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Aldosterone, Galectin-3, and NTproBNP Levels and their Values as Biomarkers in Infants with Ventricular Septal Defect

Ventriküler Septal Defektli süt Çocuklarında Aldosteron, Galectin-3 ve NTproBNP Düzeyleri ve Biomarker Değerleri

ORIGINAL ARTICLE
KLİNİK ÇALIŞMA

ABSTRACT

Objective: Galectin-3 is a biomarker used to detect cardiac remodelling and fibrosis. It could also potentially be a biomarker for developing new treatments. Aldosterone and galectin-3 levels and their relationship to N-terminal pro-brain natriuretic peptide (NT-proBNP) and left ventricular dilatation have not yet been studied in infants with ventricular septal defect (VSD). In this study, we aimed to investigate the biomarker feature of galectin-3 in infants with VSD.

Methods: Aldosterone, galectin-3, and NT-ProBNP levels were quantified and left ventricular diameters were measured with M mode echocardiography in infants with isolated VSD who had received heart failure treatment. The results were compared with those of healthy children of similar age and gender.

Results: This study included 22 infants (13 girls, nine boys) with VSD who formed the patient group and 22 healthy infants (13 girls, nine boys) who formed the control group. There was a significant difference between the two groups regarding the median left ventricular end-diastolic diameter and the median left ventricular end-systole diameter. The patient and control groups had no significant difference with respect to aldosterone levels (median values 43.5 pg/mL vs 41.3 pg/mL, respectively) (P = .851), although there was a significant difference with regard to galectin-3 levels (median values: 4 vs 2.5 ng/mL, respectively) (P = .015) and NT-proBNP levels (median values: 204.3 vs 94.2 pg/mL, respectively) (P = .003).

Conclusion: Galectin-3 increases independent of left ventricular dilatation and may have a biomarker value with similar strength as NT-proBNP in infants with VSD.

Keywords: Ventricular septal defect, aldosterone, galectin-3, NT-proBNP, children

ÖZET

Amaç: Galektin-3, kardiyak yeniden şekillenmeyi ve fibrozisi tespit etmek için kullanılan bir biyobelirteçtir. Ayrıca potansiyel olarak geliştirilebilecek tedavilerin bir biyobelirteçi olabilir. Aldosteron ve galektin-3 seviyeleri ve bunların N-terminal pro B tip natriüretik peptidi (NT-proBNP) ve sol ventrikül dilatasyonu ile ilişkileri ventriküler septal defektli (VSD) bebeklerde henüz çalışılmamıştır. Bu çalışmada, VSD'li bebeklerde galektin-3'ün biyobelirteç özelliğinin araştırılması amaçlandı.

Yöntemler: Kalp yetersizliği tedavisi alan izole VSD'li bebeklerde aldosteron, galektin-3 ve NT-ProBNP düzeyleri ölçüldü. Sol ventrikül çapları M mod ekokardiyografi ile değerlendirildi. Sonuçlar benzer yaş ve cinsiyetteki sağlıklı çocukların değerleri ile karşılaştırıldı.

Bulgular: Bu çalışmada VSD'li 22 süt çocuğu (13 kız, 9 erkek) hasta grubunu ve sağlıklı 22 süt çocuğu (13 kız, 9 erkek) kontrol grubunu oluşturdu. Sol ventrikül end diyastol diameter z skoru (ortanca değerleri 0.9 karşın -0.3) (P < .001) ve sol ventrikül end sistol diameter z skoru (ortanca değerleri 0.4 karşın -0.4) (P = .012) arasında istatistiksel olarak anlamlı fark saptandı. Hasta ve kontrol grupları arasında; aldosteron düzeyleri (ortanca değerleri 43.5 karşın 41.3 pg/mL) arasında istatistiksel olarak anlamlı fark saptanmadı (P = .851), galectin-3 düzeyleri (ortanca değerleri 4 karşın 2.5 ng/mL) (P = .015) ve NT-proBNP düzeyleri (ortanca değerleri 204.3 karşın 94.2 pg/mL) (P = .003) arasında istatistiksel olarak anlamlı fark saptandı.

