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Relationship between plasma osmolality and neutrophil/lymphocyte ratio in heart failure with reduced ejection fraction

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Abstract

Aim: Heart failure (HF), a progressive disease, is accompanied by chronic inflammation and changes in osmolality. The neutrophil-to-lymphocyte ratio (NLR) demonstrates a systemic inflammatory response in most diseases; however, the relationship between plasma osmolality and the systemic inflammatory response in HF patients is not yet clear. Therefore, we aimed to investigate the possible associations of NLR with plasma osmolality levels in patients with HF.

Materials and Methods: The present study included 189 consecutive patients with chronic HF with an ejection fraction (EF) of <40%. They were classified into four groups based on admission plasma osmolality quartiles: hypo-osmolar (first quartile), normo-hypo-osmolar (second quartile), normo-hyperosmolar (third quartile), and hyperosmolar (fourth quartile). We evaluated the relationship between NLR, plasma osmolality, type-B natriuretic peptide (BNP), and the New York Heart Association (NYHA) functional class.

Results: The hyperosmolar group had an increased NLR ($p = 0.007$). The presence of NYHA class 3–4 functional capacity, high-sensitivity C-reactive protein, and high osmolality were independent predictors of increased NLR. In correlation analysis, osmolality was significantly positively correlated with NLR ($r = 0.201$, $p = 0.011$).

Conclusion: Higher NLR values may be associated with increased plasma osmolality, which may indicate an increased inflammatory status in the HF phenomenon.

Introduction

Heart failure (HF) disease has a poor prognosis and high cardiovascular mortality rates [1]. Because of the growing elderly population and the increasing frequency of coronary artery disease (CAD), the prevalence of HF has increased. Despite the development of treatment approaches, mortality rates are still high [1]. Because of its poor prognosis, the identification of prognostic factors is critical. The evaluation of plasma osmolality is significant for the detection of the water and electrolyte poise of the human body in HF [2]. Blood urea nitrogen (BUN), plasma sodium, and glucose are essential elements that determine plasma osmolality. In previous studies, plasma osmolality was a valuable indicator of in-hospital mortality in HF patients. One study showed that hypoosmolality was linked with higher mortality in HF [3]. However, hypernatremia and hyperosmolality are related to morbidity and mortality, particularly in elderly hospitalized patients [4]. Although many prognostic factors have been associated with HF in previous studies, only a few of these factors are related to inflammation [5-6]. Chronic inflammation is general in HF and plays a key role in both the initiation and progression of HF [6].

White blood cells (WBC) and their subtypes are major inflammatory parameters in cardiac diseases. An elevated neutrophil count reflects acute inflammation, while lymphopenia is related to physiological stress. The neutrophil-to-lymphocyte ratio (NLR), a composite index and significant indicator of systemic inflammation, has served as a novel prognostic parameter for many cardiovascular diseases [7,8]. An elevated NLR is an established hematologic index of oxidative stress correlated with poor cardiovascular outcomes. Many studies have shown its prognostic importance for both cardiac and noncardiac diseases [9,10]. Previous studies have shown that neutrophilia and lymphopenia are linked to increased mortality and poor outcomes in acute coronary syndromes and HF [8,9,10].

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Elevated NLR can indicate the severity of CAD [11]. Lymphopenia indicates chronic inflammation and occurs secondary to the release of stress-related cortisol. An inverse relationship has been observed between lymphocyte counts and cardiac outcomes [12,13].

Animal data have shown that hyperosmolality triggers the release of proinflammatory cytokines from macrophages [14]. In vitro studies have demonstrated an increase in inflammatory parameters upon hyperosmolality [14,15]. The relationship between systemic inflammatory parameters and response to osmolality levels and osmotic stimulus has been investigated by in vitro, human corneal, bronchial, and intestinal cell studies [16,17,18]. These studies have shown an increased inflammatory response to higher osmolality levels with increased proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Contrary to these results, a recent study by Sailer et al. indicated that circulating inflammatory markers, including interleukins and TNF-α, decreased with osmotic stimulus in healthy volunteers [19].

