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Supportive Data for Galectin-3 as a Prognostic Factor in Coronary Artery Disease

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ABSTRACT

Introduction: Galectin-3 is known as a biomarker in patients with heart failure. This protein participates in different mechanisms involved in atherosclerosis, including inflammation and plaque formation. This study was conducted to investigate whether this factor could be a predictive biomarker for the severity of atherosclerosis.

Material and Methods: The study group consisted of 80 patients with coronary atherosclerosis referred to the Department of Cardiac Surgery of Ghaem Hospital, affiliated to Mashhad University of Medical Sciences, Mashhad, Iran. The serum level of galectin-3 was measured using a commercial enzyme-linked immunosorbent assay kit. The severity of coronary artery disease (CAD), evaluated by the serum levels of galectin-3, was expressed as the number of involved vessels.

Results: Galectin-3 concentration was directly correlated with the number of involved vessels. The serum level of galectin-3 was significantly higher in patients with four involved vessels (20.76±7.20 ng/ml) than those with three-vessel disease (14.31±4.45 ng/ml); (P<0.001). Patients with three-vessel disease had higher levels of galectin-3 than patients with one and two involved vessels (7.20±4.09 ng/ml); (P<0.001).

Conclusion: The relationship between the number of vessels involved and the concentration of Galectin-3 was statistically significant. According to the results, serum galectin-3 level could be considered as a noninvasive tool for the diagnosis of preliminary assuming the coronary artery involvement. Although this study needs further detailed investigations with preferably larger sample size, the results of the present study highlighted the importance of this factor in CAD. This protein can help in early evaluations for preliminary determining the prognosis before the complementary aggressive intervention.

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Introduction

Coronary artery disease (CAD) is one of the most common causes of mortality and morbidity with a high global burden (1, 2). The management protocols of CAD are frequently reviewed and updated. In addition, new factors are introduced in revised versions of CAD management

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guidelines (3, 4). Atherosclerosis is the ultimate result of a multifactorial pathophysiology (5).

Systemic and local inflammatory events mediate all phases of atherosclerotic plaque development from initiation to plaque rupture (6). In recent years, mediators and effectors of these phases have been vastly studied in order to improve better understanding of the mechanism behind plaque formation. In this regard, the identification of new serum biomarkers is helpful for determination of patient at risk of future vascular events (6).

Galectin-3, which is a member of galectins and a family of β-galactoside-specific lectins, regulates many aspects of the inflammatory process, as well as various biological aspects such as cell to cell, and cell-extracellular matrix adhesion, cell growth and differentiation, cell cycle, signaling, apoptosis, and angiogenesis (7). In addition, this protein influences the strength of antigen activation in dendritic cells and controls acquired immunity including both T-helper cell 1 and T-helper cell 2 responses, depending on the context of the host immune response (8). An elevated serum level of galectin-3 is associated with several inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, and Behçet's disease (9).

In atherosclerotic sites, galectin-3 is often localized in macrophages and foam cells indicating its key role in the inflammatory process for plaque formation. There is a correlation between the expression of galectin-3 and phagocytosis. Therefore, this lectin may mediate the recognition and internalization of large particles and microorganisms that could play a role in atherogenesis (10).

Galectin-3 enhances inflammation by inducing the expression of a series of well-known proinflammatory molecules in plaque pathology (11). This study was performed to investigate the possible correlation between galectin-3 and CAD severity.

Materials and Methods

Study population and ethical considerations

The study was reviewed and approved by the Local Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran (IR.MUMS.REC.1394.95). A written informed consent was obtained from each patient before enrollment. The study group included 80 patients with advanced CAD referred to the Department of Cardiac Surgery from September 2014 to November 2015 (Ghaem university hospital, Mashhad, Iran).

The participants were assigned into three groups according to CAD severity based on the number of vessels grafted by the surgeon. The group A consisted of patients with one or two involved vessels. The patients in group B suffered from three-vessel disease. In addition, group C involved the patients with four diseased vessels. A questionnaire addressing the history of any diagnosed clinical conditions including inflammatory diseases, renal failure, or malignancies was filled for each participant.