Sonuç: Galectin-3 VSD'li süt çocuklarında sol ventriküler dilatasyonundan bağımsız olarak artmaktadır ve NT-proBNP'ye benzer güçte biyomarker değerine sahip olabilir.

Anahtar Kelimeler: Ventriküler septal defekt, aldosteron, galectin-3, NT-proBNP, çocuk

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Ventricular septal defect (VSD) is the most common congenital heart disease after bicuspid aortic valve. The most important factor in the pathophysiology of the disease is the size of the defect. Left-right shunt at the ventricular level caused by a medium-to-large VSD causes pulmonary congestion, left ventricular dilatation secondary to an increased blood volume returning to the left ventricle, and reduces cardiac output. The sympathetic nervous system is activated as a compensatory mechanism, and angiotensin II production is increased by the renin-angiotensin-aldosterone (RAAS) system, which causes an increase in systemic vascular resistance and water and sodium retention via aldosterone. Although these mechanisms initially provide a temporary improvement in circulatory stability, they will adversely affect heart failure over time.[1-2]

Hypertrophy, apoptosis, profibrotic milieu, and endothelial dysfunction develop within cardiac myocytes with the contribution of activated RAAS and the resulting vasoconstriction.[3] Neurohumoral factors are increased in the circulation independent from RAAS activation and affect cardiac systolic and diastolic function adversely by their action on the remodeling process.[3] As neurohumoral factors are unstable substances and their serum levels frequently vary, it is impractical to use these factors in diagnosis and treatment monitoring.

Various stress factors affecting myocardial cells increase brain natriuretic peptide (BNP) production. BNP is mainly secreted from the ventricles in response to left and right ventricular enlargement owing to pressure and volume overload. In atrial septal defect and VSD, when the left-right shunt is excessive, the level of N-terminal pro-brain natriuretic peptide (NT-proBNP) increases.[4] Current heart failure treatment is designed to target excessive neurohumoral mechanisms. Angiotensin-converting enzyme inhibitors reduce afterload by inhibiting the RAAS pathway and have the ability to prevent myocardial remodeling. Diuretics are used in patients with fluid overload.

Galectin-3 is a galactoside-binding lectin. It is expressed in the gut, spleen, colon, kidney, and myocardium and inflammatory cells, including mast cells, neutrophils, macrophages, and fibroblasts. Its carbohydrate recognition domain consists of 130 amino acids. It produces various biological effects by binding to different b-galactosides. Galectin-3 is involved in inflammation, tissue repair through fibrogenesis, and ventricular remodeling, which is an important feature of heart failure.[5,6]

ABBREVIATIONS

BNP	Brain natriuretic peptide
CBC	Complete blood count
ELISA	Enzyme-linked immunosorbent assay
LVEDD	Left ventricular end-diastolic diameter
LVESD	Left ventricular end-systolic diameter
MCP	modified citrus pectin
NT-proBNP	N-terminal pro-brain natriuretic peptide
RAAS	Renin-angiotensin-aldosterone system
VSD	Ventricular septal defect

Clinical studies on galectin-3 inhibition in several diseases, such as pulmonary fibrosis, hepatic fibrosis, chronic kidney disease, and malignancy are ongoing.[7-9] In experimental studies, it has been shown that galectin-3 directly induces pathological cardiac remodeling, causing cardiac fibrosis and ventricular dysfunction. In experimental models of myocardial dysfunction, different carbohydrate-based ligands of galectin-3 such as N-acetyllactosamine and modified citrus pectin (MCP) have been shown to reduce cardiac fibrosis and inhibit the progression of cardiac remodeling.[10,11]

In this study, we investigated whether there was a relationship between aldosterone and galectin-3 levels in infants with VSD and clinical heart failure and whether galectin-3 could be used as a biomarker in these patients.

