Association between galectin-3 levels and isolated coronary artery ectasia

Gonul Aciksari, Turgut Uygun, Adem Atici, Kurtulus Aciksari, Aybala Erek Toprak, Imran Onur, Yusuf Yılmaz, Muhammed Esad Cekin, Emre Yalçınkaya, Ebuzer Aydin, Mustafa Caliskan

Abstract

Background: Coronary artery ectasia (CAE) is a well-recognised disorder characterised by abnormal dilation of the coronary arteries. Underlying mechanisms associated with abnormal luminal dilation in CAE remain to be elucidated. However, histopathological features resemble those of coronary atherosclerosis. Galectin-3 (Gal-3) is a valuable biomarker for both progression and destabilisation of atherosclerotic lesions. To the best of our knowledge, there is no study in the literature examining serum Gal-3 levels in patients with isolated CAE. In the present study, therefore, we aimed to investigate the possible relationship between serum Gal-3 levels and isolated CAE.

Methods: Between March 2016 and March 2017 this prospective, case-controlled study included a total of 49 consecutive isolated CAE patients (31 males, 18 females) diagnosed with CAE by coronary angiography at the catheter laboratory of Medeniyet University, Goztepe Training and Research Hospital, and 43 individuals (19 males, 24 females) with normal coronary arteries. Physical examination, medical history, blood biochemistry and transthoracic echocardiography were performed in both groups. Serum concentrations of Gal-3 were measured using blood samples.

Results: Median Gal-3 levels were significantly higher in isolated CAE patients than in the controls [23.2 (23.9 ± 7.1) vs 16.8 ng/ml (17.8 ± 7.3); \( p < 0.001 \)]. According to the Markis classification, the extent of CAE was not correlated with Gal-3 levels (\( p = 0.41 \)). Multivariate regression analysis revealed that Gal-3 concentration was an independent predictor of isolated CAE.

Conclusion: Our study results suggest that Gal-3 serum concentrations significantly increased in patients with isolated CAE, indicating that Gal-3 may be involved in the pathogenesis of isolated CAE.

Keywords: isolated coronary artery ectasia, galectin-3, atherosclerosis

Coronary artery ectasia (CAE) is defined as the dilatation of coronary arteries to a diameter of 1.5 times or greater than that of the adjacent normal coronary artery. Among patients undergoing coronary angiography, 0.3 to 4.9% have been reported to have CAE. Isolated CAE, which is an uncommon angiographic finding with varying presentation patterns, is defined as pure ectasia without significant coronary artery stenosis, accounting for 0.1 to 0.79% of all cases with CAE. More than half of the patients with CAE have coronary atherosclerosis, although concomitant connective tissue disorder or vasculitis may present in certain patients. Histopathological examination of the ectatic segments reveals extensive atherosclerotic alterations as well as disruption of the media layer of the vessel wall. Risk factors for atherosclerosis have also been found to be pertinent to patients with CAE.
findings have suggested that, despite having a varying aetiologies, CAE may be considered a variant of coronary atherosclerosis. On the other hand, underlying mechanisms associated with abnormal luminal dilation in CAE patients remain to be elucidated. In addition, CAE may lead to increased cardiac morbidity and mortality through a number of mechanisms, including low coronary flow, coronary vasospasm and dissection formation.

Galectin-3 (Gal-3) is a galactoside-binding lectin, also known as Mac-2 antigen, which is expressed by macrophages, fibroblasts, activated T-lymphocytes and endothelial cells. It is involved in a number of biological processes, including cell growth, adhesion, apoptosis and phagocytosis, as well as in pathological processes such as inflammation, fibrosis and atherosclerosis. It has been suggested that it plays a key role in atherogenesis through increased phagocytosis and induction of the proliferation of vascular smooth muscle cells (VSMCs).

