduğu görüldü.

Sonuç: Mantar zehirlenmeleri sonucunda gelişebilecek karaciğer yetmezliği veya transplantasyon ihtiyacını erken tahmin edilmesinde NLO'nun faydalı olabileceğini düşünmekteyiz.

Anahtar kelimeler: Akut karaciğer yetmezliği, mantar zehirlenmesi, nötrofil-lenfosit oranı

Yazışma Adresi/Address for Correspondence: Dr. Bengü G. Aydın, Bülent Ecevit University Faculty of Medicine, Department of Anesthesiology and Reanimation, Zonguldak, Turkey E mail:bengukoksal@gmail.com
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INTRODUCTION

Mushrooms are organisms that do not contain chlorophyll pigments for photosynthesis and which reproduce with spores[1].

There are more than 5000 kinds of mushrooms and it is known that about 50-100 of them are toxic and only about 200-300 of them can be safely eaten[2]. Mushrooms are beneficial for people through many aspects, but macro mushrooms attract more attention by people. Among them, edible and poisonous mushrooms have an important place. Forest areas, meadows and streams are suitable environments for mushrooms to grow up. When collecting wild mushrooms, it is necessary to be careful and avoid the suspicious toxic mushrooms and consult experts before collecting them. Our country is quite rich in terms of mushroom flora due to its suitable ecological conditions[3].

Zonguldak (city in the Western Black Sea Region) region is usually rainy and many kinds of mushrooms grow due to its suitable environment for mushrooms. In a study conducted in the Zonguldak region, a total of 78 taxons were identified, including 1 Ascomycetes, 76 Basidiomycetes, and 1 Gasteromycetes. 30 of these taxons are considered edible, 7 are poisonous and 41 are inedible. Nine of these species are recognized by resident individuals, but others are not. The Lactarius deliciosus species are sold in local markets[4].

Amanita phalloides is the most important one among toxic mushrooms and contains amatoxin. Amatoxin can lead to hepatic necrosis and liver failure by binding to DNA-dependent RNA polymerase B in the hepatocyte and inhibiting m-RNA formation.[5] It is important to diagnose mushroom poisoning early, because it may cause liver damage and become fatal in patients admitted with suspected poisoning. Previous studies have emphasized the importance of early diagnosis and treatment and timely liver transplantation[6,7].

The neutrophil-lymphocyte ratio (NLR) provides an indicator of the inflammatory status. It is a biomarker that can easily be calculated from readily available complete blood count[8].

Our study aimed to evaluate NLR and the demographic characteristics, together with anticipated liver failure or transplantation need as a result of mushroom poisoning.

MATERIALS AND METHODS

The local ethics committee approval with the protocol number of 2016-96-24 / 08 was obtained. Our study was carried out by retrospectively examining the files of 102 patients who had admitted to our hospital with the diagnosis of mushroom poisoning between 01.01.2004 and 31.12.2014. 18 patients with missing file information were not included in the study. Age, sex, season of hospital admission, clinical findings, presence of other poisoning cases in the family, onset time of symptoms after fungal ingestion, time to hospital admission after ingestion, poisoning severity score[9] (PSS), treatment approaches, laboratory tests (complete blood count, liver function tests, renal function tests and the coagulation parameters, neutrophil / lymphocyte ratio) were evaluated. The patients were divided into two groups according to whether the liver function tests were increased or not. NLR measurements were evaluated according to PSS and whether or not acute liver failure was present. Patients with pregnancy, trauma, sepsis, cancer, hematological disease, pediatric population, missing file information and whose files were not accessible were excluded from the study. NLR was calculated by dividing the neutrophil by the lymphocyte counts.

Table 1.. Severity grades of PSS [9]

None (Grade 0)	No symptoms or signs related to poisoning
Minor (Grade I)	Mild ,transient and spontaneously resolving
Moderate (Grade II)	Pronounced or prolonged symptoms
Severe (Grade III)	Severe or life threatening symptoms
Fatal (Grade IV)	Death

Statistical analysis

The Statistical Package for the Social Sciences (SPSS)19.0 (IBM Corp. in Armonk, NY) was used for the statistical analysis. The descriptive statistics were given with frequencies and percentages for the categorical variables, and the mean, standard deviation, median and min-max values for the continuous variables. The Shapiro Wilk test used for testing the normality. The Independent samples t test and the Mann Whitney U test were used for the normally distributed and non-normally distributed variables, respectively. The Yates chi-square and the fisher exact chi-square tests were used to test the

difference between groups in terms of the categorical variables. Receiver-operating characteristics (ROC) curves were plotted in order to determine the predictive value of the parameters for acute liver failure decided upon with liver function test. For all of the statistical comparisons, a p value below 0.05 was assumed to be statistically significant.