Methods

A prospective cross-sectional study was conducted between February 2019 and July 2019 in the pediatric cardiology outpatient clinic of Health Sciences University, Hamidiye School of Medicine, Kayseri Training and Research Hospital. The study was approved by the Erciyes University Faculty of Medicine clinical research ethics committee. The families of all the patients and control group infants included in the study provided informed consent.

Twenty-two infants with isolated VSD who received heart failure treatment formed the study group, and 22 healthy infants formed the control group. VSDs with a diameter < 3 mm were classified as small, those with a diameter of 3-5 mm as medium, and those > 5 mm diameter as large.[12] Patients with hemodynamically significant VSDs were enrolled in the study. Of the 19 patients with a medium and three with a large VSD; 15 patients had perimembranous VSD, six had muscular VSD, and one had an inlet type VSD. All the patients were older than three months and were taking heart failure (captopril and furosemide) therapy for at least one month when the blood samples were taken. Echocardiographic data were obtained for the study. The control group consisted of healthy children evaluated for a murmur and whose echocardiographic examination was normal.

The exclusion criteria were small VSD, VSD accompanied by other cardiac pathologies, presence of active infection, chronic disease and medication use, and additional pathologies that caused feeding difficulties such as cleft lip and palate, prematurity, history of surgeries, and syndromes.

Echocardiographic examinations were performed by the same pediatric cardiology physician using Vivid Pro 7 (GE, Vingmed Ultrasound, Horten, Norway) echocardiography device using two dimensional, color Doppler, continuous wave Doppler, and M mode echocardiographic examinations. Left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) z scores were calculated using the parameter z score software.

For complete blood count (CBC), 2 cc blood samples were taken into a tube containing ethylenediamine tetraacetic acid. Blood

samples were studied on the same day with a Sysmex branded XN-9000 model device at Kayseri Education Research Center Biochemistry Laboratory. The 2 cc blood samples taken for NT-proBNP measurement were centrifuged to separate their sera. NT-proBNP measurements were performed according to standard procedures using sandwich enzyme-linked immunosorbent assay (ELISA) method developed by Roche Diagnostics (Elecsys® pro-BNP II, Cobas, Mannheim, Germany). The analytical sensitivity of the kit is 5 pg/mL, assay range 5-35000 pg/mL, and the intra-assay CV is < 2%.

Serum samples of the patient and control groups were stored at -80°C until the day of analysis. According to the manufacturer's instructions, serum aldosterone levels were measured by competitive inhibition ELISA method using a commercial kit (Cloud-Clone Corp, TX, USA). Aldosterone level was expressed as pg/mL. Detection range and intra/inter-assay CV for serum aldosterone are 24.69-2000 pg/mL and <10%, respectively. According to the manufacturer's instructions, serum galectin-3 levels were studied by sandwich ELISA method using a commercial kit (Cloud-Clone Corp, TX, USA). The galectin-3 level was expressed as ng/mL. Detection range and intra/inter-assay CV for serum galectin-3 are 0.156-10 ng/mL and <10%, respectively. For all parameters, the concentrations of the samples were calculated using calibration curves obtained from study standards with known levels. The relationship between aldosterone, galectin-3, NT-proBNP, and left ventricular diameters was examined.

Statistical analysis

The normality of the distribution of the study data was tested with a histogram, Q-Q graphics, and the Shapiro-Wilk test. Homogeneity of variance was tested with Levene's test. Comparisons between the two groups were performed with the Mann-Whitney U test and independent two-sample t-test for quantitative variables and Pearson chi-squared test for qualitative variables. The Kruskal-Wallis H test was used for comparisons among more than two groups. Receiver operating characteristic (ROC) analysis determined both the area under the curve for galectin-3 and NT-proBNP levels. The cut-off point of each marker was determined according to the Youden index. Sensitivity, specificity, and the positive and negative predictive values were calculated with a confidence interval of 95%. The DeLong test was used to compare the area under the ROC curve. The relationship between the quantitative data was evaluated using Spearman correlation analysis, where $0.0 \leq r < 0.20$ indicated a very weak relationship or almost no relationship, $0.20 \leq r < 0.40$ a weak relationship, $0.40 \leq r < 0.60$ a moderately good relationship, $0.60 \leq r < 0.80$ a good relationship, and $0.80 \leq r < 1.00$ an excellent relationship. Data analysis was performed with the SPSS version 24 software (IBM Corp., Armonk, NY, USA). The significance level was set at $p<0.05$.

