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Assessment of the Relationship between Galectin-3 and Ejection Fraction and Functional Capacity in the Patients with Compensated Systolic Heart Failure

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ABSTRACT

Background: Galectin-3 is a soluble ß-galactoside–binding lectin released by activated cardiac macrophages. Galectin-3 has been proposed for diagnosis and prognosis of HF patients.
Objectives: The present study aimed to investigate the relationship between galectin-3 as a biomarker and ejection fraction and functional capacity in the patients with compensated systolic heart failure.
Patients and Methods: In this study, serum levels of Galectin-3 were measured in 76 patients with compensated heart failure with New York Heart Association class I–IV and left ventricular ejection fraction < 45%. Galectin-3 was measured by an ELISA kit. Besides, echocardiography was used to evaluate left ventricular ejection fraction. Additionally, functional capacity was determined based on the patients' ability to perform a set of activities. After all, the data were analyzed used t-test, Kruskal-Wallis, one–way ANOVA, and chi-square test. $P < 0.05$ was considered as statistically significant.
Results: The patients' age ranged from 45 to 75 years, with the mean age of 63.85 ± 9 years. In addition 57.9% of the patients were male. The results revealed no significant correlation between Galectin-3 and age, body mass index, and estimated glomerular filtration rate. Also, no significant correlation was observed between Galectin-3 levels and left ventricular ejection fraction ($P = 0.166$) and functional capacity ($P = 0.420$). Yet, a significant difference was found between males and females regarding the mean of Galectin-3 ($P = 0.039$).
Conclusions: The study results suggested that Galectin-3 could not be used as a marker of disease progression in the patients under treatment, which could probably be the result of medication use in these patients.

►*Implication for health policy/practice/research/medical education:*
In order to prevent the progression of heart failure in patients with compensated systolic heart failure appears helpful to use biomarkers such as Galectn-3 along with echocardiography.

1. Background

Heart Failure (HF) remains one of the most prevalent and challenging medical conditions. Despite adoption of guideline–based therapy, HF is associated with high morbidity and mortality rates; such a way that 80% of men and 70% of women aged 65 years or above die within 8 years after the initial diagnosis. HF is also one of the most costly medical conditions (1, 2).

This deleterious condition is associated with progressive ventricular dysfunction and cardiac remodeling (3, 4).

Changes in cardiac structure and function often occur before the symptoms appear, resulting in difficulty in prediction of clinical outcomes. Thus, many patients require

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specialized imaging techniques, such as cardiac magnetic resonance imaging, which are not always available (5, 6).

Risk factors, such as age, diabetes, and smoking, and severity of symptom are indicative of at risk patients, but are not enough to risk stratify patients (7). The disease may even progress in the patients who are under treatment; therefore, it is necessary to monitor these patients during the course of treatment.

HF biomarkers have growing importance in daily clinical practice as well as in clinical trials. Biomarkers can help in monitoring of response to therapy, prediction of patients outcome in clinical practice, and appropriate patient stratification (8). Several biomarkers are used for diagnosis and prognosis of HF patients. Recently, Galectin-3 has been proposed for diagnosis and prognosis of HF patients.

Galectin-3 (also known as Mac-2, CBP-35, L29, LBP or eBP) belongs to the family of β-galactoside binding proteins with an extended N terminal region composed of tandem repeats of short amino acid segments and C terminal domain which is responsible for lectin activity (8, 9).

Galectin-3 is a product of active macrophages and plays a pivotal role in pathogenesis of remodeling, including inflammation and fibrosis, in HF patients (10, 11).

Galectin-3 is not only prognosticate, but it also plays a direct role in HF progression making it a potential target for acute or chronic intervention (7).

2. Objectives

In this study, serum Galectin-3 concentration was measured in compensated HF patients. Besides, distribution of the patients in different New York Heart Association (NYHA) functional classes I, II, III and IV and Ejection Fraction (EF) groups (44 – 35%, 34 – 25%, < 25%) was determined by quartile of Galectin-3 levels. Then, the associations between Galectin-3 levels and EF and Functional Capacity (FC) were assessed in these patients.