However, most studies were conducted with human cell cultures or laboratory animals. Human in vivo studies investigating the relationship between plasma osmolality levels and inflammatory systems are scarce. Accordingly, there is insufficient data on the relationship between osmolality and inflammation in a patient population. However, one study observed an increased inflammatory response to hyperosmolality in systemic inflammatory response syndrome [20].

No available studies have investigated the relationship between osmolality and NLR in HF patients. Therefore, we aimed to determine possible associations of systemic inflammation depicted by NLR with the osmolality of body fluids, N-terminal prohormone B-type natriuretic peptide (NT-proBNP) levels, and clinical parameters.

Materials and Methods

Study population

A total sample of 189 consecutive patients with chronic HF with reduced ejection fraction (HFrEF $<$ 40%) from two HF centers was prospectively collected from the outpatient clinic when the patients had their cardiac status checked at our hospitals between November 2017 and January 2020. We included some New York Heart Association (NYHA) class IV chronic HF patients who were not hospitalized for acute decompensated HF as well as other NYHA functional capacity classes. All participants signed written informed consent forms before enrolling, according to the Declaration of Helsinki. The local ethics committee approved this observational study (Decision no: 2017/116/12/04).

The exclusion criteria were acute decompensated HF, acute coronary syndromes within a three-month period, chronic renal disease (estimated glomerular filtration rate [eGFR] $<$ 60 mL/min/1.73 m2), patients younger than 18 years, serum NT-proBNP concentration $<$ 125 pg/mL, chronic pulmonary disease, chronic liver disease, infectious disease, chronic inflammatory or rheumatic diseases, pregnant women, malignancy, thyroid disorder, all conditions that may impair plasma osmolality levels, those who had taken any anti-inflammatory drugs within the last month, and those with missing laboratory data within the first eight hours of hospital admission.

Study design

The patients' demographic and current medication data were obtained upon admission. Body mass index (BMI) was calculated from the ratio of body weight (kg) to meters squared (m^2). Blood samples were obtained within 8 hours of admission, with minimal tourniquet application, to determine hemogram and routine biochemistry between 08.00 a.m. and 11.00 a.m. after 10 hours of fasting. Complete blood counts covered total WBC, neutrophil and lymphocyte counts, and routine biochemical analyses, such as BUN, creatinine, sodium, blood glucose, high-sensitivity C-reactive protein (hs-CRP), and serum NT-proBNP were determined. The NLR was obtained by dividing the neutrophil count by the lymphocyte count from the same blood sample collected at hospital admission. The neutrophil and lymphocyte counts had inter-assay coefficients of variation (CV) of 2.5% and 3%, respectively. A routine electrocardiogram was obtained upon admission to determine the rhythm.

Diabetes mellitus (DM) was detected by a fasting plasma glucose $>$126 mg/dL or the usage of any antidiabetic drug. Systolic blood pressure $\geq$140 mm Hg and/or diastolic pressure $\geq$90 mm Hg during office measurements or the use of any antihypertensive medication determined a diagnosis of hypertension. Hyperlipidemia was represented by total cholesterol $\geq$ 200 mg/dl, low-density lipoprotein cholesterol (LDL-C) $\geq$ 130 mg/dl, triglyceride (TG) $\geq$ 150 mg/dl, and high-density lipoprotein cholesterol (HDL-C) $\leq$ 40 mg/dl, as described earlier [21]. Based on their functional capacity classification, which is accepted as a simple method in terms of HF symptoms, the patients were classified into four functional capacities [22]. All the patients underwent a detailed transthoracic echocardiographic examination (Vivid S5; GE Healthcare, Vingmed Ultrasound AS, Horten, Norway) at the left-side decubitus position by an experienced cardiologist blinded to the study population. The biplane Simpson's method for left ventricular ejection fraction (LVEF) measurements was performed using the techniques recommended by the American Society of Echocardiography [23].