Results

This study included 22 infants (13 girls, nine boys) with VSD who formed the patient group and 22 healthy infants (13 girls, 9 boys) who formed the control group. The mean age of the patient group was 6.55 ± 2.52 months, and the mean age of the control group was 7.86 ± 2.61 months. There was no significant difference between the patient and control groups in terms of sex distribution (P = .999), mean age (P = .096), mean body weight z scores (P = .313), and mean body height z scores (P = .725) of the patient and the control groups.

A significant difference was found between the hemoglobin values of the patient (11.10 ± 0.97 gr/dL) and control groups (11.72 ± 0.94 gr/dL) (P = .035). No significant difference was found between the patient and control groups for aldosterone levels. There was a significant difference between the two groups in terms of galectin-3 and NT-proBNP levels (Table 1).

The LVEDD z and LVESD z scores of the patient group were significantly greater than those of the control group. In the patient group, two patients had an LVEDD z score, and four patients had an LVESD z score >2 (Table 2).

A moderately significant negative correlation was found between body weight z score and LVEDD z score (r = -0.458). There was a weakly significant negative correlation between body weight z score and LVESD z score (r = -0.316).

A moderately significant positive correlation was found between NT-proBNP and LVEDD z score (r = 0.417). There was no significant correlation between galectin-3, aldosterone and NT-proBNP in the patient group (P > .05). Correlations between other variables were not statistically significant (P > .05). The area under the ROC curve for the galectin-3 level was 0.715 (P < .05). The area under the curve for the NT-proBNP level was 0.765 (P < .05). No significant difference was found between the predictive performances of galectin-3 and NT-proBNP levels (P = .653). The optimal cut-off value for galectin-3 level according to Youden index was 3.62 ng/mL (> 3.62 ng/mL for patients, ≤ 3.62 ng/mL for controls); the optimal cut-off value for NT-proBNP level was 96.42 pg/mL (> 96.42 pg/mL for patients, ≤ 96.42 pg/mL for controls). Thirteen (59%) patients in the patient group and three (18.1%) children in the control group had a galectin-3 level above the cut-off level. Twenty (90.9%) patients in the patient group and eight (36.3%) children in the control group had an NT-proBNP level above the cut-off level.

In the patient group, two patients with NT-proBNP below the cut-off level had galectin-3 levels below the cut-off level. In the healthy control group, galectin-3 level was above the cut-off level in one child among 14 patients whose NT-proBNP was below the cut-off level. In the patient group, NT-proBNP level was above the cut-off level in seven of nine patients whose galectin-3 level was below the cut-off level. In the healthy control group, NT-proBNP level was above the cut-off level in seven of 19 patients whose galectin-3 level was below the cut-off level. In four children with a LVESD z score > 2, NT-proBNP was elevated in all of them, whereas galectin-3 was elevated in two children.

The sensitivity and specificity of galectin-3 were 59.1% and 86.4%, respectively. For a galectin-3 level >3.62 ng/mL, 59.1% of individuals who were actually sick could be predicted, and 86.4% of individuals who were actually not sick could be predicted. The sensitivity and specificity of NT-proBNP were 90.9% and 63.6%, respectively. For an NT-proBNP level >96.42 pg/mL, 90.9% of individuals who were actually sick could be predicted, and 63.6% of individuals who were actually not sick could be predicted (Table 3).

The area under the ROC curve was 0.715 for galectin-3 and 0.765 for NT-proBNP. No significant difference was found between the predictive performances of galectin-3 and NT-proBNP levels. The optimal cut-off value was 3.62 ng/mL for galectin-3 and 96.42 pg/mL for NT-proBNP levels.