3. Patients and Methods

In this study, 76 patients (age range of 45 to 75 years) diagnosed with chronic HF class I–IV (according to NYHA classification) and Left Ventricular Ejection Fraction (LVEF) < 45% were selected from the patients who regularly visited the heart failure clinic in Shahid Beheshti Hospital, Kashan, Iran. All the patients were examined by a cardiologist and underwent detailed echocardiographic examination. A full clinical history was also obtained. Baseline demographic data, functional status, cardiovascular risk factors, and medications were recorded, as well. These patients were receiving proper medication for HF and blood pressure.

The exclusion criteria of the study were suffering from renal failure and chronic inflammation disease. Serum creatinine (Cr) levels were measured by routine laboratory methods. Then, male patients with Cr > 1.3 and female ones with Cr > 1.2 and positive C-Reactive Protein (CPR) were excluded from the study. It should be mentioned that written informed consents were obtained from all the patients.

At baseline, 5 mL blood was taken from the patients after an overnight fasting. The blood samples were then centrifuged at 2000 – 3000 rpm for 20 minutes. Afterwards, the serum was stored at −20°C until analysis. Galectin-3 was measured by an enzyme-linked immunosorbent assay kit (SHANGHAI CRYSTAL DAY BIOTECH C). Calibration of the assay was according to the manufacturer's protocol. Values were normalized to a standard curve. The intra-assay and inter-assay variances for Galectin-3 were < 10% and < 12%, respectively. Moreover, echocardiography was used to evaluate LVEF with reference to Braunwalds' classification of systolic HF (12).

Furthermore, FC was determined based on the patients' ability to perform a set of activities. It should be noted that Galectin-3 levels (pg/mL) were divided in quartiles as follows: first quartile: < 441, second quartile: 441 - 501, third quartile: 501 – 560, and fourth quartile > 560. After all, the data were entered in to the SPSS statistical software (v. 16) and analyzed using t-test, Kruskal-Wallis, one–way ANOVA, and chi-square test. $P < 0.05$ was considered as statistically significant.

4. Results

A total of 76 baseline serum samples from HF patients were available for analysis. The characteristics of the study groups according to the Galectin-3 levels have been presented in Table 1. As the table depicts, the patients' age ranged from 45 to 75 years, with the mean age of 63.85 ± 9 years. In addition, 57.9% of the patients were male. The mean age of male and female patients was 63.77 ± 8.5 and 63.98 ± 9.5 years, respectively (P = 0.950). Besides, 35.5%, 44.7%, and 19.7% of the patients were in the NYHA functional class I, II, and III, respectively. It should be noted that due to the small number of subjects with class IV, classes III and IV were combined. Co-morbidities included diabetes mellitus in 25% and myocardial infarction in 35.5% of the patients. Additionally, the mean serum Cr level was 0.9 ± 0.1 mg/dL.

After removal of the outliers (the variables' values greater than 3 IQR are assumed as outliers) from the data, Galectin-3 showed normal distribution and the mean Galectin-3 level was 490 ± 95 pg/mL (Figure 1). The study results revealed no significant correlation between Galectin-3 levels and age (r = 0.108, P = 0.358), Body Mass Index (BMI) (r = -0.029, P = 0.807), and estimated Glomerular Filtration Rate (eGFR) (r = -0.070, P = 0.550). The patients' mean of BMI was 27.11 ± 4.5. Moreover, the mean of Galectin-3 was 666 ± 467 pg/mL in males and 499 ± 199 pg/mL in females and the difference was statistically significant (P = 0.039). However, no significant correlation was found between Galectin-3 levels and LVEF (P = 0.166) and FC (P = 0.420) in the HF patients.