Serum NT-proBNP concentrations were measured using a proBNP sandwich immunoassay (Elecsys 2010; Roche Diagnostics). According to the manufacturer's instructions, these ranged between 5.1 and 35.000 pg/mL. The inter-assay and intra-assay CV of NT-proBNP in the low and high ranges were reported as 8.8%–11.6% and 9.9%–12.2%, respectively. The ELISA kit used for the hs-CRP (DRG International Inc., NJ, USA) had inter-assay and intra-assay CVs of $<$4.1% and $<$7.5%, respectively.

Grouping

The study population was categorized into four groups based on admission plasma osmolality quartiles: hypo-osmolar (first quartile), normo-hypo-osmolar (second quartile), normo-hyperosmolar (third quartile), and hyper-osmolar (fourth quartile) (Table 1). We calculated plasma

osmolality (milliosmoles per kilogram) using the formula (2 × Na) + (BUN/2.8) + (Glucose/18), which is known as the Worthley equation [24]. This difference is called a serum/plasma osmolal gap, which under physiological conditions is <10 mOsm/kg. However, in many pathological conditions, including HF, this osmolal gap is increased. Although there is strong evidence that the osmolal gap is increased in lactic acidosis, ketoacidosis, severe chronic kidney disease, and as a result of intravenous mannitol infusion, the validation of this simple equation is considered good [25]. However, the present study did not include patients with the above conditions; therefore, values between 275 and 295 mOsm/kg were accepted as normal [26]. However, we used only the osmolality quartiles of the specific study population.

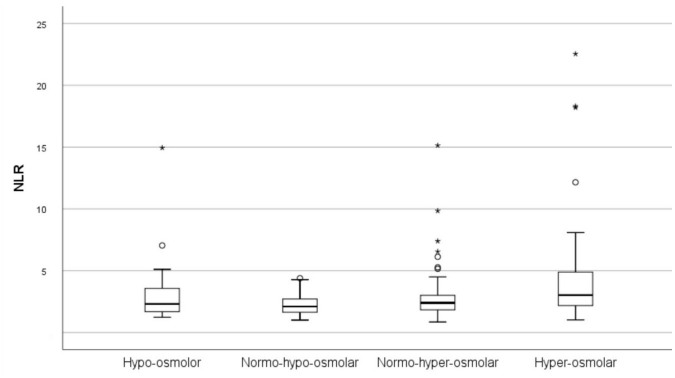

Figure 1. Relationship between neutrophil-to-lymphocyte ratio (NLR) and plasma osmolality.

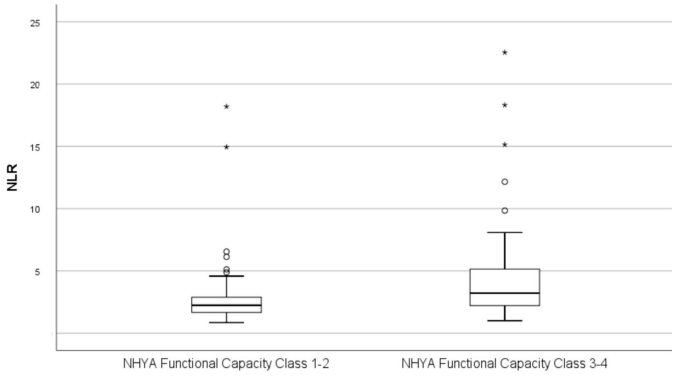

Figure 2. Relationship between neutrophil-to-lymphocyte ratio (NLR) and NYHA functional capacity classification.

Statistical analysis

All statistical tests were conducted using the Statistical Package for the Social Sciences (IBM SPSS Inc., Chicago, IL, USA), version 20 software for Windows. The Kolmogorov–Smirnov test was used to show the normality of the data. The continuous variables were expressed as mean ± standard deviation, and the categorical variables were presented as numbers (i.e., percentages). Analysis of variance (ANOVA) was used to compare the parametric variables of the four patient groups, and the Kruskal–Wallis test was used to compare the non-parametric variables. For cases with significant deviations, as determined by ANOVA, post hoc analyses were performed using Tukey or Tamhane's tests, depending on the homogeneity of the variances. Similarly, Dunn's test was used for non-parametric pairwise multiple comparisons following the Kruskal–Wallis test.

