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Galectin-3: A biochemical marker to detect paroxysmal atrial fibrillation?

Abstract

Purpose: Atrial fibrillation (AF) is the most common form of arrhythmia. AF leads to electrical remodelling and fibrosis of the atria; however, the mechanism(s) remain poorly understood. Galectin-3 is a potential mediator of cardiac fibrosis. The present study aimed to examine the relationship between serum galectin-3 levels and paroxysmal AF.

Methods: Forty-six patients with paroxysmal AF and preserved left ventricular systolic function, and 38 age- and gender-matched control subjects, were involved in the study. Serum galectin-3 levels were analyzed with an enzyme-linked immunosorbent assay (ELISA).

Results: Serum galectin-3 levels (median 1.38 ng/mL; 1.21 ng/mL-1.87 ng/mL; $p < 0.001$) were significantly elevated in patients with paroxysmal AF compared with the control. Left atrial diameter was significantly higher in patients with paroxysmal AF (41.2 ± 3.0 mm *vs.* 39.6 ± 3.3 mm). Left atrial diameter was found to be significantly correlated with serum galectin-3 levels in patients with paroxysmal AF ($r = 0.378$, $p = 0.001$).

Conclusion: Serum galectin-3 levels are significantly elevated and significantly correlated with left atrial diameter in patients with paroxysmal AF.

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Atrial fibrillation, the most common arrhythmia encountered in clinical practice, is a worldwide health care problem due to its constantly increasing prevalence, high morbidity and mortality. It affects about 1.5% of the population in the developed world [1]. Atrial fibrillation (AF) may present as paroxysmal (self-terminating AF within seven days), persistent (lasts greater than seven days) or a long-standing persistent AF (continuous AF for 12 months). Patients with AF are at increased risk of severe complications, including dementia, heart failure, stroke and premature death, thus generating a substantial clinical and economic burden on the community and the health care systems [2,3].

Pathophysiological mechanisms that dominate cardiac remodelling and heart failure also underlie the remodelling of atrial structure. In particular, atrial interstitial fibrosis is a key contributor to AF substrate. The exact pathways leading to atrial fibrosis remain undefined, but a number of studies have shown involvement of the renin angiotensin axis and transforming growth factor $\beta 1$ (TGF- $\beta 1$); a key factor in the development of myocardial fibrosis [4-7].

Galectin-3 is a member of the beta-galactosidase-binding lectin family, which plays numerous important regulatory roles in inflammation, immunological response and cancer [8-9]. Galectin-3 also functions in a number of fibrotic processes, including cardiac fibrosis. The role of galectin-3 in the pathogenesis of cardiac fibrosis involves recruitment of macrophages, myofibroblasts and fibroblasts into the myocardium, resulting in cellular proliferation and collagen accumulation. A number of studies have focused on the association between galectin-3 levels and cardiac fibrosis in heart failure [10-11] and another study has shown elevated plasma galectin-3 to be associated with incident heart failure [12]. Recent reports show higher circulating galectin-3 concentrations are associated with increased risk for AF development [13,14]. Although the association between AF and atrial fibrosis has been highlighted in the literature, the role of galectin-3 in the pathogenesis of these conditions has not yet been soundly elucidated [13,14]. The present study focused on determining whether serum galectin-3 levels were elevated in patients with paroxysmal AF and/or preserved left ventricular function.

Materials and Methods

Forty-six patients with paroxysmal AF and preserved left ventricular (LV) function and thirty-eight age- and gender-matched control subjects were enrolled in the study. Paroxysmal AF, also termed intermittent AF, is defined as an episode of AF that terminates spontaneously or with

intervention in less than seven days. The control group consisted of subjects without histories of AF who underwent routine screening visits in the outpatient clinics. The participants had a comprehensive clinical examination during the study visit. Exclusion criteria included left ventricular ejection fraction (LVEF) <50%, moderate to severe valvular disease, pericardial disease, renal disease, diabetes mellitus, gastrointestinal disease, pulmonary disease, acute infection, chronic inflammation, congenital heart disease, alcohol consumption, abnormal thyroid function and cancer. All baseline characteristics and biochemical analyses (serum creatinine, total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high density lipoprotein cholesterol [HDL-C], triglyceride [TG], and blood cell count) were recorded before the study.

A total of participants included in this analysis also had routine M-mode and two-dimensional echocardiography. LV end-diastolic diameter, LV end-systolic diameter, interventricular septum thickness, posterior wall thickness, and left atrial (LA) diameter were determined from 2D targeted M-mode echocardiography based on the criteria of the American Society of Echocardiography. LV ejection fraction (EF) was calculated using the biplane method of disks (modified Simpson's rule) [15].