In addition, Gal-3 has been shown to play a central pathophysiological role in the development of cardiovascular diseases by enhancing cardiac hypertrophy, fibrosis, arterial stiffness, inflammation and oxidative stress during cardiovascular remodelling. Recent studies have demonstrated not only the potential role of Gal-3 in atherogenesis, but also an association between increased Gal-3 expression and the development of atherogenesis.

In the literature, there are several studies carried out in animal and human models. In an animal model, inhibition of Gal-3 was found to be associated with decreased atherosclerotic plaque volume in mice with apolipoprotein E deficiency. In an animal model, inhibition of Gal-3 was found to be associated with decreased atherosclerotic plaque volume in mice with apolipoprotein E deficiency. In a recent study, the utility of Gal-3 as a diagnostic and prognostic marker for cardiovascular conditions was reported. In the light of these data, Gal-3, which is associated with inflammation and atherosclerosis, may play a major role in coronary artery disease (CAD) as well as in CAE, which is considered to represent a variant of CAD and to have a similar aetiopathology and clinical course.

To the best of our knowledge, there is no study in the literature examining serum Gal-3 levels in patients with isolated CAE. In this study therefore we aimed to investigate the possible relationship between serum Gal-3 levels and isolated CAE.

**Methods**

In this prospective, case-controlled study, we included a total of 49 consecutive isolated CAE patients diagnosed with CAE by coronary angiography at the catheter laboratory of Medeniyet University, Göztepe Training and Research Hospital between March 2016 and March 2017. The control group consisted of a total of 43 individuals with normal coronary arteries. Detailed demographic data were obtained from each participant. Physical examination, medical history, blood biochemistry and transthoracic echocardiography were performed in both groups to rule out systemic conditions.

Hypertension was defined as a systolic blood pressure of ≥ 140 mmHg and/or a diastolic blood pressure of ≥ 90 mmHg or current use of hypertensive agents. Diabetes was defined as fasting blood glucose of > 126 mg/dl (6.99 mmol/l) or current use of a diet or oral antidiabetic agents to lower blood glucose levels. The use of anti-hyperlipidaemic agents or a fasting plasma total cholesterol of > 200 mg/dl (5.18 mmol/l) or a low-density lipoprotein cholesterol (LDL-C) of > 130 mg/dl (3.37 mmol/l) were considered to denote hyperlipidaemia.

Exclusion criteria were as follows: the presence of acute coronary syndrome, left ventricular dysfunction (ejection fraction < 50%), left ventricular hypertrophy, valvular heart disease, peripheral vascular disease, congenital cardiac disease, hepatic, renal, inflammatory or connective tissue, infectious or autoimmune disorders and malignancy.

A written informed consent was obtained from each participant. The study protocol was approved by the local ethics committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Morning venous blood samples were obtained after 12 hours of fasting. Serum glucose, creatinine, high-sensitivity C-reactive protein (hs-CRP), total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using standard laboratory methods. Additional blood sampling was performed to measure serum Gal-3 concentrations.

Blood samples were immediately centrifuged at 1 000 μg for 15 minutes and sera were separated and stored at −80°C until Gal-3 assays. Serum Gal-3 concentrations were analysed in a blinded manner using a commercial enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer’s instructions (EBioscience, CA, USA). The values were normalised to the standard curve. Intra-assay and inter-assay variance for Gal-3 at a Gal-3 concentration of 1.5 ng/ml were 6.4 and 11.4%, respectively.

Indications for coronary angiography were the presence of typical angina pectoris symptoms or suspicious or positive test results in non-invasive methods to assess coronary ischaemia (dobutamine stress echocardiography, treadmill test or myocardial perfusion scintigraphy). Coronary angiography was performed using the Judkins technique with left heart catheterisation and without the use of nitroglycerine (Siemens, Medical Solutions 2007, Munich, Germany). The angiography results were based on agreement between two experienced angiography specialists blinded to the study groups. Isolated CAE was defined as the dilatation of coronary arteries with a diameter of 1.5 times or greater than that of the adjacent normal coronary artery without significant stenotic lesions.