The chi-squared test was used for intergroup analysis. For pairwise comparisons of the categorical variables, a p-value of 0.017, adjusted by the Bonferroni method, was used. Correlation coefficients were calculated to determine whether there were correlations among the osmolality and other continuous variables by Pearson and Spearman correlation analyses. The cutoff level for increased NLR was determined to be 3.41 because the cutoff point for the fourth NLR quartile in the study population was 3.41. For the comparison of the four subgroups, variables with a p-value of <0.2 were used in univariate and multivariate logistic regression models to quantify the variables related to increased NLR. The results are presented as odds ratios with 95% confidence intervals (CIs).

Consecutive patients were recruited until 50 individuals were reached in the hyperosmolar group which included fewer participants than the other groups. Therefore, this number was exceeded for extra confidence. The power of the study was 82.6%, with a reliability of 95%. A p-value of <0.05 was accepted as statistically significant.

Results

The demographic data and baseline clinical characteristics, medications, and laboratory results of the study population are presented in Table 1. No significant differences were found between the four subgroups in terms of age, sex, smoking status, hypertension, DM, CAD, HF duration, resting heart rate, systolic and diastolic blood pressure, atrial fibrillation, and LVEF. The mean age of the total sample was 68 ± 10.7 years. Of the total population of 189, 138 were male, and 51 were female. The mean EF was 30 ± 4%. The median concentration of NT-proBNP was 2040 pg /mL.

The mean plasma osmolality values were classified into quartiles: first quartile 282 ± 4.5 mOsm/kg, second quartile 289 ± 0.9 mOsm/kg, third quartile 292.2 ± 1.3 mOsm/kg, and fourth quartile 299.7 ± 3.7 mOsm/kg. The first quartile was also identified as the hypo-osmolar group (n = 53), the second and third quartiles represented normo-osmolar groups (n = 86), and the fourth quartile represented the hyperosmolar group (n = 50) (Table 1). The EF and NT-proBNP levels were not significantly different between the subgroups. The presence of NYHA Class 3–4, fasting plasma glucose, BUN, creatinine, sodium, LDL cholesterol, TG, lymphocytes, and NLR were found to be significantly different among the four osmolality subgroups. The fourth quartile group had increased NLR values with a higher NYHA functional capacity than the other quartiles (Table 1 and Figure 1). No difference was seen in terms of medications among the groups, such as antiplatelet agents, anticoagulant agents, beta-blockers, renin-angiotensin system blockers, digoxin, and diuretics (all p-values > 0.05) (Table 1).

Table 1. Baseline characteristics, laboratory findings and medications of study population.

