ORIGINAL ARTICLE

Prognostic effect of Nesfatin-1 on the diagnosis and staging of acute cholecystitis

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ABSTRACT

BACKGROUND: Gallbladder diseases are an important health concern affecting approximately 20% of the population in developed countries. Acute cholecystitis is the most common complication of gallstones. The aim of our study is to determine the use of Nesfatin-1, which is an easily applicable and fast resulting and is thought to have an association with inflammatory events, in the diagnosis and grading of acute cholecystitis.

METHODS: Patients who admitted and were hospitalized and treated with the acute cholecystitis diagnosis in İstanbul Training and Research Hospital between July 1, 2020, and December 1, 2020, were included in the study. The patients were divided in threemain groups as mild, moderate, and severe according to Tokyo Guidelines 2018 based on their routine blood tests and imaging results. All patients who are included in the study were tested for their blood leukocyte, neutrophil, lymphocyte, Nesfatin-1 levels, and neutrophil/lymphocyte ratios within the first 24 h of their hospitalization.

RESULTS: With at least 15 patients in each group, 61 volunteers in total were included in the study as healthy volunteers, mild, moderate, and severe cholecystitis. The average age of the participants were 58.11±19.76 years. About 47.54% of the participants were female and 52.46% weremale. In the study, Nesfatin-1 levels in the patient groups were found to be lower than the control group. In the subgroup analyzes, Nesfatin-1 values in the middle patient group were found to be significantly lower than the control group; however, there was no statistically significant relationship between the severity of the disease and Nesfatin-1.

CONCLUSION: Nesfatin-1 may guide the clinician for the diagnosis of the disease; however, no significant relationship was found between Nesfatin-1 and the severity or stage of the disease.

Keywords: Acute cholecystitis; gallbladder stone; Nesfatin-1.

INTRODUCTION

Gallbladder diseases pose an important health problem, which affects approximately 20% of the population in developed countries.[1] Many patients with gallstones are asymptomatic; however, 10% of patients will develop symptoms in 5 years, and 20% will develop symptoms in 20 years after gallstone diagnosis.[2] Acute cholecystitis is the most common complication of gallstone disease and develops typically in patients who have a history of symptomatic gallstones. Less frequently, acute cholecystitis can also develop without gallstones.[3]

Tokyo Guideline 2018 is one of many other guidelines employed in the diagnosis and staging of acute cholecystitis. According to this guideline, the staging of the severity of acute cholecystitis is done with physical examination, laboratory tests, imaging methods, disease duration, and organ failure.[4] Nesfatin-1 is a polypeptide that has 82 amino acids derived from the precursor protein nucleobindin 2. The middle part of Nesfatin-1 that has 29 amino acids (30–59) is defined as the active nucleus of Nesfatin-1 and is also known to exhibit an anorexigenic effect.[5] Nesfatin-1 was first detected in the brain nuclei, which regulates food intakes, such as the para-

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ventricular nucleus and arcuate nucleus.[6] It was shown in the previous studies conducted on rats and mice that Nesfatin-1 is found in many areas of the brain. Nesfatin-1 was detected to exist in the periphery, gastric mucosa, adipose tissue, pancreatic beta-cells, testicles, ovaries, uterus, epididymis, and cardiomyocytes.[7] The stomach is defined as the main source of peripheral Nesphatin-1, and the Nesfatin-1 levels, here, are higher than in other peripheral organs or the brain.[8]

Studies have been published in recent years reporting that Nesfatin-1 is a protective peptide in cases of heart, kidney, and intestinal ischemia.[9,10] There are many studies in the literature reporting that Nesfatin-1 has pro-inflammatory or anti-inflammatory functions.[11–14] However, although there are publications, which show that there is a relation between Nesfatin-1 and inflammatory events, there is no study showing its relations with acute cholecystitis, or examining its use in the diagnosis and staging of acute cholecystitis.

The purpose of our study was to evaluate the availability of Nesfatin-1, which is a marker easy to apply, providing quick results, and considered to be related to inflammatory events, in the diagnosis and staging of acute cholecystitis.