The conventional cardiovascular risk factors including dyslipidemia, hypertension (defined as systolic blood pressure higher than 140 mmHg or diastolic blood pressure higher than 90 mmHg), and diabetes were evaluated for each patient. In order to check heart failure, ejection fraction was recorded from the patient's medical record.

Blood sampling and biochemical analysis

Blood samples were collected and sera were kept at -70°C until analysis. Thereafter, lipid profile comprising total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c), as well as high-sensitivity C-reactive protein (hsCRP) were determined for each patient.

Galectin-3 measurement

The serum concentration of galectin-3 was measured using an enzyme-linked immune-sorbent assay (ELISA) kit (Catalog #: ELH-Galectin-3) (RayBio, Norcross, United States) according to the manufacturer's instructions. Briefly, serum galectin-3 was captured firstly by the anti-galectin-3 antibody in pre-coated microplates. Then, the secondary anti-galectin-3 antibody was bound in a sandwich format by the captured galectin-3.

Afterward, a horseradish peroxidase (HRP)-conjugated streptavidin reagent was added to the wells and 3,3′,5,5′-Tetramethylbenzidine was used as the HRP substrate. The colorimetric reaction was measured at the wavelength of 450 nm using an ELISA plate reader (PerkinElmer, United States). The limit of detection was 0.6 ng/ml, and the reproducibility coefficient of variation of intra-assay and inter-assay was less than 10% and 12%, respectively.

Statistical Analysis

Data analysis was performed using Kolmogorov-Smirnov, one-way analysis of variance (ANOVA), independent samples t-test, Pearson's correlation coefficient, and Spearman's rank-order correlation tests in SPSS software, version 20. The normally distributed continuous variables were expressed as the mean and standard deviation. In all measurements, P-value less than 0.05 was considered statistically significant.

Results

The study population consisted of 52 males and 28 females with the mean age of 62.58±10.13

Table1. Clinical characteristics of the participants

Variable	One and two-vessel disease	Three-vessel disease	Four-vessel disease	P-value
Age (year) (mean±SD)	63.95±9.82	62.29±10.64	58.89±8.64	0.46*
Male (%)	15 (68.2%)	28 (59.6%)	7 (77.8%)	0.52**
Diabetes mellitus (%)	7 (35%)	15 (31.9%)	2 (33.3%)	0.97**
Hypertension (%)	12 (60%)	27 (60%)	4 (66.7%)	0.95**
Ejection fraction (%) (mean±SD)	39.55±13.88	39.33±9.45	34.44±9.50	0.44*
Triglyceride (mg/dl) (mean±SD)	144.55±71.63	147.40±61.08	158.89±51.18	0.84*
Total cholesterol (mg/dl) (mean±SD)	152.18±48.42	162.66±52.64	184.22±24.61	0.26*
HDL-C*** (mg/dl) (mean±SD)	26.18±9.75	26.64±9.74	32.89±9.83	0.18*
LDL-C**** (mg/dl) (mean±SD)	96.05±38.46	94.45±45.29	122.22±20.14	0.18*

* Analysis of variance, ** Chi-squared, *** High-density lipoprotein cholesterol, **** Low-density lipoprotein cholesterol

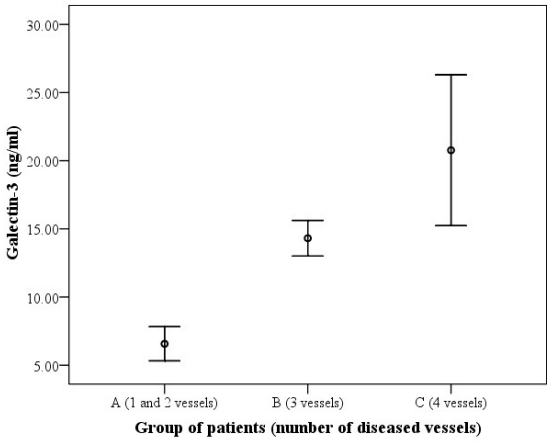

Figure 1. Levels of galectin-3 in the patients' sera (The values are mean (95% CI)