5. Discussion

In this study, Galectin-3 levels were divided into four quartiles. The study results revealed no significant relationship between Galectin-3 and EF and FC in any of the quartiles. Galectin-3 is a novel biomarker that has been shown to mediate fibrosis in a variety of organs, including the heart. The role of Galectin-3 (a β-galactoside-binding lectin macrophage product) in HF was initially described in rat models, in which Galectin-3 expression was found to be up-regulated in endomyocardial biopsies of the rats with cardiac hypertrophy that subsequently progressed

Table 1. Demographic and Clinical Characteristics of the Study Population Based on the Quartiles of Galectin-3 Levels

Variables	Quartiles of Galectin-3 (pg/mL)				
	Quartile 1 (< 441)	Quartile 2 (441 – 501)	Quartile 3 (501 – 560)	Quartile 4 (> 560)	P value
N	19	19	19	19	
Age (years)	63 ± 7	62 ± 10	65 ± 10	65 ± 9	0.738
Male %	47.4	52.6	63.2	68.4	0.041
NYHA (I / II / III) %	42 / 47 / 11	42 / 32 / 26	26/ 58 / 16	32 / 42 / 26	0.420
LVEF (1 / 2 / 3) %	42/ 21 / 37	37 / 37 / 26	36 / 32 / 32	26 / 26 / 48	0.166
BMI (kg/m^2)	26.31 ± 3.3	28.13 ± 3.3	27.71 ± 5.6	27.31 ± 5.7	0.655
Myocardial infarction%	5	8	10	4	0.810
Diabetes (%)	42	10.5	37	10.5	0.035
Medication					
ACE inhibitors (%)	24	36	24	16	0.385
ARB (%)	31	21	25	23	0.333
β- Blocker (%)	24.6	26	24.6	26	0.956
Diuretics (%)	28.6	22	24.5	24.5	0.779
Digoxin (%)	31	25	19	25	0.223
Astatine (%)	25	23	28	23	0.593
Galectin-3, mean ± SD	384 ± 41	476 ± 14	527 ± 20	1147 ± 800	

Abbreviations: NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; BMI, body mass index; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; LVEF 1: 44 - 35%, LVEF 2: 34 - 25%, LVEF 3: < 25%

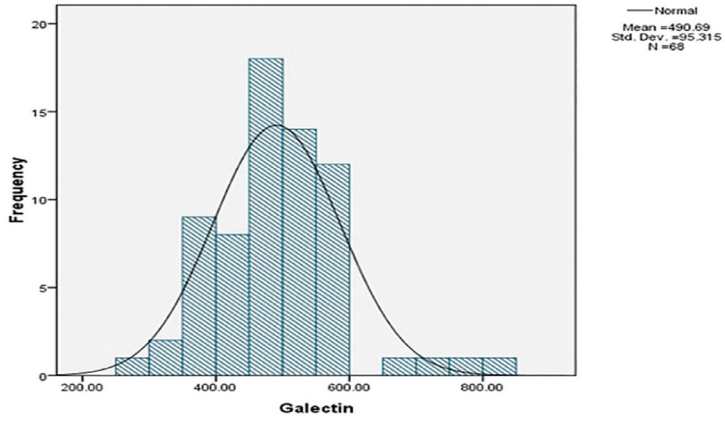

Figure 1. Histogram of Normal Distribution of Galectin-3

to HF. Mechanistically, Galectin-3 is a product of active macrophages with binding sites on cardiac-resident fibroblasts, leading to an increase in myocardial collagen expression and interstitial fibrosis, transformation of growth factor-β activation, and subsequently LV dysfunction. Hence, Galectin-3 may play a pivotal role in the response to injury and inflammation during HF, including being an important part of ventricular remodeling (6).

Evidence from animal experiments has been supported by observations in humans. Galectin-3 was found to be significantly up-regulated in hypertrophied hearts of the patients with aortic stenosis (10). After initiated systolic HF, several compensatory mechanisms are activated, including enlargement of the heart, increased thickness of the heart muscle fibers, and increased heart rate, that cause symptoms partial recovery. Yet, drug therapy can be effective in improvement of cardiac symptoms. The activation of compensatory mechanisms and drug therapy may improve symptoms in many patients. Nonetheless, even though some cases present no symptoms, the disease may still progress. Based on our findings, serum levels of Galectin-3 could not predict EF and FC in the treated patients. Therefore, serum levels of Galectin-3 cannot be a sign of disease progression or severity in these patients.