MATERIALS AND METHODS

Patients admitting to İstanbul Training and Research Hospital with acute cholecystitis between July 1, 2020, and December 1, 2020, and hospitalized in General Surgery Ward were included in the present study. Patients were divided into three groups as mild, moderate, and severe cholecystitis according to Tokyo Guidelines 2018 classification based on routine blood tests (hemogram, AST, ALT, glucose, urea, creatinine, and bilirubin), and imaging methods (abdominal ultrasonography or abdominal MRI). Control patients were selected from among healthy volunteers who did not have any known diseases and who did not have gallstones.

The inclusion criteria of patients were determined as having acute cholecystitis due to the gallbladder and being between the ages of 18 and 80. Exclusion criteria were cholecystitis developing without gallstones, cholecystitis associated with pancreatitis, diabetes, having any cancer history, pregnancy, being under 18 years of age, being over 80 years of age, having a history of immunodeficiency, and refusing to participate in the study.

Patients were excluded from the study and new patients who met the inclusion criteria were included in the study if they stated that they wanted to be excluded from the study if they were newly diagnosed with diabetes or cancer.

A 10 ml tube of blood was taken from the patients who met the inclusion criteria for the study during their hospitalization. The tubes were centrifuged at 1300xg for 10 min in 24 h and were then plasmas were separated, which were taken into Eppendorf Tubes, encoded, and stored in the storage cabinet at -80°C.

The age, gender, blood white sphere values, duration of hospitalization of the patients, and the treatment methods applied were noted at the time of hospitalization. Nesfatin values examined were added to this information later.

The study was approved by the ethics committee of university of health sciences, İstanbul Training and Research Hospital, and informed consent forms were received from the volunteers.

Measurement of Nesfatin-1 Level

After an adequate number of patients was achieved, all the blood samples, including the control group, were dissolved only once on the day of analysis. The measurement of Nesfatin-1 level was carried outusing BT-LAB brand, commercial kit with the catalog number of E3063Hu, and by working with enzyme-linked immunosorbent assaymethod.

Statistical Analysis

Statistical analyses were made with the SPSS version 17.0 Program. The normal distribution of the variables was examined with Histogram Graphs and with the Kolmogorov–Smirnov Test. Mean and standard deviation values were used when descriptive analyses were presented. Nonparametric twogroups were compared with the Mann–Whitney U Test, and more than twogroups were compared with the Kruskal–Wallis Test. Spearman Correlation Test was used in the analysis of the measurement data. When P-value was below 0.05, it was evaluated as a statistically significant result.

RESULTS

A total of 61 patients were included in the present study. The control group was determined as 15 volunteers. Patient groups were separated as mild, moderate, and severe based on Tokyo Guideline 2018. There were 15 patients in the mild cholecystitis group, 16 in the moderate cholecystitis group, and 15 in the severe cholecystitis group. The mean age of the study participants was 58.11±19.76, the female rate was 47.54%, and the male rate was 52.46%. The demographic data and the distribution of leukocyte, neutrophil, lymphocyte, and neutrophil/lymphocyte of all volunteers who participated in the study according to groups are given in Table 1 as a result of the blood count.

In this respect, significant differences were detected in terms of age, leukocyte, neutrophil, lymphocyte, and neutrophil/lymphocyte distributions between the groups ($p=0.047$, $p<0.001$, $p<0.001$, $p=0.01$, and $p<0.001$, respectively) (Table 1). According to posthoc analysis, severe group had higher leukocyte, neutrophil, and neutrophil/lymphocyte than control group ($p<0.001$, $p<0.001$, and $p<0.001$, respectively); moderate

Table 1. The blood counts (leukocyte, neutrophil, lymphocyte levels and neutrophil/ lymphocyte ratio) and demographic data distribution of the volunteers participating in the study according to the groups