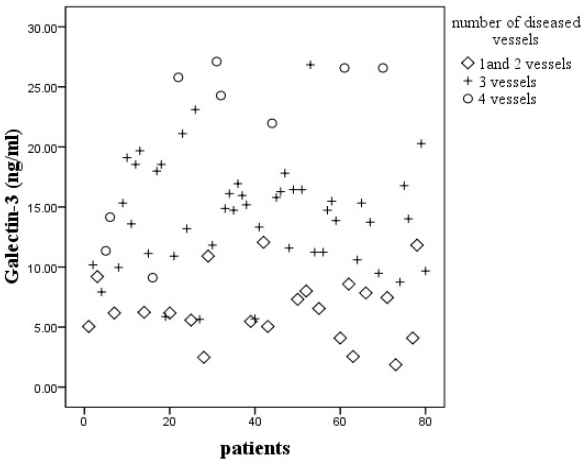

Figure 2. Scatter plot of galectin-3 in patients with coronary artery disease according to the number of diseased vessels

Table 2. Linear regression analyze for predictive effect of CAD severity on galectin-3 regarding confounding factors

Predictors	Unstandardized B	Std. Error	P value	95.0% Confidence Interval for B	
				Lower Bound	Upper Bound
Diseased vessels	7.25	0.84	0.001	5.521	8.81
Sex (male)	0.8	1.05	0.43	-1.26	2.94
age	-0.06	0.05	0.18	-0.15	0.03
hsCRP	-0.001	0.006	0.87	-0.01	0.01
Triglyceride	0.01	0.009	0.11	-0.006	0.03
Total cholesterol	0.009	0.01	0.54	-0.02	0.04
HDL-C	0.018	0.06	0.77	-0.1	0.1
LDL-C	0.01	0.01	0.41	-0.02	0.05

years old. The demographic characteristics of patients are shown in Table 1. The mean serum level of galectin-3 was 12.87±6.26 ng/ml (ranging between 1.87 and 27.11 ng/ml). As shown in Fig. 1, ANOVA analysis showed a significant difference between the groups in terms of galectin-3 concentration (P<0.001).

In addition, a direct correlation was found between the serum level of galectin-3 and the number of diseased vessels (P<0.001; r=0.676; Figure 2). The results of regression analyze displayed that several factors such as age and gender, as well as the serum levels of LDL-c, HDL-c, total cholesterol, triglyceride, and hsCRP, had no effect on the relationship between galectin-3 concentration and CAD severity (Table 2).

There was a direct correlation between the serum levels of galectin-3 and triglyceride (P=0.02, r=0.257), total cholesterol (P=0.002, r=0.340), and LDL-c (P=0.004, r=0.317). No significant differences were found in galectin-3 levels in terms of gender and the history of diabetes and hypertension (P=0.474, P=0.08, and P=0.96, respectively). Additionally, there was no significant correlation between age, sex, ejection fraction and the serum levels of HDL-c, hsCRP, and galectin-3. Regarding the obtained results, no significant correlation was observed between serum levels of hsCRP and other variables.

Discussion

The results of the current study revealed a correlation between the serum levels of galectin-3 and severity of CAD, which is expressed by the number of diseased vessels. Patients with one- or two-vessel disease were grouped together

because of the limited number of these patients. It was reported that galectin-3 concentration is increased in several other clinical conditions including malignancies, inflammatory diseases, and Alzheimer's disease (10-12).

We excluded these confounders in our patients using a questionnaire checklist addressing any diagnosed diseases affecting our results.

Galectin-3 was previously suggested as a possible prognostic marker of acute coronary syndrome (ACS). The study reported rather higher plasma levels of galectin-3 in patients with significant stenosis based on ACS (13); however, our study was conducted according to the number of the diseased vessels (13).