N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), a monocyte-derived anti-inflammatory and antifibrotic peptide that is reduced by Angotansin Converting Enzyme (ACE), inhibits Galectin-3 expression in the left ventricle and reduces Galectin-3–induced macrophage activation and migration (13, 14). In a rat model, intrapericardial infusion of Ac-SDKP mitigated many of the adverse effects of Galectin-3, leading to reduced myocardial macrophage and mast cell density, decreased left ventricular collagen burden, and improved diastolic and systolic function. These findings suggested that the adverse consequences associated with Galectin-3 might be modifiable (13).

In the present study, the patients were under treatment with ACE inhibitors, which perhaps could explain low Galectin-3 levels in these patients. In contrast, the results of a survey conducted by Wilson et al. on the patients with coronary disease demonstrated a weak negative correlation between Galectin-3 and EF (r = -0.31, P = 0.0002) (13). Similarly, the results of a research conducted by Chen on the patients diagnosed with Chronic Heart Failure (CHF) and coronary heart disease with stable angina showed that plasma Galectin-3 level was inversely correlated to LVEF (r = –0.683, P < 0.01) (15). This difference might be due to the fact that unlike our study, the patients in the two above-mentioned studies had not received any treatments. The difference might have also resulted from the difference in the etiologies of the disease in these studies.

In contrast to other studies, we found no increase in Galectin-3 levels. It seems that after long-term treatment, Galectin-3 level returns to its initial value. Consequently, it can be used for monitoring the patients during the course of treatment.

The findings of the current study indicated no significant correlation between Galectin-3 and age. These results were not in agreement with those of other studies, which might be because of participation of younger subjects in this study compared to other studies (4, 10) or the effect of drugs. Although numerous studies have shown that Galectin-3 level is increased substantially in acute or decompensated chronic heart failure, it cannot be a suitable marker for detection of HF. It is yet more efficient as a marker of disease progression. The findings of the study Lok et al. conducted on 232 patients with chronic heart failure indicated that Galectin-3 levels were elevated in only half of the patients with advanced but stable HF. However, 90% of the patients with HF showed abnormal levels of Brain Natriuretic Peptide (BNP), indicating its poor diagnostic property unlike its high prognostic value as a biomarker for detection of Galectin-3 (4, 16). Moreover, Van Kimmenade and colleagues studied 209 patients with HF and showed that Galectin-3 had lower specificity and sensitivity in identification of HF compared to amino terminal pro-brain natriuretic peptide (NT-proBNP) (17), but serum Galectin-3 levels were elevated and showed a better predictive value of prognosis compare to NT-proBNP and apelin (17).

Several studies conducted on the correlation between Galectin-3 and HF have given rise to more questions instead of answering the existing questions. Therefore, further studies are necessary to investigate the use of Galectin-3 in clinics to determine whether it can be used as a biomarker for detection of HF or only as a tool for monitoring the progression of the disease.

Our study had several important limitations. First, this study was cross-sectional and was conducted on a relatively small number of patients. Thus, further follow-up studies are required to demonstrate the prognostic value of Galectin-3. In addition, our study was a correlational study and was not sufficient to make mechanistic conclusions. Furthermore, an interval was there between performance of echocardiography and taking blood samples in some patients. Since Galectin-3 half-life was not identified in our patients, this time interval might have affected the results. However the process of myocardial fibrosis and remodeling (putatively reflected by galectin-3 concentrations) is not evanescent, and is likely to have been reflected in the associations between Galectin-3 values on admission and echocardiographic findings.

The findings of the present study showed no significant association between serum Galectin-3 concentration and EF and FC in the patients with compensated systolic HF. Therefore, our findings suggested that Galectin-3 could not be used as a marker of disease progression in the treated patients, which might be the result of medication use in these patients.

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Authors' Contribution

Roya Atabakhshian: Study concept and design and Drafting of the manuscript, Faranak Kazerouni: Analysis and interpretation of data, Fariba Raygan: Study concept and design, Hushang Amirrasouli and Ali Rahimipour: Study supervision, Nezhat Shakeri: Statistical analysis

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