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RESEARCH ARTICLE

The relationship between the amount of edema and functional capacity and annexin V and ICAM 1 in patients with heart failure

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ABSTRACT

Objective: Edema, one of the common findings of congestive heart failure, can be seen in the form of pretibial, scrotal or pulmonary edema and always undesired changes the quality of life of patients. Some studies have previously demonstrated the correlation of some cytokines and inflammation markers with heart failure. This study purposed to investigate the relevance of the amount of edema and functional capacity with ICAM 1 and annexin V in heart failure patients. **Methods:** The study included a total of 40 individuals, 20 of whom were diagnosed with heart failure and 20 of whom were enrolled as the control group. Edema grades and functional capacities were scored and noted. All patients underwent transthoracic echocardiography. ICAM 1 and annexin V levels were compared between the patient and control groups. The correlation between the grade of edema and functional capacity and ICAM 1 and annexin V levels was examined. ROC curve analysis was used to determine the cut-off point. **Results:** There was a significant difference in both ICAM 1 and annexin V levels in the patient group compared to the control group (ICAM 1 58.14±25.65 ng/mL, 36.04±14.14 ng/mL, $p<0.05$; annexin V 9.84±1.86 ng/mL, 8.39±0.61 ng/mL, $p<0.05$). Analyzes showed a correlation between increased degrees of edema and decreased functional capacity and levels of ICAM 1 and annexin V. **Conclusion:** The results of this study may contribute to the prediction that ICAM 1 can be a new goal for the treatment of heart failure and that annexin V can be an auxiliary test for the diagnosis. It is believed that this contribution can be expanded by studies with a larger sample size.

Keywords: ICAM 1, annexin V, heart failure, edema, functional capacity

ÖZET

Kalp yetmezliği hastalarında ödem miktarı ve fonksiyonel kapasite ile annexin V ve ICAM 1 arasındaki ilişki

Amaç: Konjestif kalp yetmezliğinin yaygın bulgularından biri olan ödem pretibial, skrotal ya da pulmoner ödem şeklinde görülebilmekte ve hastaların yaşam standartlarını daima olumsuz yönde etkilemektedir. Bazı sitokin ve inflamasyon markerlarının kalp yetmezliği ile korelasyonu daha önce kimi çalışmalarda gösterilmiştir. Amacımız; kalp yetmezliği hastalarında fonksiyonel kapasite ve ödem miktarı ile ICAM 1 ve annexin V arasında ilişkiyi incelemektir. **Yöntem:** Çalışmaya kalp yetmezliği tanısı almış 20 ve kontrol grubu olarak belirlenmiş 20 olmak üzere toplam 40 kişi alındı. Ödem dereceleri ve fonksiyonel kapasiteleri skorlanarak not edildi. Tüm hastalara transtorasik ekokardiografi yapıldı. Hasta ve kontrol grubu arasında ICAM 1 ve annexin V düzeyleri karşılaştırıldı. Ödem derecesi ve fonksiyonel kapasite ile ICAM 1 ve annexin V düzeyleri arasındaki korelasyona bakıldı. Kesim noktasının belirlenmesi için ROC Curve analizi kullanıldı. **Bulgular:** Hem ICAM 1 hem de annexin V düzeylerinde hasta grupta kontrol grubuna kıyasla anlamlı fark oluştu (ICAM 1 58.14±25.65 ng/mL, 36.04±14.14 ng/mL, $p<0.05$; annexin V 9.84±1.86 ng/mL, 8.39±0.61 ng/mL, $p<0.05$). Analizler ödem dereceleri artması ve fonksiyonel kapasitenin azalması ile ICAM 1 ve annexin V seviyeleri arasında bir korelasyon gösterdi. **Sonuç:** Bu çalışmanın sonuçları, ICAM 1'in kalp yetmezliği tedavisi için yeni bir hedef olabileceği ve annexin V'nin tanı için yardımcı bir test olabileceği öngörüsüne katkıda bulunabilir. Bu katkının daha geniş örneklemli çalışmalarla genişletilebileceği düşünülmektedir.