RESULTS

A total of 84 patients, aged 16-85 years, were included in the study. Of these, 21 (25%) were male and 63 (75%) were female. The mean age of the patients was 44.2 ± 17.3 years. When the hospital admission periods were evaluated, we found that the most frequent admissions were made in October (n=36, 42.9%) and November (n=20, 23%). The most common complaints were nausea- vomiting (n=63, 75.1%), diarrhea (n=23, 27.4%), and abdominal pain (n=15, 17.9%). 40 (47.6%) patients had a positive family history (at least two or more poisonings in their family or relatives). According to the onset of symptoms, 74 (88.1%) patients had presented early (within 6 hours) and 10 (11.9%) patients had presented late (> 6 hours). In addition to symptomatic treatment, active charcoal was used in 68 patients (81.1%), penicillin G in 17 patients (20.4%), N-acetyl cysteine in 6 patients (20.4%), multivitamins in 10 patients (11.9%) and legalon in 10 patients (12%). In 69 (82.1%) of the patients, no laboratory abnormalities were detected, whereas 15 patients (17.9%) had elevated liver function tests, 2 patients (2.4%) had impaired renal function test and 6 patients (7.14%) had a hematological abnormality. The creatinine values of two patients with renal dysfunction were 4.6 mg/dl and 2.4 mg/dl, respectively. High international normalized ratio (INR) was seen as a hematological abnormality. Twenty (24%) patients had presented to the hospital within 6 hours, 46 (52.9%) patients had presented in 6-12 hours, 14 (16.8%) patients had presented in 12-24 hours, and 4 patients (4.8%) had presented after the first 24 hours of symptom onset. The average NLR value of 84 patients was 6.58 ± 5.92. The complaints of patients who had died due to mushroom poisoning were determined to have begun in the late phase after ingestion and had presented to the hospital 12 and 48 hours after the ingestion, respectively (4,76%). Six patients (7.14%) with acute liver failure need transplantation and the two of these patients died (%2.38). The Neutrophil/lymphocyte ratio was 10.82±8,7 in patients with impaired liver function tests and statistically significant compared to patients with normal liver function tests (5.66±4.71), (p= 0.042). The comparison of variables of normal and impaired liver function tests has been demonstrated in Table 2. NLR was statistically significantly higher in patients who developed acute liver failure and in those with high poisoning severity score (p = 0.026 and p <0.001, respectively) (Table 3). Mean of NLR of 2 patients who died was 20.67±1.52.

Table 2. Comparison of the variables of normal and impaired liver function tests.

	General distribution	Normal liver function tests (n=69)	Impaired liver function tests (n=15)	p
Male/Female	21/63	16/53	5/10	0.411
Age	44.2±17.3	43.55±17.47	47.33±16.87	0.447
Onset time of symptoms First 6 hour After 6 hour	 74 10	 63 6	 10 5	 *0.010
AST	194.72±829,51	25.77±12.59	971.89±1814.07	*<0.001
ALT	230.40±1132.06	24,49±14.72	1177.57±2538.08	*<0.001
GGT	28.42±49.88	17.17±10.00	80.1±103.8	*<0.001
Albumin	4.28±0.42	4.3±0.33	4.16±0.73	0.263
Total bilirubin	0.89±0.99	0.69±0.52	1.78±1.87	*<0.001
INR	1.10±0.74	0.99±0.18	1.55±1.25	*<0.001
NLR	6.58±5,92	5.66±4.71	10.82±8.77	*0.042
Severity grades of PSS1/2/3/4	71/7/4/2	65/2/2/0	6/5/2/2	*<0.001

*p<0.05 ,Data have been presented as number and mean ±standard deviation;AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, NLR: Neutrophil lymphocyte ratio, GGT:Gama glutamyl transferase, INR:International normalized ratio

Table 3. Neutrophil lymphocyte ratio according to groups

	NLR	p
Severity grades of PSS 0(n:0) 1(n:71) 2(n:7) 3(n:4) 4(n:2)	 5.59±4.69 4.85±2.26 15.12±9.74 20.67±1.52	 *<0.001
Outcome Discharged (n:78) Acute liver failure (n:6)	 5.72±4.64 17.77±9.44	 *0.026
Liver function tests Normal (n:69) İmpaired(n:15)	 5.66±4.71 10.82±8.77	 *0.042

*p<0.05

DISCUSSION

The main reason for mushroom poisoning in Turkey, especially in rural areas, is the widespread eating habits of mushrooms picked up from the forests or grasslands. Mushrooms may cause toxic hepatitis like medicines and chemical substances. Toxic hepatitis is a pathological condition that frequently leads to liver dysfunction. Acute hepatitis represents 90% of toxic liver diseases and may be in the form of morphological changes or as fulminant hepatitis[10].