Variables	Hypo-osmolar (n=53)	Normo-osmolar		Hyper-osmolar (n=50)	P value
		Normo-hypo-osmolar (n=32)	Normo-hyper-osmolar (n=54)		
Age (years)	65.3±12.6	65.7±11.2	69.8±9.5	69.5±8.8	0.065
Male (%)	39(73.5)	24(75)	39(72.2)	36(72)	0.989
Hypertension n (%)	27(50.9)	22(68.7)	36(66.6)	30(60)	0.283
Hyperlipidemia n (%)	14(26.4)	8(25)	17(31.4)	11(22)	0.031
Diabetes mellitus n (%)	14(26.4)	11(34.3)	17(31.4)	22(44)	0.290
CAD n (%)	34(64.1)	23(71.8)	37(68.5)	36(72)	0.822
Disease duration (months)	5.75(0-18)	4.67(0-20)	4.77(0-20)	6.61(0-18)	0.416
Resting heart rate (bpm)	84.7(56-126)	80(48-118)	81(51-140)	86.5(56-168)	0.439
Systolic BP (mm Hg)	118.8±13.3	115.6±17	117±11.5	118±16.8	0.743
Diastolic BP (mm Hg)	62.2±6.4	60.6±7.2	61±7.3	63.8±8.5	0.160
NYHA class 3–4 n (%)	13 (24.5)	10 (31.2)	20 (37)	21(42)	0.025^{c*}
AF n (%)	24(45.2)	13(40.6)	20(37)	24(48)	0.687
LVEF (%)	30.6±4.1	30.5±4.3	30.5±4.8	29.8±4.5	0.763
Osmolality (mOsm/kg)	282±4.5	289±0.9	292.2±1.3	299.7±3.7	$<0.001^{a,b,c,d,e,f*}$
Fasting glucose (mg/dL)	102(57-194)	119(81-215)	115(70-307)	130(51-325)	$0.008^{a,c*}$
BUN (mg/dL)	17.1(7-44)	19.5(11-34.5)	22.5(11.7-52)	29.7±14.9	$<0.001^{b,c,e*}$
Creatinine (mg/dL)	1.03±0.2	1.11±0.3	1.20±0.5	1.36±0.4	$<0.001^{b,c,d*}$
Uric acid (mg/dL)	7.14±2.3	6.8±2.2	7.3±2.2	8.1±2.4	0.076
Sodium (mEq/L)	135±2.8	137.5±1.43	138±1.95	141±3.1	$<0.001^{b,c,d*}$
Potassium (mEq/L)	4.3±0.5	4.4±0.5	4.4±0.5	4.3±0.6	0.799
hs-CRP (mg/dL)	7.14(3.5-8)	6.5(0.1-9)	7.3(0-8)	9.5(3.9-9)	0.638
NT-proBNP (pg/mL)	3329(136-19600)	2503(153-8870)	4735(147-26700)	6642(170-35000)	0.107
TC (mg/dL)	185±57.5	173±47.1	183±52.3	161±50	0.102
HDL-C (mg/dL)	41.6±13.7	40.5±9.6	39.8±12.3	38.1±12.1	0.518
LDL-C (mg/dL)	116.6±48.4	101.3±36.3	118.2±42	96.1±37	0.023^{e*}
TG (mg/dL)	129(43-682)	154(47-368)	126(36-323)	124(43-544)	0.032
ALT (IU/L)	23(6-123)	16.4(3-39)	21.8(6-263)	40.8(5-796)	0.065
AST (IU/L)	25(12-128)	20.4(12-43)	23.3(9-245)	40.3(9-826)	0.488
WBC (10^9/L)	7.1±2.1	7.7±2.1	7.3±2.6	7.7±2.1	0.401
Neutrophils (10^9/L)	4.5(2.1-8.8)	4.6(1.9-8.9)	4.9(2.4-11.5)	5.0(2.0-11.8)	0.452
Lymphocytes (10^9/L)	1.8±0.6	2.2±0.95	1.95±0.75	1.50±0.72	$<0.001^{a,c,d*}$
NLR	2.86(1.2-15)	2.26(1-4.4)	3.04(0.85-15.1)	4.44 (1-22.5)	0.007^{e*}
Hemoglobin (g/dL)	13.2±1.8	13.3±2.2	13.1±1.8	12.3±1.7	0.126^{c*}
Hct (%)	40.5±5.0	40±5.8	40±4.8	38±4.8	0.094
MCHC (g/dL)	32.8±1.02	33.1±1.2	32.8±1.1	32.2±1.4	0.110^{e*}
Platelet (10^9/L)	235(94-619)	239(118-520)	236(116-432)	209(94-399)	0.146
Antiplatelet agents n(%)	50(94.3)	32(100)	51 (94.4)	47(94)	0.585
Anticoagulant agents n (%)	6(11.3)	8(25)	10 (18.5)	9(18)	0.440
Beta-blockers n (%)	45(84.9)	28(87.5)	47(87)	44(88)	0.853
ACEi /ARB n (%)	34(64.1)	21(65.6)	39(72.2)	32(64)	0.738
Digoxin n (%)	14(26.4)	5(15.6)	6(11.1)	9(18)	0.303
Diuretics n (%)	34(64.1)	25(78.1)	34(62.9)	40 (80)	0.253
MRA n (%)	34(66)	19 (59.3)	28(51.8)	28(56)	0.500