	Group 1 (Control, n=15)	Group 2 (Mild cholecystitis, n=15)	Group 3 (Moderate cholecystitis, n=16)	Group 4 (Severe cholecystitis, n=15)	p-value
Age (years)	58.27±11.11	50.60±23.09	54.69±23.06	69.13±15.60	0.047[1]
Sex (n)					
Woman	7	6	9	7	0.840[2]
Man	8	9	7	8	
Leukocyte (10^9/L)	7.73±1.46	13.05±2.84	22.10±4.71	22.55±5.39	<0.001[1]
Neutrophil (10^9/L)	4.43±1.27	10.30±3.39	19.34±5.04	19.36±4.62	<0.001[1]
Lymphocyte (10^9/L)	2.48±0.87	1.53±0.88	1.47±0.68	1.64±0.84	0.010[1]
Neutrophil/Lymphocyte	2.08±1.25	11.14±10.03	17.54±12.48	17.22±17.12	<0.001[1]

[1]Kruskal Wallis Test; [2]Chi-Square Test.

group had higher leukocyte, neutrophil, and neutrophil/lymphocyte then control groups (p<0.001, p<0.001, and p<0.001, respectively); and mild groups had higher leukocyte, neutrophil, and neutrophil/lymphocyte than control group (p<0.001, p<0.001, and p<0.001, respectively). Control group had higher lymphocyte values than mild, moderate, and severe groups (p=0.019, p=0.002, and p=0.007, respectively). According to Spearman correlation test, the age, leukocyte, and neutrophil values had significant relations of the severity of disease (p=0.012, p<0.001, and p<0.001, respectively) (Table 2).

The distribution of Nesfatin-1 levels of all volunteers participating in the study according to the groups is given in Table 3. The Nesfatin-1 values of the patient group were lower compared to the control group. In the subgroup analyses, statistically significant differences were detected between the control group and the moderate group (p=0.011). However, no statistically significant data were detected in the four-group analysis (p=0.069). In addition to this according to Spearman correlation test, no statistically significant difference was found between Nesfatin-1 levels and the severity of disease. (p<0.857) (Table 2).

Table 2. Spearman correlation test for acute cholecystitis

	Acute cholecystitis	
	r	p
Age	0.366	0.012
Leukocyte	0.712	<0.001
Neutrophil	0.717	<0.001
Lymphocyte	0.024	0.872
Neutrophil/Lymphocyte	0.230	0.124
Nesfatin-1	-0.027	0.857

With the direction of these results, the ability of predicting all patients and moderately ill patients with nesfatin-1 levels were analyzed with ROC analysis and significant cutoff values were researched. When the cutoff for predicting moderate illness was taken, as <5.54 93.75% sensitivity, 66.67% specify, 75% PPD, and 90.91% NPD were found. No significant cutoff has been found for predicting all patients (Table 4). The graphic for the ROC analysis of control and moderate groups for Nesfatin-1 levels is shown in Figure 1.

DISCUSSION

The present study of ours is the first one in the literature evaluating the relation between acute cholecystitis and Nesfatin. Nesfatin-1 values were found to be lower in the patient groups compared to the control group; and it is considered that it may be a significant marker in diagnosing acute cholecystitis. No significant differences were detected in the subgroup analyses between the groups; and adequate evidence

Table 3. Nesfatin-1 distribution of the volunteers participating in the study according to the groups

	Group 1 (Control, n=15)	Group 2 (Mild cholecystitis, n=15)	Group 3 (Moderate cholecystitis, n=16)	Group 4 (Severe cholecystitis, n=15)	p-value
Nesfatin-1	16.42±21.38	9.57±9.69	4.89±0.68	12.50±20.54	0.069

[1]Kruskal Wallis Test.

Table 4. The ROC analysis for control–all patients and control–moderate illness groups for nesfatin-1 levels

Nesfatin-1	Area	Standard error	p	95% Confidence interval	
				Lower limit	Upper limit
All patients-Control	0.661	0.086	0.063	0.492	0.830
Moderate-Control	0.767	0.092	0.011	0.586	0.947

ROC: Receiver Operating Characteristic.

could not be obtained to show that the level of Nesfatin-1 played a role in determining the severity of the disease.