Informed consent was obtained from each patient before enrollment. The study was in compliance with the principles outlined in the Declaration of Helsinki. The study protocol was reviewed and approved by ethics committee of Turgut Ozal University, and each patient signed an informed consent form before the procedure.

Blood samples were collected via venipuncture, centrifuged and frozen at -80°C until the assays were performed. Serum galectin-3 concentrations were analyzed in the plasma with an enzyme-linked immunosorbent assay (ELISA, BG Medicine Inc., Waltham, MA, U.S.A). The assay quantitatively measured the concentration of human galectin-3 levels in EDTA serum, had high sensitivity, and exhibited no cross-reactivity with collagens or other members of the galectin family. Commonly-used medication, including ACE inhibitors, beta blockers, acetylsalicylate, warfarin and digoxin, do not interfere with the assay. Calibration and standardization of the assay were carried out according to the manufacturer's protocol. The mean intra-assay coefficient of variation was <7%.

Normally distributed continuous parameters are presented as mean $\pm$ SD. Other continuous variables are presented using median (minimum-maximum), and categorical variables are given as percentages. The Shapiro-Wilk test was used to verify

the normality of distribution of continuous variables; the Mann–Whitney U test was used for continuous variables; the χ^2 test was used for categorical variables. Correlations were assessed using the Spearman rank test. Univariate and multivariate logistic regression analyses were performed to determine the independent predictors of paroxysmal AF. Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cut-off value of galectin-3 with maximum sensitivity and specificity for the diagnosis of paroxysmal AF patients. For all statistical analyses, a *p* value of <0.05 was considered significant. All analyses were performed using SPSS® software (version 16.0; SPSS Inc., Chicago, Ill., U.S.A).

Results

A total of 84 patients, consisting of 46 with paroxysmal AF with preserved LV function and 38 without paroxysmal AF (PAF) as control, were included in the study. The mean age of AF patients was 59 years, and the mean age of the control was

59 years; there was no significant difference between the groups (59.1 ± 7.7 *vs.* 59.8 ± 7.3 , $p=0.787$). Baseline demographic, clinical and laboratory data of the study population are listed in Table 1. There were no statistical differences between the two groups in terms of gender, body mass index (BMI) and smoking. Hypertension was also an unchanged parameter (PAF patients *vs.* no PAF controls). In terms of antihypertensive drugs, no statistical difference was detected between the two groups (PAF patients *vs.* no PAF controls, angiotensin converting enzyme (ACE) inhibitors-angiotensin II receptor blockade; 60% *vs.* 68%, $p=0.5$, beta blockers; 100% *vs.* 94% $.7$, $p=0.2$, calcium channel blockers; 10.9% *vs.* 10.5%, $p=0.9$).

Serum levels of galectin-3 (median 1.38 ng/mL; 1.21 ng/mL-1.87 ng/mL; $p<0.001$) and left atrial diameter were significantly elevated in patients with paroxysmal AF compared to the control (Figure 1, 41.2 ± 3.0 mm *vs.* 39.6 ± 3.3 mm, $p=0.02$). In addition, echocardiographic parameters of the study population are listed in Table 2.

TABLE 1. Baseline demographic, clinical and laboratory parameters of the study population (n=84)

	Paroxysmal Atrial Fibrillation group (n= 46)	Control group (n=38)	p
Age (years)	59 (59.1 ± 7.7)	59 (59.8 ± 7.3)	0.78
Gender			
Males	22	15	NS
Females	24	23	0.44
Body mass index (kg/m ²)	24.4 ± 1.6	24.3 ± 1.5	NS
Active smoker %	23.9	39.5	0.15
Hypertension %	47.8	68.4	0.07
Serum biochemical markers			
Hemoglobin (g/dL)	13.6 ± 1.5	13.5 ± 1.5	0.72
White blood cell count ($\times 10^6$ /mL)	7.8 ± 1.7	8.1 ± 1.7	0.42
Platelets ($\times 10^3$ /uL)	217 ± 61	228 ± 74	0.48
Creatinine (mg/dL)	0.88 ± 0.17	0.96 ± 1.16	0.03
Total cholesterol (mg/dL)	189 ± 33	160 ± 37	0.02
HDL cholesterol (mg/dL)	42 ± 7	37 ± 10	0.01
LDL cholesterol (mg/dL)	117 ± 35	95 ± 36	0.02
Triglycerides (mg/dL)	157 ± 63	144 ± 36	0.46

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. NS, non-significant

FIGURE 1. Comparison of plasma galectin-3 levels among groups

FIGURE 2. Graph showing the correlation between left atrial diameter and serum galectin-3 levels

In Spearman correlation analyses, left atrial diameters were found to be in significant correlation with serum galectin-3 levels in patients with AF ($r = 0.378$, $p = 0.001$) (Figure 2). In addition, total cholesterol and low density lipoprotein cholesterol (LDL-C) were found to be positive correlation with serum galectin-3 levels in patients with paroxysmal AF ($r = 0.28$, $p = 0.008$; $r = 0.27$, $p = 0.01$). There was no correlation between serum galectin-3 level and age ($r = -0.04$, $p = 0.73$).