Abbreviations: ACEi, angiotensin- converting enzyme inhibitor; AF, atrial fibrillation; ALT, alanine transaminase ; ARB, angiotensin receptor blocker; AST, aspartate transaminase; BP, blood pressure; BUN, blood urea nitrogen; CAD, coronary artery disease; Hct, hematocrit; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein ; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MCHC, mean corpuscular hemoglobin concentration; MRA, mineralocorticoid receptor antagonist; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; NYHA, New York Heart Association; TC, total cholesterol; TG, triglyceride; WBC, white blood cell.
*If there is p<0.05 as the significance level, Pa: Hypo-osmolar vs Normo-hypo-osmolar, Pb: Hypo-osmolar vs Normo-hyper-osmolar, Pc: Hypo-osmolar vs hyper-osmolar, Pd: Normo-hypo-osmolar vs normo-hyper-osmolor, Pe: Normo-hypo-osmolar vs Hyper-osmolor, Pf: Normo-hyper-osmolor vs Hyper-osmolor.

Table 2. Correlations between plasma osmolality and other variables.

	Correlation coefficients (r)	P value
Age (years)	0.186	0.010
hs-CRP (mg/dL)	0.016	0.845
NT-proBNP (pg/mL)	0.075	0.348
TC (mg/dL)	-0.110	0.136
HDL-C (mg/dL)	-0.103	0.161
LDL-C (mg/dL)	-0.107	0.150
TG (mg/dL)	-0.025	0.736
WBC (10^9/L)	0.007	0.924
Neutrophils (10^9/L)	0.098	0.179
Lymphocytes (10^9/L)	-0.244	0.002
NLR	0.201	0.011
Hemoglobin (g/dL)	-0.163	0.025
Hct (%)	-0.139	0.056
MCHC (g/dL)	-0.166	0.022
Platelet (10^9/L)	-0.107	0.142

Abbreviations: Hct, hematocrit; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein ; LDL-C, low-density lipoprotein cholesterol; MCHC, mean corpuscular hemoglobin concentration; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; TC, total cholesterol; TG, triglyceride; WBC, white blood cell.

Table 3. Correlations between NT-proBNP and other variables.

	r value	P value
LVEF (%)	-0.281	<0.001
hs-CRP (mg/dL)	0.435	<0.001
Osmolality (mOsm/kg)	0.130	0.078
WBC (10^9/L)	0.006	0.939
Neutrophils (10^9/L)	0.105	0.155
Lymphocytes (10^9/L)	-0.247	<0.001
NLR	0.276	<0.001
Hemoglobin (g/dL)	-0.356	<0.001
Hct (%)	-0.293	<0.001
MCHC (g/dL)	-0.325	<0.001
Platelet (10^9/L)	-0.038	0.610

Abbreviations: Hct, hematocrit; hs-CRP, high-sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; MCHC, mean corpuscular hemoglobin concentration; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; WBC, white blood cell.

The relationship between osmolality and NLR and other variables was examined using correlation analysis (Table 2). Osmolality was negatively correlated with lymphocyte count, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration (MCHC) ($r = -0.244$, $p = 0.002$; $r = -0.163$, $p = 0.025$; $r = -0.139$, $p = 0.056$; and $r = -0.166$, $p = 0.022$, respectively), but it was significantly positively correlated with age and NLR ($r = 0.186$, $p = 0.010$; $r = 0.201$, $p = 0.011$, respectively). NT-proBNP concentrations were negatively correlated with lymphocytes ($r = -0.247$; $p < 0.001$), hemoglobin ($r = -0.356$; $p < 0.001$), hematocrit ($r = -0.293$; $p < 0.001$), MCHC ($r = -0.325$; $p < 0.001$), and LVEF (%) ($r = -0.281$; $p < 0.001$) and significantly positively correlated with hs-CRP ($r = 0.435$; $p < 0.001$) and NLR ($r = 0.276$; $p < 0.001$), respectively (Table 3). However, the correlation between NT-proBNP and hs-CRP was greater than that between the other variables (Table 3).