Table 1. Comparison of aldosterone, galectin-3, and NT-proBNP values between groups

	Groups		
	Patients (n = 22)	Control (n = 22)	*P*
Aldosterone (pg/mL)	43.5 (27.0/80.2)	41.3 (28.8/68.5)	.851
Galectin-3 (ng/mL)	4.0 (2.6/5.5)	2.5 (2.0/3.5)	.015
NT-proBNP (pg/mL)	204.3 (124.6/332.7)	94.2 (70.7/176.5)	.003

Data are expressed as mean ± standard deviation, median (first quarter/third quarter), and n (%).
NT-proBNP, N-terminal pro-brain natriuretic peptide.

Table 2. Comparison of M-mode echocardiography parameters between groups.

	Groups		
	Patients (n = 22)	Control (n=22)	*P*
LVEDD (mm)	26.00 ± 3.02	23.64 ± 2.22	.005
LVEDD z score	0.9 (0.3/1.4)	-0.3 (-0.9/-0.1)	< .001
LVESD (mm)	16.91 ± 2.76	15.36 ± 1.87	.035
LVESD z score	0.4 (0.1-1.6)	-0.4 (-0.7/0.6)	.012
EF (%)	68.23 ± 6.70	67.36 ± 7.44	.688
FS (%)	35.64 ± 4.61	34.41 ± 4.56	.380

Data are expressed as mean±standard deviation, median (first quarter/third quarter), and n (%).
EF, ejection fraction; FS, fractional shortening; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter.

Discussion

According to results from our study, galectin-3 and NT-proBNP levels were increased, although aldosterone levels did not change in infants with medium and large VSDs who were receiving heart failure treatment. It was found that NT-proBNP level increased in proportion to left ventricular dilatation, whereas there was no correlation between aldosterone, galectin-3 levels, and left ventricular dilatation.

Galectin-3 is a useful biomarker in prognostication and risk stratification in patients with adult heart failure A recently published meta-analysis involving 18 studies on 32,350 subjects showed that galectin-3 level predicts all-cause mortality and new-onset heart failure in the general population.[13] Although many studies have demonstrated the usefulness of galectin-3 in predicting prognosis, mortality, and re-hospitalization in acute heart failure, several other studies have emphasized that galectin-3 is not a heart-specific biomarker.[6,14]

The American College of Cardiology Foundation/American Heart Association, heart failure treatment guidelines 2013 recommend using the galectin-3 level for risk stratification and determining the prognosis in patients with moderate or severe heart failure (class IIb). The European Society of Cardiology 2016 guidelines on heart failure, however, do not recommend the use of galectin-3 in clinical practice.[6]

In cardiac stress, increased serum and tissue galectin-3 levels cause the differentiation of cardiac fibroblasts to active myofibroblasts leading to increased collagen production. As a result of this process, aggregation, maturation, and cross-linking of collagen, regulated by different genes, occur in the interstitium.[7-11] The conditions that result in cardiac stress in adults are hypertension, coronary artery disease, myocardial infarction, and borderline or reduced left ventricular function. In children, this process is likely to occur in congenital and acquired heart diseases.[15] In our patients with VSD, the increase of galectin-3 irrespective of LV dilatation and the increase of NT-proBNP in relation to LV dilatation, may have resulted from the production of these biomarkers through different pathways.

Some experimental studies[9,16] have provided evidence that galectin-3 increases via aldosterone and that hyperaldosteronism causes cardiac fibrosis by inducing inflammatory profibrotic phenotypes and inhibiting antifibrotic factors, although other studies have shown that the increase in galectin-3 cannot be explained solely by aldosterone and suggested that the process

Table 3. ROC analysis results and diagnostic statistics of Galectin-3 and NT-proBNP markers between groups.