Anahtar kelimeler: ICAM 1, annexin V, kalp yetmezliği, ödem, fonksiyonel kapasite

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INTRODUCTION

Heart failure is still the cause of a significant proportion of mortality and morbidity in the world. Despite the late advancements in treatment methods, its incidence and mortality increase with age. While the incidence of heart failure for all age groups in Europe is 0.3%, the incidence for the adult population is 0.5% [1,2]. Edema, one of the common findings of congestive heart failure, can be seen in the form of pretibial, scrotal or pulmonary edema and always adversely affects the living standards of patients. Some studies have previously demonstrated the correlation of some cytokines and inflammation markers such as interleukin-6 (IL-6), IL 2, IL-1, tumor necrosis factor-alpha, transforming growth factor-beta (TGF-beta) and intercellular cell adhesion molecule 1 (ICAM 1) with heart failure [3-5].

ICAM 1, which mediates the transendothelial migration of leukocytes from the blood to the inflammation site, is an immunoglobulin-like molecule [6] and is highly expressed in the human intramyocardial vascular endothelium during myocardial infarction [7]. An animal experiment with an induced heart failure model showed an increase in the amount of ICAM 1 in the vascular endothelium with left ventricular systolic dysfunction [8]. An experimental study investigating the effect of ICAM 1 on cardiac remodeling in animals with induced left ventricular dysfunction showed that those with ICAM 1 deficiency were preserved from negative cardiac remodeling and heart failure through mechanisms involving cardiac fibrosis and leukocyte-mediated cardiac inflammation [9]. These results have made ICAM 1 a target for the treatment of heart failure patients.

Annexin V is a single-chain protein that binds to calcium-mediated phosphatidylserine of the annexin gene superfamily with high affinity [10]. A prospective study showed that annexin V was secreted in high amounts from the liver, kidney, lung and spleen in heart failure and significantly increased the diagnostic efficacy of NT-pro-BNP as a precursor of mortality in heart failure [11]. These results have led to the emergence of the opinion that annexin V can be used as a diagmnostic tool in heart failure.

The aim of this study is to examine the relationship of heart failure stages and edema amount with ICAM 1 and annexin V in patients with heart failure.

MATERIALS and METHODS

Our study was approved by Ethics Committee of Clinical Research of Çanakkale Onsekiz Mart University (Date: March 11, 2020, and Decision No. 2011-KAEK-27/2020-E.2000030326).

Patients

The study counted in a sum of 40 individuals, 20 of whom were diagnosed with heart failure and 20 of whom were enrolled as the control group with the same sociodemographic characteristics. In this study, heart failure was defined as heart failure with low left ventricular ejection fraction (LVEF), LVEF <40%, as specified in the European Society of Cardiology (ESC) “Guidelines for Acute and Chronic Heart Failure". The stages of heart failure were determined based on the New York Heart Association (NYHA) classification and the grades of edema in the body were noted. The functional capacities of the patients with heart failure in the study were in the NYHA II-IV class. Under 18 years of age, advanced valve pathology, connective tissue disease, history of autoimmune disease, multiple sclerosis, active cancer, active infection and history of myocardial infarction in the last 6 months were considered the exclusion criteria. The severity of edema was graded on a scale from 4 to 1, considering lung edema as 4 points, scrotal edema as 3 points, 3 or 4+ pretibial edema as 2 points, 1 or 2+ pretibial edema as 1 point, and its correlations with ICAM 1 and annexin V were examined. An equal number of patients in each group were included in our study. Pretibial edema was classified using the classical method based on the indentation of 2,4,6,8 mm, which corresponds to 1+, 2+, 3+, and 4+, respectively. The correlation of the functional capacity levels of the patients classified as NYHA II, III, IV with ICAM 1 and annexin V was also examined. There were 7 patients in the NYHA II class, 9 patients in the NYHA III class, and 4 patients in the NYHA IV class.

Echocardiography

LVEF, the presence of valve disease, and pulmonary arterial systolic pressure (PASP) of all subjects included in the study were evaluated with a GE Vivid S8 echocardiography device and recorded. PASP was calculated from the formula jet velocities previously determined in the literature. All calculations were made using the average of three TRV measurements. RA pressure was calculated by adding 10 mmg Hg when the inferior vena cava diameter was greater than 20 mm, and by adding 5 mm Hg if it was small [12]. Echocardiographic imaging was performed according to current recommendations as previously described. LVEF, were calculated from the apical 2- and 4-chamber views using the modified Simpson method [13]. Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), interventricular septal diastolic thickness, and LV posterior wall diastolic thickness were measured in the parasternal long-axis view using 2-dimensional methods.