Many markers were examined in the literature to determine the prognosis of acute cholecystitis. The most commonly used one was C-reactive protein (CRP). A retrospective study[15] conducted by Gurbulak et al. showed that CRP can be considered to be a powerful predictor of different degrees of the disease. A prospective study conducted with 556 cases[16] conducted by Bouassida et al. showed that CRP is the best biomarker for severe acute cholecystitis and converting to open surgery.

There are also publications that examine plasma procalcitonin levels to determine the prognosis of acute cholecystitis. It was shown in the study of Sakalar et al.[17] with 95 patients and in the study of Yuzbasioglu et al.[18] with 200 patients, it was shown that procalcitonin levels determined the severity of acute cholecystitis effectively and significantly.

Patients were divided into two groups as severe and non-severe acute cholecystitis according to Tokyo Guidelines 2018

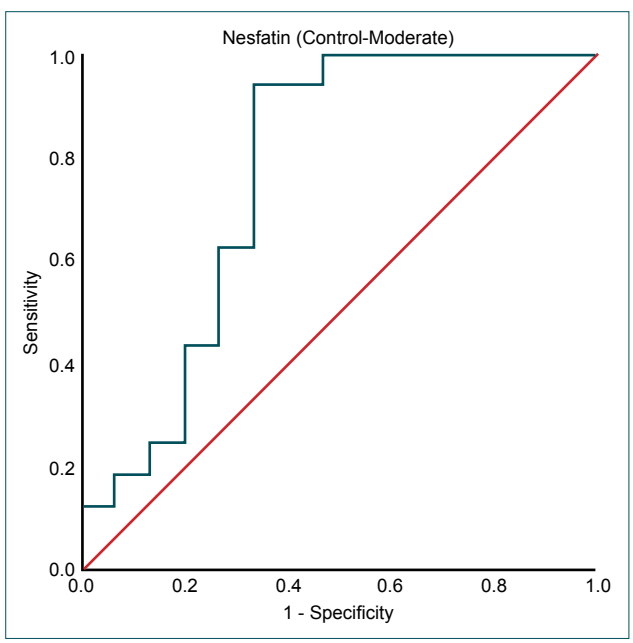

Figure 1. The graphic for the ROC analysis of control and moderate groups for Nesfatin-1 levels.

in a retrospective study[19] conducted by Lee et al. that included 379 patients admitting to the emergency department diagnosed with acute cholecystitis. The WBC, CRP, and delta neutrophil index levels were evaluated during their admissions. The delta neutrophil index was shown to be among the early markers of severe cholecystitis with a significantly higher predictive value than WBC and CRP levels to detect severe cholecystitis.

There are studies reporting that the neutrophil lymphocytes ratiocan also be used as a predictive marker. In a retrospective study[20] conducted with 632 patients who underwent cholecystectomy due to acute cholecystitis, patients were divided into two groups as simple and severe cholecystitis, and NLO>3 was shown to be associated with severe cholecystitis. In another study[21] conducted by Sato et al., 262 patients diagnosed with acute cholecystitis were divided into three groups according to Tokyo 2018 Guideline; and NLO and CRP/Alb values were measured. As a result, it was shown that these values can show the degree of severity independently in acute cholecystitis patients and can be used as a predictor.

There are also novel markers in the literature examined to determine the severity and prognosis of acute cholecystitis. In a study conducted with 60 patients diagnosed with acute cholecystitis,[22] patients were divided into two groups with and without gallbladder perforation; and the serum pentraxin 3 and pro-Adrenomedullin levels were measured. These two markers, which are among the new acute phase reactants, were found to be significantly higher in patients with gallbladder perforations. In a study conducted by Park et al.,[23] visfatin levels were examined in patients who underwent cholecystectomy due to acute and chronic cholecystitis, and it was found that visfatin is a pro-inflammatory marker reflecting the severity level of cholecystitis increasing in a short-time during acute cholecystitis.