In the absence of an identifiable adjacent normal segment, the mean diameter of the corresponding coronary segment in the control group was accepted as the normal value. The severity of ectasia was defined according to the Markis classification on the basis of the extent of ectatic involvement, as follows, in decreasing order of severity: diffuse ectasia in two or three vessels (type 1); diffuse involvement in one vessel and segmental involvement in another vessel (type 2); diffuse involvement in a single vessel (type 3); and segmental or localised involvement (type 4).

**Statistical analysis**

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 22.0 software (IBM Corp, Armonk, NY, USA). Descriptive data are expressed as mean ± standard deviation (SD), median (min–max) or number and frequency. The distribution of variables was analysed using the Kolmogorov-Smirnov test. The Mann–Whitney U-test and independent samples t-test were used for the analysis of
Glycated haemoglobin (HbA1c) and Gal-3 levels were significantly higher in patients with isolated CAE compared to the controls ($p < 0.001$). 

Ectasia occurred most frequently in the left anterior descending artery (32%), followed by the circumflex artery (30%), right coronary artery (26%) and left main coronary artery (15%). Type 4 was the most common type of ectasia according to the Markis classification in 36% of patients. Median Gal-3 levels were significantly higher in isolated CAE patients than in the controls [23.2 (23.9 ± 7.1) vs 16.8 ng/ml (17.8 ± 7.3); $p < 0.001$ (Table 1, Fig. 1).

In patients with isolated CAE, there was no significant association between Gal-3 levels and Markis classification or the number of involved vessels ($p = 0.41$ and 0.093, respectively; Table 3). In univariate analysis, natural log (In) Gal-3 was found to have a significant impact for differentiating controls and patients ($p < 0.05$). Multivariate logistic regression analysis demonstrated that concentrations of In Gal-3 were an independent predictor of CAE, coronary artery ectasia.

**Discussion**

In this study, we investigated the relationship between serum Gal-3 levels and isolated CAE. Our results showed significantly increased Gal-3 levels as a novel cardiac biomarker among quantitative data. The chi-squared test was used for analysis of qualitative data, and Fisher’s exact test was used when the chi-squared test was not suitable. The impact level and cut-off values were assessed using receiver operating characteristic (ROC) curves. The impact level was examined using univariate and multivariate logistic regression analyses. A $p$-value of $< 0.05$ was considered statistically significant.

### Results

In the study population there were 31 males and 18 females in the isolated CAE group and 19 males and 24 females in the control group. There was no significant difference in age, gender, systolic arterial pressure, diastolic arterial pressure, body mass index, hypertension, hyperlipidaemia, cigarette and alcohol use and family history between the groups ($p > 0.05$). However, median glycated haemoglobin (HbA1c) and Gal-3 levels were significantly higher in patients with isolated CAE compared to the controls ($p < 0.05$). Demographic, clinical and laboratory characteristics of the study population are shown in Table 1.

Table 2 shows frequency of distribution of ectatic coronary arteries and the Markis classification in isolated CAE patients.
isolated CAE patients, compared to controls. However, there was no significant association between serum Gal-3 levels and the extent of isolated CAE.

Despite uncertainties regarding the pathophysiological mechanisms of CAE, the frequent occurrence of concurrent CAD and the presence of atheromatous ulcerations in ectatic segments suggest an important role for atherosclerosis in the development of CAE.\(^2\) Degeneration in the media layer of the coronary artery, a common denominator of all conditions resulting in coronary ectasia, has been reported to be associated with advanced atherosclerosis.\(^2^3\)