A logistic regression analysis was performed to detect the factors affecting the development of paroxysmal AF, in which risk of development of male sex paroxysmal AF increased 5.9 fold ($B = 1.7$, $p = 0.047$, odds ratio: 5.9). A unit decrease in serum galectin-level reduced the risk of paroxysmal AF by 2.8%. ($B = -0.029$, $p = 0.001$). The left atrial diameters were found to be statistically significant effect of galectin-3 by linear regression analysis ($R^2 = 0.216$, $p < 0.001$).

Serum galectin-3 levels alone predicted 89.7% of paroxysmal AF patients (area under the curve, $AUC = 0.897$, $p < 0.001$, 95% CI). ROC curve analysis suggested that the optimal galectin-3 cut-off point for diagnosis of paroxysmal AF was 1.28 ng/mL with acceptable sensitivity and specificity (Figure 3).

Discussion

The most important observation from the current study is the serum galectin-3 significantly increased in patients with paroxysmal AF with normal left ventricular function. In addition, the levels correlated with left atrial diameters in the whole group.

Atrial fibrillation has been shown to lead to electrical remodelling and fibrosis of atria [16], and atrial electrical remodelling and functional changes in subcellular atrial myocyte function in short term lead to abbreviation of atrial action potential duration and refractory period [17]. Histological abnormalities observed (atrial myocyte

TABLE 2. Serum galectin-3 levels and echocardiographic parameters of the study population (n=84)

	Paroxysmal Atrial Fibrillation group (n= 46)	Control group (n=38)	p
Galectin-3 (ng/mL)	1.38 (1.21-1.87)	1.21 (0.88-1.37)	<0.001
Left atrial diameter (mm)	41.2±3.0	39.6±3.3	0.02
Left ventricular end-diastolic pressure (mmHg)	4.6±0.4	4.6±0.3	NS
Diameter (cm)			
Left ventricular ejection fraction (%)	63.7±3	63.8±3	NS
Thickness of interventricular septum (mm)	9.5±1.2	9.4±1.2	NS

NS: non-significant

FIGURE 3. ROC curve analysis to determine optimal cut-off value for plasma galectin-3 in paroxysmal AF

degeneration and necrosis and patchy fibrosis) may represent an organic substrate for the electrogenic mechanisms involved in paroxysmal AF [18]. Fibrosis of left atrial wall have been found by delayed-enhancement MRI in patients with paroxysmal AF [19].

Paroxysmal AF commonly precedes chronic AF. AF is initiated by rapid firing (or triggers) from the pulmonary veins. The left atrial free wall around the pulmonary vein area presents particularly strong interstitial fibrotic changes [20]. Catheter ablation of AF depends in large part on the electrical isolation of the pulmonary veins from the remainder of the atrium. Patients with AF also display increased atrial fibrous tissue content, increased expression of collagen I and III, up-regulation of matrix metalloproteinase protein-2 (MMP-2) and down-regulation of the tissue inhibitor of metalloproteinase. Expression of the active form of MMP-9 and of monocyte chemoattractant protein-1, an inflammatory mediator, is increased in AF patients [21,22].

Galectin-3, a macrophage-derived mediator, is definitely a potential mediator of cardiac fibrosis. Previous studies indicate that an early increase in galectin-3 expression identifies failure-prone hypertrophied hearts. Galectin-3 induces cardiac fibroblast proliferation, a consequent collagen deposition, and finally ventricular dysfunction [23]. Gurses *et al.* carried out a case-control study in patients with paroxysmal or persistent atrial fibrillation and preserved left ventricular systolic

function. The serum levels of galectin-3 were found to be higher in patients with atrial fibrillation than in healthy control subjects [14]. Recently, Yalcin *et al.* performed a nested-case control study including patients with paroxysmal AF who underwent delayed-enhancement magnetic resonance imaging prior to cryoballoon-based AF ablation. A significant correlation was found between serum levels of galectin-3 and extent of left atrium fibrotic burden [24]. The results of the present study showed that galectin-3 levels increased somewhat in paroxysmal AF patients. Furthermore, a significant correlation was also found between galectin-3 values and left atrial diameters. These results strongly suggest that serum galectin-3 levels may be an indicator for AF. The levels, in particular, may reflect the structural remodelling of left atrial atrium in paroxysmal AF.

A limitation of the study is that the galectin-3 concentrations reflect other fibrotic conditions, since it is not specific to the cardiovascular system.

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