In our study, significant differences were detected in WBC, neutrophil, lymphocyte, and NLO values in the examination in the control and patient groups. WBC, neutrophil, and NLO values were significantly higher and lymphocyte levels were low in the patient groups compared to the control group. It was also observed that WBC and neutrophil val-

ues increased at significant levels as cholecystitis severity increased. It can be argued that these findings are compatible with the literature data. These parameters can help the clinician in determining the diagnosis and severity of acute cholecystitis.

Nesfatin-1 is a satiety peptide consisting of 82 amino acids and were first discovered in 2006. It is found in many nuclei of the hypothalamus, including the paraventricular nucleus. In the previous studies, the effects of Nesfatin-1 on neuroendocrine regulation, autonomic control of visceral functions, development and differentiation of adipose tissue, inflammation, thermoregulation, pancreatic insulin secretion, homeostasis of glucose in CC, sleep, attention, anxiety, and stress. It was also reported that it regulates gastric ejaculation, gastric acid secretion, gastric motility, and reproductive functions.[9]

Studies have been published recently reporting that Nesfatin-1 is a protective peptide in cardiac, renal, intestinal ischemia cases.[9] In a study conducted by Tatar et al.,[9] rats with induced mesenteric ischemia had significantly higher Nesfatin-1 values than the control group. In another study, patients who had acute myocardial infarction and patients who had angina pectoris were compared with the control group; and it was found that plasma Nesfatein-1 levels were lower at statistically significant levels in the acute myocardial infarction group. It was also reported that lower concentrations of Nesfatin-1 might play very important roles in the development of acute myocardial infarction.[10]

A study conducted by Özsavci et al.[11] based on the subarachnoid hemorrhage model reported that Nesfatin-1 has an anti-apoptotic and anti-inflammatory structure in rats. It was shown that there are higher improvement levels in neurological disorders and oxidative brain damage in the group administered with Nesfatin-1, and the plasma pro-inflammatory cytokines due to subarachnoid hemorrhage were suppressed. Another study conducted by Buzcu et al.[12] based on the acute pancreatitis model showed that Nesfatin-1 has anti-inflammatory effects on acute pancreatitis, basically affecting melanocortin receptors. In another study conducted by Karatay et al.,[13] the performance of Nesfatin-1 in separating celiac disease and diarrhea-weighted irritable bowel syndrome, which is an inflammatory disease, was investigated, and significantly higher Nesfatin-1 levels were detected in cases with Celiac Disease.

On the other hand, a study conducted by Leivo-Korpela et al.[14] to examine emphysema-type COPD patients suggested that Nesfatin-1 may be a novel factor associated with systemic inflammation in COPD.

As it is seen, although there are publications in the literature reporting the relations of Nesfatin-1 with inflammatory events, there are no studies examining the relations between Nesfatin-1 and acute cholecystitis. Although many markers were investigated for the prognosis and staging of acute cholecystitis, there are no studies reported in the literature examining the availability of Nesfatin-1 in the severity and staging of acute cholecystitis.

In our study, Nesfatin-1 values were found to be lower in patient groups compared to the control group, which can be considered valuable for the diagnosis of acute cholecystitis. Nesfatin-1 values lower than normal may guide the clinician in the diagnosis of acute cholecystitis. Subgroup analyses showed that there were significant differences between the control group and the moderate cholecystitis group. However, no statistically significant relations were detected between acute cholecystitis severity and nesfatin-1 in 4-group analyses. According to these data, Nesfatin-1 has no significant role in determining the severity or stage of acute cholecystitis and in predicting its prognosis.

In our study, low Nesfatin-1 levels were detected gradually when compared to the control group in mild and moderate cholecystitis. In severe cholecystitis, it was noted that Nesfatin-1 levels started to increase, but remained low when compared to the control group. If we are to make conclusions based on the available data and previous studies, the levels of Nesfatin-1, which has significant anti-inflammatory features, decrease after the onset of any inflammation in our body, and pro-inflammatory markers increase. Inflammation progresses with the decrease in Nesfatin-1 levels. In this way, body homeostasis is disrupted, Nesfatin-1 levels increase, and organ ischemia or failure occurs. Based on another point of view, the body starts to increase Nesfatin-1 levels to balance severe inflammation. It progresses at high levels in chronic inflammatory events. In a different interpretation, severe inflammation and organ failure may be developing with low Nesfatin-1 levels. Furthermore, Nesfatin-1 levels may vary in the manifestation of severe inflammation and organ failure, which is specific to a tissue. More studies are needed to understand this process better.