Atherosclerosis typically presents itself as a narrowing of the vessel lumen. However, post mortem and intravascular ultrasound (IVUS) studies have demonstrated that atherosclerotic plaque may also advance into the medial layer and external elastic membrane (EEM) without a marked narrowing in the vessel lumen, indicating that the vessel wall may react to atherosclerosis in two different ways.\(^2\) While negative remodelling results in reduced vessel lumen diameter, positive remodelling is associated with the propagation of plaque towards the EEM. The latter may result in dilation without significant lumen narrowing and obstructive CAD.\(^2\) Formation of foam cells is directly linked to the weakened connective tissue of the arterial wall. Macrophages secrete elastase in response to endocytosis of modified LDL-C. Weakening of the coronary artery, particularly caused by protease activity, may lead to positive remodelling.\(^2^3\)\(^2^4\) It has been proposed that CAE may represent an exaggeration of positive remodelling. Other studies examining the pathogenesis of CAE have shown that endothelial injury due to atherosclerosis may lead to degeneration in the media layer of the vessel via activation of macrophages and inflammatory mediators such as metalloproteins and that these structural changes may result in segmental vessel dilation through the release of nitric oxide and other vasodilator agents from the endothelium.\(^2^5\) In a post mortem case report by Markis \textit{et al.},\(^2\) diffuse hyalinisation, fatty accumulation, disrupted intima and media layers, focal calcification and fibrosis, cholesterol crystals and intramural haemorrhage were found, while CAE was not present in areas where the media layer was grossly intact.

Gal-3 belongs to the family of soluble β-galactoside-binding lectins. Although Gal-3 is primarily released by activated macrophages, it can be also synthesised by T-lymphocytes, endothelial cells and fibroblasts.\(^2^\) Gal-3 also plays a role in the conversion of monocytes to macrophages and macrophages to foam cells. It has been found to be expressed in foam cells and macrophages in atherosclerotic lesions.\(^2^\)\(^3\)\(^7\)\(^2\)\(^8\) It also enhances entry of this cell into the arterial wall, resulting in intracellular cholesterol deposition through augmentation of the internalisation of advanced glycation end-products and endocytotic uptake of modified lipoproteins.\(^2^\)\(^9\)\(^3\)\(^0\) Furthermore, Gal-3 aggravates vascular inflammation, leading to the expression of a series of chemokines and other pro-inflammatory molecules from macrophages.\(^3\)

In addition, an important process that contributes to plaque instability and the progression of atherosclerotic lesions is the phenotypic switch of VSMCs from a differentiated state to a dedifferentiated state. \textit{In vitro} experiments have shown that Gal-3 plays a role in the phenotypic switch of VSMCs.\(^2\)\(^7\)\(^2\) Due to the aforementioned mechanisms, Gal-3 is recommended as a biomarker for the progression and imbalance of atherosclerotic plaques.\(^3\)\(^3\)\(^3\)

The impact of Gal-3 on both atherosclerotic plaque formation and destabilisation has been confirmed in several studies.\(^2^\)\(^6\)\(^3\)\(^2\)\(^3\)\(^4\) In one study, MacKinnon \textit{et al.}\(^3\)\(^7\) reported that pharmacological inhibition of Gal-3 in a well-characterised mouse model of atherosclerosis reduced plaque development. In another study, Tsai \textit{et al.}\(^3\)\(^7\) found a significant increase in serum Gal-3 levels in patients with ST-segment elevation myocardial infarction (STEMI). In addition, patients with STEMI undergoing primary

---

**Table 3. Galectin-3 levels according to the number of affected ectatic arteries and Markis classification**

<table>
<thead>
<tr>
<th>Markis classification</th>
<th>Galectin-3</th>
<th>1-vessel disease</th>
<th>2-vessel disease</th>
<th>3-vessel disease</th>
<th>4-vessel disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Median</td>
<td>Min–Max</td>
<td>p-value</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Type I</td>
<td>22.4 ± 5.5</td>
<td>22.7</td>
<td>12–34</td>
<td>0.418</td>
<td>25.6 ± 7.3</td>
</tr>
<tr>
<td>Type II</td>
<td>25.4 ± 7.2</td>
<td>25.1</td>
<td>18–39</td>
<td></td>
<td>25.4 ± 7.2</td>
</tr>
<tr>
<td>Type III</td>
<td>19.5 ± 6.4</td>
<td>18.2</td>
<td>10–34</td>
<td></td>
<td>19.5 ± 6.4</td>
</tr>
<tr>
<td>Type IV</td>
<td>22.9 ± 7.3</td>
<td>21.8</td>
<td>10–42</td>
<td></td>
<td>22.9 ± 7.3</td>
</tr>
</tbody>
</table>

*Kruskal–Wallis test, SD: standard deviation.