Blood collection and biochemistry

Using standard procedures, blood was drawn from each patient in biochemistry tubes, centrifuged at 4000 rpm at 4°C for 10 minutes, plasma aspirated and kept at -80°C until the study. In line with the manufacturer's directives, the blood was studied using the Enzyme-Linked Immunosorbent Assay method and ICAM 1 (Thermo Fisher Pub no: MAN0016468, Waltham, Massachusetts, USA) and annexin V (Thermo

Fisher Pub no: MAN0016625, Waltham, Massachusetts, USA) levels were examined.

Statistical analysis

The study data were transferred to SPSS version 22.0 (IBM, USA) statistical software, and data control and analysis were performed with this software. In statistical evaluations, the significance of the differences between the annexin V and ICAM 1 levels of the patient and control groups was examined. The correlations between the severity of heart failure and annexin V and ICAM 1 were examined, and ROC Curve analysis was used to define the cut-off point. Normal distribution analysis was performed and the Pearson method was used for correlation. Weak correlation was considered for 0-0.25 correlation coefficient, moderate correlation for 0.26-0.5, strong correlation for 0.56-0.75, and very strong correlation for 0.76-1 The significance of biochemical data was determined using the independent t-test method and the data were evaluated. The level of statistical significance was set at $p<0.05$.

RESULTS

A total of 40 individuals, 20 patients and 20 controls, were counted in the study. The clinical and demographic characteristics of the patients are demonsrated in Table 1. There was no significant difference in terms of the presence of diabetes and arterial hypertension and biochemical and complete blood count results regarding the medical history before admission. There was a significant difference in terms of coronary artery disease, atrial fibrillation (AF), alcohol use, age, ejection fraction (LVEF) and pulmonary artery pressure (PASP) parameters in the patient group (respectively $p<0.05$, $p<0.05$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.001$). The ICAM 1 results showed a significant difference between the patient and control groups with an increase in favor of the heart failure group (58.14 ± 25.65, 36.04 ± 14.14, $p<0.05$) (Figure 1). The annexin V results also revealed a significant difference between the patient and control groups, with an increase in favor of the heart failure group (9.84 ± 1.86, 8.39 ± 0.61, $p<0.05$) (Figure 2). As the functional capacities of the patients in the heart failure group decreased (as the NYHA classification increased), annexin V and ICAM 1 values increased, which showed a correlation between them (respectively 0.67 and 0.81). Moreover, as the edema amount of the patients in the heart failure group increased, annexin V and ICAM 1 values increased, which revealed a correlation between them (respectively 0.68 and 0.81). ROC curve analysis was performed to demonstrate the cut-off points for ICAM 1 and annexin V. The cut-off values for both markers were determined according to the results of the study (Table 2, Figure 3).

DISCUSSION

The important findings of our study can be listed as

Table 1. Clinical and demographic characteristics of the patients

	Patient (n=20)	Control (n=20)	P values
Age (year)	68.45±9.59	53.85±5.86	<0.001
Smoking	14 (%70)	9 (%45)	0.115
Alcohol	10 (%50)	3 (%15)	0.018
Hypertension	12 (%60)	10 (%50)	0.537
Diabetes mellitus	6 (%30)	7 (%35)	0.744
CAD	16 (%80)	7 (%35)	0.003
Atrial fibrillation	10 (%50)	3 (%3)	0.012
Urea (mg/dL)	42.81±15.51	32.3±10.13	0.161
Creatinine (mg/dL)	1±0.4	0.84±0.22	0.673
Glucose (mg/dL)	134.5±43.35	122.2±36.63	0.358
ALT (U/L)	18.1±9.2	22±10.7	0.216
AST (U/L)	21.6±6.2	22.7±8.7	0.879
HDL (mg/dL)	40±11	48.8±19.31	0.1
LDL (mg/dL)	113.4±45.91	105.9±27.8	0.573
TG (mg/dL)	133.8±48.5	149.38±74.9	0.478
Hemoglobin (g/dL)	12.79±1.95	13.95±1.45	0.356
Thrombocyte (10^3/µL)	222.75±79.87	234.7±46.1	0.561
Leukocyte (10^3/µL)	7.79±1.93	8.48±2.33	0.311
Neutrophil (10^3/µL)	4.51 ±1.37	5.18±1.73	0.184
Lymphocyte (10^3/µL)	2.11±1.31	2.31±0.81	0.553
Sodium (mEq/L)	138.5±1.95	139.8±2.18	0.049
Potassium (mEq/L)	4.38±0.31	4.5±0.41	0.216
LVEF (%)	29.1±7.81	59.6±9.5	<0.001
PASP (mmHg)	41.1±5.3	20.4±2.1	<0.001
Annexin V (ng/mL)	9.84±1.86	8.39±0.61	0.003
ICAM 1 (ng/mL)	58.14±25.65	36.04±14.14	0.002