After determining the NLR quartiles, we deemed that NLR values >3.41 represented the highest fourth quartile and increased NLR. Table 4 presents the univariate and multivariate logistic regression analyses for the prediction of increased NLR (NLR > 3.41). In our logistic regression model, the presence of NYHA class 3–4 functional capacity (OR = 1.629, 95% CI 1.210–1.974, $p = 0.002$), the presence of a lower lipid profile (OR = 0.307, 95% CI 0.104–0.913, $p = 0.034$), high osmolality (OR = 1.581, 95% CI 1.301–1.792, $p = 0.028$), and hs-CRP (OR = 2.581, 95% CI 2.127–2.891, $p < 0.001$) were found to be independent predictors of increased NLR. Figure 2 shows the NLR values of patients with NHYA 1–2 and NYHA 3–4 functional capacity.

Discussion

This study found that hyperosmolality was associated with increased NLR in patients with HFrEF. To our knowledge, this is the first study to specifically address the relationship between NLR and hyperosmolality in patients with HFrEF. High osmolality on admission was an independent predictor of increased NLR in the patient population, which also included more patients with NYHA class 3–4 functional capacity, a higher loop diuretic use ratio, and a lower percentage of hyperlipidemia. We know that functional capacity is an effective indicator of hospitalization, and it determines routine loop diuretic usage.

Although not statistically significant, the fourth quartile had higher hs-CRP, NT-proBNP, and diabetes ratios. In our study, NLR was found to be associated with plasma osmolality and hs-CRP; however, there was a lack of correlation between hs-CRP and osmolality. Although this finding may be related to the limitations of the study, such as sample size, it potentially suggests that hs-CRP may not be a good inflammatory marker for chronic HF, unlike acute HF and acute coronary syndrome. This remarkable finding needs to be confirmed by further studies.

In addition, plasma osmolality was negatively correlated with hemoglobin, hematocrit, and MCHC, direct markers of anemia. In this study, the correlation between NT-proBNP and osmolality, lower hemoglobin, higher hs-CRP, and NLR levels proves that increased NT-proBNP acts as a valuable indicator of HF.

The published literature favors a relationship between increased NLR and mortality due to HF; however, data on the mechanism of such an association between WBC and HF are limited. Neutrophilia, lymphocytopenia, and increased NLR in HF result from downregulation of the differentiation and proliferation of lymphocytes, neurohumoral activation, and lymphocyte apoptosis, respectively [8,9,27]. Cardiac decompensation and venous congestion induce a hypoxic state through low cardiac output. This activates the levels of proinflammatory cytokines, such as TNF-α, which amplify major proinflammatory pathways

Table 4. Factors predicting increased NLR on logistic regression analysis.

	Univariate Analysis			Multivariate Analysis		
Variables	OR	95% CI	P	OR	95% CI	P
Age	1.028	0.986-1.072	0.200			
NYHA class 3-4 presence	1.279	1.124-1.626	0.001	1.629	1.210-1.974	0.002
Presence of lower lipid profile	2.975	1.211-7.30	0.017	0.307	0.104-0.913	0.034
High Osmolality presence	1.106	1.020-1.199	0.015	1.581	1.301-1.792	0.028
hs-CRP	1.052	1.020-1.084	0.001	2.581	2.127-2.891	<0.001
NT-proBNP	0.997	0.989-1.001	0.091			

Abbreviations: 95% CI, 95% confidence interval; hs-CRP, high-sensitivity C-reactive protein; NLR, neutrophil-to-lymphocyte ratio; NYHA, New York Heart Association; NT-proBNP, N-terminal prohormone B-type natriuretic peptide; OR, odds ratio.

and IL-6-inducing hs-CRP [28,29]. This proinflammatory state creates a vicious cycle by causing leukocytes to produce more cytokines [30-31].