**Table 4. Variables associated with CAE according to univariate and multivariate logistic regression analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate model</th>
<th>Multivariate model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Ln age</td>
<td>1.05</td>
<td>1.00–1.11</td>
</tr>
<tr>
<td>Ln gender</td>
<td>2.18</td>
<td>0.94–5.02</td>
</tr>
<tr>
<td>Ln HbA(_1\c))</td>
<td>1.13</td>
<td>0.74–1.74</td>
</tr>
<tr>
<td>Ln Gal-3)</td>
<td>1.13</td>
<td>1.06–1.21</td>
</tr>
</tbody>
</table>

*ORs for continuous variables are expressed in per one standard deviation change in the natural log-transformed variables. Ln, natural log; CI, confidence interval; OR, odds ratio; HbA\(_1\c), glycated haemoglobin; Gal-3, galectin-3.*
percutaneous coronary intervention had higher Gal-3 levels compared to healthy controls, and Gal-3 levels were found to have a predictive value for major adverse cardiac events on day 30. Furthermore, Falcone et al. found higher serum Gal-3 levels in patients with unstable angina pectoris compared to those with stable angina pectoris, with a significant correlation between Gal-3 levels and the number of diseased vessels.

In another study, type 2 diabetes mellitus patients were found to have higher Gal-3 levels compared to type 2 diabetes without CAD. The authors also found a significant correlation between serum Gal-3 levels and the total number of diseased vessels and plaques, as well as the type of calcified plaques. A cross-sectional study also demonstrated higher plasma Gal-3 levels in patients with carotid atherosclerosis than in those without. These findings indicate the role of Gal-3 in atherosclerotic plaque instability, macrophage activation and increased monocyte recruitment. Furthermore, studies involving long-term follow up have suggested that Gal-3 levels may represent an independent predictor of cardiovascular events and mortality in patients with CAD. Finally Gal-3 plays a key role in vascular inflammation and fibrosis induced by aldosterone.

In light of these data, it appears reasonable to assume a cause-and-effect relationship between elevated serum Gal-3 levels in isolated CAE patients and the development of ectasia through weakening of the arterial wall as a result of a number of mechanisms including atherosclerosis, vascular inflammation and oxidative stress.

Furthermore, in our study, patients with isolated CAE were further classified into four groups based on the extent of ectasia. Serum Gal-3 levels did not differ significantly among these four groups, suggesting that Gal-3 may be associated with the atherosclerotic process rather than the severity of ectasia. However, the number of patients in each subgroup was too low to draw a firm conclusion. Considering several studies examining the physiopathology of CAE and showing a more intense inflammatory process compared to those with CAD, similar fibrinogen and hs-CRP levels in our study as biomarkers of inflammation may be explained on the basis of a subtler inflammatory process.

There are some limitations to this study. Its small sample size and single-centre design are the main limitations. Another limitation involves the fact that normal coronary arteries were defined on the basis of contrast angiography of the lumen without using IVUS. Therefore, the presence of underlying atherosclerotic plaques might have been overlooked. In addition, normal epicardial arteries in the control group were not able to be evaluated for vasospasm or microvascular dysfunction with an independent method, which can be deemed another limitation.

Conclusion
This study is the first to show a significant increase in serum Gal-3 levels in patients with isolated CAE, indicating that Gal-3 may play a role in the pathogenesis of CAE, similar to CAD. Based on our study results, we suggest that increased Gal-3 levels may affect coronary remodelling in a way that favours the development of ectasia. However, further studies at a cellular level in larger series are warranted to gain a better understanding of the role of Gal-3 in patients with isolated CAE.

References