In line with our results, it was proposed that serum levels of galectin-3 might be a useful biomarker of coronary atherosclerotic involvement in patients undergoing diagnostic coronary angiography with the suspicion of CAD for the first time. Based on the angiography report, the galectin-3 level was correlated with the number of diseased vessels (14). It was shown that the circulating levels of galectin-3 is directly associated with type II diabetes and could be reduced by anti-diabetic medications (15).

A significant correlation was reported between the serum levels of galectin-3 and the number of diseased vessels and plaques (16). Moreover, a relationship between galectin-3 levels and vascular complications in diabetic patients, including diabetic nephropathy and peripheral vascular disease was observed (17). Nevertheless, in this study, no difference in the level of galectin-3 was found between diabetic and non-diabetic participants.

According to the literature, the levels of galectin-3 in normal populations are between the ranges of 3 and 10 ng/ml (18-20). The correlation between the serum level of galectin-3 with gender remains controversial; however, a higher concentration of galectin-3 in elderly has been shown (21, 22). Consistent with the findings obtained by Ginsberg et al., we observed higher levels of galectin-3 in males. Nevertheless, there was no correlation between the serum levels of galectin-3 and age in our study..

This result might be due to the limited age range of our study population. In line with the evidence, the results of this study demonstrated no association between galectin-3 and ejection fraction in patients with CAD (13, 23). However, the relationship between galectin-3 concentration and heart failure is confirmed, and this factor is a marker in these patients (24, 25).

Triglyceride and cholesterol levels were directly correlated with galectin-3 concentrations (P=0.002). This correlation might be as a result of the fact that higher triglyceride and cholesterol levels trigger molecular events leading to increasing galectin-3 production. Nevertheless, the exact underlying molecular mechanisms involved in such a process remains to be further elucidated; though several mechanisms have been suggested.

Atherosclerosis is recognized as a chronic inflammatory condition. Macrophages recruited to the vascular intima absorb lipoprotein particles such as LDL-c and form foam cells. These cells coordinate lesion development by promoting inflammation and smooth muscle cell proliferation (26). In vitro studies determined that the expression of galectin-3 is up-regulated when monocytes differentiate to macrophages and when macrophages are loaded with lipids and transformed into foam cells (27). Therefore, the increased levels of lipids might result in the production of galectin-3.

The presence of galectin-3 in most foam cells in the atherosclerotic injuries in hypercholesterolemic rabbits suggests a connection between lipid profile and serum level of galectin-3 (28). In addition to the previous mechanism, macrophage foam cells may secrete galectin-3, which is a strong chemoattractant for monocytes and macrophages, leading to the migrations of these cells to the artery wall.

Galectin-3 chemotactic activity along with the endocytosis of modified lipoproteins results in intracellular cholesterol reposition and binding that has a remarkable role in atherosclerosis (28). We found higher serum amounts of galectin-3 in patients with the multi-vessels disease compared to those reporting one- or two-vessel disease. Accordingly, it seems reasonable that galectin-3 may play a continuous role in developing plaque and CAD. Regardless of the mechanistic issues, the increased galectin-3 levels in CAD patients should be further investigated in larger studies, particularly focusing on its possible clinical applications as a promising diagnostic and prognostic biomarker.

Strengths and Limitations of the Study

One of the limitations of this study was a small sample size, which was due to the limited number of patients admitted to the department. Secondly, the lack of repeated measurements of galectin-3 over the time was another limitation. Third, as many patient-based studies, the lack of information on possible ignored underlying conditions that may contribute to the elevation of galectin-3 might be considered as confounding factors. However, we addressed the main possible confounders in our inclusion criteria and it seems unlikely that minor remaining conditions could entirely affect the results.

Conclusion

Regarding the results of the present study, significant correlations were found between the serum level of galectin-3 and the number of diseased vessels. This factor has an important role in the progression of atherosclerosis in patients with CAD. In addition, it can be considered as a biomarker in the management of these patients. Further studies with larger sample size are recommended to assess the clinical utility of galectin-3 as a prognostic biomarker.

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Conflict of Interest

The authors declare no conflict of interest.

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