It has been reported that hepatocyte damage, necrosis, lobular and portal inflammation, bubble degeneration in hepatocytes and apoptotic bodies are observed together with focal necrosis foci, and inflammatory cells in the portal area, including neutrophil and eosinophils in case of liver failure resulting from toxic causes[11-13].

In addition to the inhibitory effects on DNA-dependent RNA polymerase-II, Amatoxins have been shown to inhibit rapid protein synthesis and to affect transcription, particularly by causing fragmentation in all nuclear components of hepatic and renal cells, even at low doses, while other mechanisms may be the production of reactive oxygen species, which cause oxidative stress-related injury[15]. Although the underlying mechanism is not yet known, amatoxins have been shown to be able to act synergistically with tumor necrosis factor (TNF) to induce apoptosis[14]. In another study, it was shown that high NLR was associated with an increase in TNF alpha and various interleukins (IL-6, IL -7, IL-8, IL-12, IL-17)[16,17].

NLR is an easy, cheap, non-invasive and widely used laboratory indicator of systemic inflammation[18]. NLR is a combination of two independent inflammatory markers. While the high number of neutrophils reflects inflammation, low lymphocyte counts reflect an inadequate health status[19]. It has been shown that NLR is superior to white blood cell count in predicting adverse events in various inflammatory and surgical conditions[20].

In a healthy, adult, non-geriatric population, normal NLR values range from 0.78 to 3.53[21]. Likewise, it has been used to predict the prognosis in various types of cancer, major cardiac events, and pesticide intoxication. Sun et al[22] reported that pre-treatment NLR was an independent prognostic factor in patients with nasopharyngeal carcinoma and that an NLR of ≥ 2.7 was associated with a short survival time. Although the mechanism of this condition in patients with a tumor has not been clearly defined yet, since neutrophils are inflammatory cells, it has been suggested that they may support tumor angiogenesis at any step of tumor development by producing cytokines and secreting angiogenic factors such as the vascular endothelial growth factor[23,24].

In a retrospective study of pesticide intoxications, NLR was shown to be significantly higher in patients who required mechanical ventilation (16.6±10.1) and those who had a fatal course (11.8±3.6)(p <0.002). These patients had cut-off values of 8.4 and 7.1, respectively[25] .Similarly, another study reported that when free radicals production in pesticide intoxication exceeds the patient's antioxidant capacity, leukocytosis and neutrophilia can be seen in the complete blood count at the early stage[26].

In their study conducted on patients having hemoperfusion need or not due to mushroom poisoning, Koylu et al.[27] found that NLR was significantly higher in the group receiving hemoperfusion (9.91±111.72) (p = 0.002).

In our study, NLR was significantly higher in patients with acute hepatic failure compared to patients discharged with complete healing (17.77±9.44 vs. 5.72±4.64, p = 0.007). We also found the cut-off value as 9.65. Consistently, NLR was significantly higher in patients with impaired liver function tests (p = 0.033).

Our study was performed retrospectively and also the mushroom type was not identified. The number of patients presenting with acute hepatic failure was significantly low compared to the total patient number. And furthermore, we could not obtain information about the survival of the patients who

were referred to centers with transplantation capability due to liver transplantation need.

Considering all these mechanisms, we believe that NLR, an easy, cheap and readily available laboratory test, may be useful in the early prediction of liver failure or transplantation need, which may develop as a result of mushroom poisoning.

Yazar Katkıları: Çalışma konsepti/Tasarımı: BGA, HA; Veri toplama: ÖP, RDO; Veri analizi ve yorumlama: BGA, GK; Yazı taslağı: BGA; İçeriğin eleştirel incelenmesi: HA, RDO, BA; Son onay ve sorumluluk: BGA, GK, ÖP, BA, RDO, HA; Teknik ve malzeme desteği: ÖP, BA; Süpervizyon: HA, GK; Fon sağlama (mevcut ise): yok.
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