Worsening renal function in chronic HFrEF patients mainly occurs due to renal hypoperfusion. Renal hypoperfusion and excessive diuretic use result in increased BUN levels, which are linked to lymphopenia [31]. The activation of the sympathetic system and inflammatory cytokines increases endogenous cortisol levels, which may contribute to lymphocytopenia, neutrophilia, and hyperglycemia [32,33]. In this study group, hyperosmolality was mainly explained by hypernatremia, increased BUN levels, and hyperglycaemia. Hypernatremia mainly occurs due to dehydration, especially in elderly patients, and increased renin-angiotensin-aldosterone system activation, which stimulates sodium retention. Increased catecholaminergic, inflammatory activity, and renin-angiotensin system activation may have caused hyperosmolality. Willermain et al. reported that hyperosmolar stress can aggravate oxidative stress, cell membrane damage, and apoptosis by inducing proinflammatory cytokines [34]. Hyperosmolality has also been reported as responsible for tumor progression [35].

Inflammatory cytokines can cause the homing of blood lymphocytes to lymph nodes or inflamed tissues, and these processes can create inflammatory lymphopenia. In previous studies, high NLR values have been shown to be strongly correlated with cytokine levels, such as IL-2, IL-6, IL-8, and vascular epidermal growth factor [36]. Therefore, we may speculate that hyperosmolality may create an inflammatory environment that leads to higher NLRs in HF patients. In addition, some studies have revealed that both low and high osmolality are significantly linked with higher mortality rates in patients with HFrEF [3].

In our study, lower lipid levels were also found to be associated with NLR, which is a notable finding. High LDL cholesterol levels are not frequently associated with HFrEF, and symptomatic HF patients who have NYHA 3–4 functional capacity often have low concentrations of LDL cholesterol. There is, however, a link between lower LDL cholesterol concentrations and worse prognosis in HFrEF patients [37]. Lower lipid levels in HFrEF patients with an NYHA 3–4 functional capacity may occur due to impaired hepatic lipid metabolism, poor lipid absorption, and poor diet, which are consequences of HF-related congestion. Chronic sluggish hepatic vasculature flow and chronic congestion of the liver are very common during congestive HF. The relationship between increased NLR and lower lipid status in HF patients may be a confounder in these patients. Except for the present study, no previous study has addressed a possible relationship between increased NLR and lower lipid levels in HFrEF patients. Considering these findings, lipid levels may also affect the NLR in HFrEF patients.

NLR and plasma osmolality are inexpensive and easily obtainable clinical parameters, and the interaction between these parameters should be considered in clinical practice. Delcea et al. showed that NLR and NT-proBNP were independent factors of functional status and mortality in HF patients, and NLR values were correlated with serum NT-proBNP levels [9]. Therefore, plasma osmolality levels, NT-proBNP, and increased NLR could be associated with mortality in HF.

Conclusion

Although studies investigating the relationship between inflammation and osmolality in some patient populations are scarce, the current study suggests that the systemic inflammation depicted by NLR is affected by osmolality levels in patients with HFrEF. Additionally, there was a correlation between NLR, hs-CRP, and NT-proBNP levels. In conclusion, plasma osmolality can be considered an important clinical variable that affects inflammatory status in the HF phenomenon.

Limitations of the study

This study has some limitations. First, we used NLR and hs-CRP levels to indicate inflammation; however, the evaluation of TNF-α and interleukins would have produced more validated results. There is no available data in the literature regarding the relationship between systemic inflammation depicted by NLR and plasma osmolality in HF patients. However, we have attempted to explain the possible relationship between them as a concomitant finding. Second, in particular, we excluded acute patients to obtain a homogeneously chronic HF study group. The assessment of plasma osmolality without knowing the hydration status of the body and the simultaneous evaluation of water and electrolyte balance may lead to erroneous conclusions; therefore, it would be better if we had categorized the study group according to the Forrester classification. Third, the major limitation of the study is the absence of endpoints, such as mortality, hospitalization, and severity

or prognosis of HF. Finally, we considered only the osmolality quartiles of the specific study population; we did not use the normal range of 275 to 295 mOsm/kg. Further investigation is required to validate our results.

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Ethics approval
The study protocol was approved by the local Ethics Committee of Namık Kemal University (Decision no: 2017/116/12/04).

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