	ROC results		Diagnostic Statistics			
	AUC	*P*	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Galectin-3> 3.62 (ng/mL)	0.715	.008	59.1 (38.7-76.7)	86.4 (66.7-95.3)	81.3 (57.0-93.4)	67.9 (49.3-82.1)
NT-proBNP> 96.42 (pg/mL)	0.765	<.001	90.9 (72.2-97.5)	63.6 (43.0-80.3)	71.4 (52.9-84.8)	87.5 (64.0-96.5)

AUC, area under curve; ROC, receiver operating characteristic.

has more complex aspects, including inflammation and oxidant stress, implying that cardiac fibrosis does not occur solely by the action of galectin-3.[17,18] In our study, an increased galectin-3 level without a simultaneous increase in aldosterone levels in patients with VSD favors the second interpretation. The absence of an increase in aldosterone level in our patients may be because of the fact that all our patients received captopril, an angiotensin-converting enzyme inhibitor.

Kotby et al.,[19] in a study conducted on 45 children with dilated cardiomyopathy, congenital heart disease, or rheumatic heart disease, found that patients with chronic heart failure had significantly higher galectin-3 levels compared with those of the healthy control group (9.46 ± 5.43 ng/mL vs. 1.5 ± 0.66 ng/mL). The authors concluded that galectin-3 level could be a useful biomarker for determining the severity and stage of the disease. In a study conducted on 184 children with surgically corrected congenital heart disease, Dudnyk et al.[20] found evidence for the use of tissue Doppler imaging and galectin-3 levels to evaluate myocardial function.

In a study involving 602 adult patients with congenital heart, Baggen et al.[21] reported that galectin-3 increased in 7% of patients. The high galectin-3 level was associated with older age, a higher NT-proBNP, a higher NHYA class, loss of sinus rhythm, systolic and diastolic ventricular dysfunction, and adverse cardiac events. However, galectin-3 appeared to have a limited additional predictive value as the more traditional risk marker NT-proBNP. Opotowsky et al.[22] found that galectin-3 levels were high in 70 adult Fontan patients, stating that this finding was associated with an increased incidence of adverse outcomes, non-elective cardiovascular hospitalization, and death.

The presence of a correlation between NT-ProBNP and left ventricular dilatation but the lack of a correlation between galectin-3 and left ventricular dilatation suggest that galectin-3 plays a role in the pathophysiology via distinct pathways than NT-ProBNP. Medical treatment in infants with a VSD aims to reduce the symptoms of heart failure, thereby optimizing the infant's nutrition and protecting it against lower respiratory tract infections. Thus, it is aimed to provide an infant with a good health performance during the period required for the shrinkage/closure of the defect or at the time of surgery should the defect size remain significant. Captopril, which we administer to our patients with VSD, reduces angiotensin II and aldosterone levels. Furosemide treatment, however, reduces the volume and salt overload via diuresis. At present, treatments aimed at inflammation, oxidant stress, and fibrosis, which are thought to be important in the pathophysiology of heart failure, are not used because these pathways are not fully elucidated, and effective treatments targeting them have not been developed.

Limitations

The limitations of our study include the small size of the patient group, the lack of measurement of biomarkers before and after treatment, the lack of evaluation of diastolic function, the lack of angiographic measurement of pulmonary artery pressure, and the low number of patients with a left ventricular dilation z score > 2.

Conclusion

Galectin-3 is a biomarker and bio target of cardiac remodeling and fibrosis in heart failure. Future studies will focus on the inhibition of galectin-3 on the pathological processes that develop through this molecule. Additional evidence from clinical studies is needed to introduce galectin-3 inhibition as a specific therapy in treating and preventing heart failure.

In conclusion, galectin-3 increases independent of left ventricular dilatation in infants with VSD and may have a similar biomarker value as NT-proBNP.

Ethics Committee Approval: Ethics committee approval was received for this study from the Erciyes University Clinical Research Ethics Committee (Approval Date: February 20, 2019; Approval Number: 2019/131).

Informed Consent: Informed consent was obtained from the families of the patients and the control group who participated in this study.

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