LVEF: Left ventricle ejection fraction, PASP: Pulmonary artery sistolic pressure, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglycerides, CAD: Coronary artery disease.

follows: ICAM 1 and annexin V were found to be

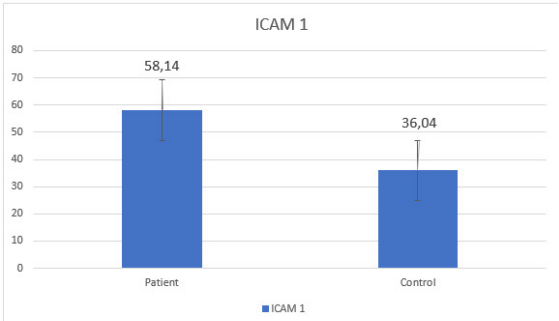

Figure 1. The results of ICAM 1.

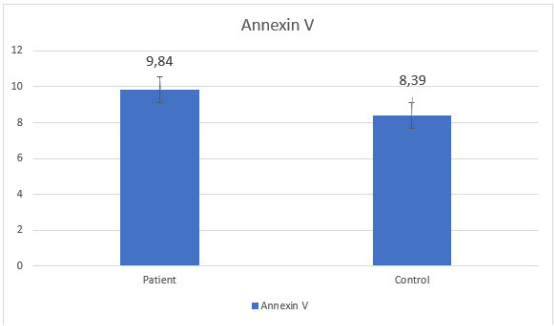

Figure 2. The results of annexin V.

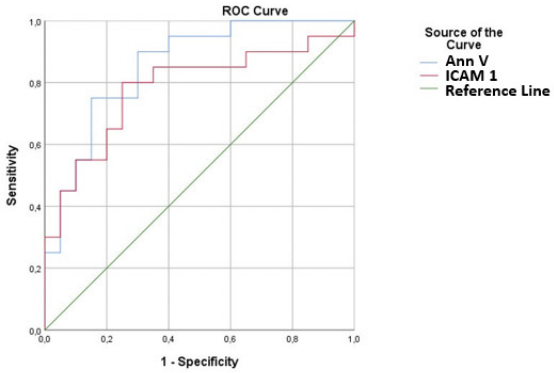

Figure 3. ROC-curve analysis.

significantly higher in heart failure patients, correlation between annexin V and ICAM with increased amount of edema and decreased functional capacity.

Heart failure is still among the important reasons for death in the world despite the advancements in medical and device treatments. Parameters that may be useful for the diagnosis of heart failure have been investigated by evaluating with many biochemical or PCR methods. There are also experimental studies conducted to develop novel diagnostic tools for heart failure [11]. Studies on both experimental and patient groups and the presented results aim to offer new approaches to the diagnosis and treatment of heart failure.

A study by Lino et al. comparing post-MI heart failure patients with patients without heart failure showed higher IL-6, VCAM-1, and ICAM-1 levels in patients with heart failure than in those without heart failure [14]. A study by Pate et al. on young adults reported an association between increased levels of ICAM 1 and worse left ventricular functions [15]. ICAM 1 has been shown to increase fibrosis and inflammation in myocardial tissue after myocardial infarction, predicting that it may be a target for treatment [7] and similarly, in our study, ICAM-1 was obtained to be significantly higher in the heart failure group. As a progressive disease, heart failure should be treated at an early stage and therefore new early detection markers and new treatment targets are being studied to reduce mortality. In an experimental study, it was shown that ICAM-1 blockade prevented cardiac remodeling induced by angiotensin 2 [16]. Reducing the effect of ICAM 1, which acts as an inflammatory mediator and leukocyte migration molecule, on cardiac remodeling also suggested that it may be a new target in the treatment of hypertrophic heart diseases. In another study, the relationship between coronary artery disease and ICAM 1 was examined and it was shown to be a risk factor in patients with coronary artery disease [17]. Heart failure is a disease with a wide etiology due to many causes. In our study, it was observed that ICAM 1 was significantly higher in the heart failure group. Considering all these, it made us think that it can be a treatment option for both ischemic and non-ischemic heart failure patients.