In our study, blood samples were collected from the patients 24 h following their hospitalization. Examining earlier and longer-term Nesfatin-1 levels, especially for patients with severe cholecystitis, may contribute to our study and literature. The detection of significant differences between the control group and the moderate cholecystitis group is encouraging for future studies. The limitation of our study might be considered as the small number of patients. A study with multiple centers over a wider time period may contribute to the literature.

Conclusion

As a conclusion, Nesfatin-1 can guide the clinician for the diagnosis of the disease; however, no significant relations were detected between Nesfatin-1 and the severity or stage of the disease. Our findings are promising for future studies.

Ethics Committee Approval: This study was approved by the İstanbul Training and Research Hospital Clinical Research Ethics Committee (Date: 12.06.2020, Decision No: 2398).

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ORİJİNAL ÇALIŞMA - *ÖZ*

Akut kolesistit tanı ve evrelendirmesinde Nesfatin-1'in prognostik etkisi

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AMAÇ: Safra kesesi hastalıkları gelişmiş ülkelerde nüfusun yaklaşık %20'sini etkileyen önemli bir sağlık sorunudur. Akut kolesistit, safra taşı hastalığının en yaygın komplikasyonudur ve tipik olarak semptomatik safra taşı öyküsü olan hastalarda gelişir. Çalışmamızın amacı kolay uygulanabilen, hızlı sonuç veren ve enflamatuvar olaylarla ilişkisi olduğu düşünülen bir belirteç olan nesfatin-1'in; akut kolesistitin tanısında ve evrelendirilmesinde kullanılabilirliğini değerlendirmektir.

GEREÇ VE YÖNTEM: Çalışmaya İstanbul Eğitim ve Araştırma Hastanesi'ne 01.07.2020 ile 01.12.2020 tarihleri arasında akut kolesistit ile başvuran ve hastaneye yatırılarak tedavi gören hastalar alındı. Hastalar rutin kan incelemeleri ve görüntüleme yöntemleri baz alınarak yapılan Tokyo Guidelines 2018 sınıflamasına göre hafif, orta ve ağır şiddette kolesistit olarak üç ana gruba ayrıldı. Çalışmaya dahil edilen tüm hastalardan yatış sonrası 24 saat içinde kan alınarak kanda lökosit, nötrofil, lenfosit, nötrofil/lenfosit ve nesfatin-1 seviyeleri ölçüldü.

BULGULAR: Her grupta en az 15 kişi olmak üzere sağlıklı gönüllüler, hafif kolesisit, orta kolesistit ve ağır kolesistit olarak toplam 61 gönüllü çalışmaya dahil edildi. Çalışmaya katılanların ortalama yaşı 58.11±19.76 yıl, kadın oranı yüzde 47.54 ve erkek oranı 52.46 idi. Çalışmada hasta gruplarındaki nesfatin-1 değerleri kontrol grubuna göre düşük saptanmıştır. Alt grup analizlerinde orta hasta grubundaki nesfatin-1 değerleri kontrol grubuna göre anlamlı olarak daha düşük bulunmuştur; ancak hastalığın şiddeti ile nesfatin-1 arasında anlamlı istatistiksel bir ilişki görülmemiştir.

TARTIŞMA: Sonuç olarak nesfatin-1 hastalığın tanısı için klinisyeni yönlendirebilir; ancak nesfatin-1 ile hastalığın şiddeti veya evresi arasında anlamlı ilişki bulunmamıştır. Bulgularımız gelecekteki çalışmalar için umut vericidir.

Anahtar sözcükler: Akut kolesistit; nesfatin-1; safra kesesi taşı.

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