The processes involved in the development and progression of heart failure require an early treatment strategy to prevent deterioration of heart function. Many studies are conducted to determine the efficacy and adequacy of new diagnostic markers in order to determine and guide the diagnosis and prognosis of heart failure [18]. N-terminal proBNP (NT-proBNP) are widely used as diagnostic biomarkers for heart failure (HF) and cardiac dysfunction in clinical medicine. A prospective study by Schurgers et al. showed that annexin V was secreted in high amounts from the liver, kidney, lung and spleen in heart failure and significantly increased the diagnostic efficacy as a mortality precursor in addition to NT-pro-BNP [11]. It is important that annexin V is found to be high in isolated heart tissue in the study, such as NT-proBNP, which is a marker used in the diagnosis of heart failure. A study by Ravassa et al. investigating the level of hypertensive heart disease according to its severity showed that annexin V level increased as the level of systolic function decreased [19]. In another study examining the level of annexin A1, another protein from the annexin family, it was found to be associated with an increase in the amount of mortality and congestion in patients with acute heart failure [20], and similarly, in our study, annexin V was obtained to be significantly higher the heart failure group. Considering the results of these studies, it was concluded that annexin V could be a supportive parameter that can be used as a diagnostic parameter for heart failure. As in the study in which annexin A1 level was examined,

Table 2. The cut-off values with ROC-curve analysis.

Risk factor	AUC (%95)	Cut-off	p	Sensitivity (%)	Specifitify (%)
Annexin V	0.855 (0.739-0.971)	8.78	<0.001	75	75
ICAM 1	0.783 (0.634-0.931)	45.02	<0.05	75	75

only acute heart failure patients were not included in our study, but patients with chronic heart failure followed up were included in a mixed form. More clear results will be obtained in a future study in which patients with acute heart failure can be recruited.

Congestion is a classic clinical feature of HF patients. In some patients, pulmonary edema develops very rapidly due to the sudden increase in left ventricular filling pressures, and a triggering factor such as acute myocardial infarction is often recognized [21]. Chronic fluid accumulation accounts for a significant proportion of hospitalizations and defines patients with a worse prognosis than those presenting with acute heart failure due to the sudden increase in LV filling pressures [22]. The comparison with the 4-point classification of the amount of edema revealed that as the amount of edema increased, both ICAM 1 and annexin V levels increased in correlation. As a result of the correlation of both markers with the amount of edema, we consider that a decrease in hospitalization rates can be achieved when they are used in diagnosis and treatment. The fact that it was found to be higher in patients with increased edema made us think that ICAM-1 may be associated with the progression of heart failure and strengthened the opinion about its being a treatment target.

The NYHA classification continues to be the most important determinant of prognosis and life expectancy in heart failure. As the functional capacity decreases, the death rate increases. It has been shown that life expectancy is shorter especially in class IV patients compared to other groups. [23,24]. It has been shown that both markers are correlated with functional capacity according to the NYHA classification. This means that the amount of both ICAM 1 and anexxin V increases as the prognosis progresses. It is known that as NYHA classification decreases, mortality rates decrease and life expectancy increases. When ICAM 1 is used as a treatment target and annexin V is used in diagnosis, it makes us think that the prognosis can be positively affected.

One of the limitations of our study was the relatively low number of cases and the fact that it was a single center after the wide exclusion criteria. Another limitation is that, since blood could not be drawn after treatment in the heart failure group, the evaluated parameters can only be evaluated at the first hospitalization, that is, before the treatment.

As a result, heart diseases remain the leading cause of death all over the world, with heart failure accounting for the biggest share. Many studies have been complited to develop the diagnostic and treatment methods for this condition. Our study evaluated ICAM and annexin V. Our results supported the potential of ICAM 1 as a new treatment target and annexin V as a novel diagnostic aid. Since the studies on both annexin V and ICAM 1 in heart failure are not many yet, studies with more patients and in more than one center may offer new approaches.

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