Annals of Clinical Case Reports

Association between Galectin-3 and Oxidative Stress Parameters with Coronary Artery Disease in Tunisian Population

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Abstract

Background: Galectin-3 (Gal-3), as a mediator of inflammation and fibrosis, has been reported to be a biomarker of severity in Coronary Artery Disease (CAD). However, the relationship between oxidative stress and Gal-3 in CAD is less clear. The study aimed to assess the relationships between Coronary Artery Disease (CAD) and risk factors, including parameters of oxidative stress and inflammation, in Tunisian patients with stable and unstable CAD.

Aims: The goal of this study was to examine the relationships between Coronary Artery Disease (CAD) and risk factors, including oxidative stress parameters and galectin-3as an inflammation marker, in Tunisian patients with stable and unstable CAD.

Methods: The study enrolled 257 patients affected by CAD. Serum Gal-3 was measured using ELISA, stress oxidative parameters were assayed in erythrocytes, and assessed the presence of CAD by coronary angiography and the severity of coronary atherosclerosis with the Gensini score.

Results and Conclusion: Unstable patients had a higher plasma Galectin-3 level in comparison to the stable subjects. We hypothesize that Gal-3 could be a useful biomarker of atherosclerotic plaque and in particular of its destabilization. We conclude that oxidative stress could be an important supplemental parameter to predict CAD.

Keywords: Galectin-3; Coronary artery disease; Coronary severity score; Score Gensini; Oxidative stress

Introduction

Coronary Artery Disease (CAD), macrovascular and microvascular complications are major causes of morbidity and mortality. Atherosclerosis is an inflammatory process, which involves in the development of CAD [1-4] Another pathway in the pathogenesis of atherosclerosis, associated with inflammation processes, is vascular fibrosis [5]. Many reports have also suggested a link between biomarkers and CHD Of these biomarkers, Galectin-3 is a β-galactoside binding lectin playing an important regulatory role in inflammation, immunity, and fibrosis [6]. Galectin-3 is expressed in leucocytes, mast cells and various organ tissues [7,8] and secreted by activated macrophages. It directly induces transformation of reactive oxygen species, fibroblasts, proliferation, secretion of matrix metalloproteinases and tissue inhibitors of metalloproteinases and deposition of type I collagen in extracellular matrix, which results in adverse matrix remodeling [9]. Many studies have implicated Gal-3 in an atherogenesis and the progression of heart failure, Gal-3 was shown to have predictive value in several heart failure cohorts [10,11]. Another case control study showed that Gal-3 is a predictive marker for coronary vascular disease and might be a useful biomarker of CAD [12]. The known traditional risk factors for CAD are smoking, obesity, hypertension, a family history of CAD, diabetes mellitus, and hyperlipidemia. In addition to the traditional CAD risk factors, enhanced oxidative stress is a novel risk factor of CAD. Increased oxidative stress is associated with the pathogenesis of CAD [13,14]. Clinical trials have revealed that oxidative stress may increase free

Oxygen Reactive Species (ROS) formation and reduce antioxidant defenses. However, oxidative stress plays an important role in the development of many pathologies [15,16]. Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical-mediated damage and oxidative stress [17]. Antioxidant enzymes such as Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) protect cells against oxidative stress [18]. We hypothesize that Gal-3, as a biomarker linking inflammation and fibrosis, could provide useful information for the prediction of CAD. The purpose of the present study was to verify the hypothesis that an elevated Gal-3 level is a useful biomarker in CAD and to explore antioxidant status by determining the SOD, GPx and MDA activity among coronary artery disease patients.

Materiel and Methods

Population

The studied population (N=361) consisted of 193 patients with Stable Coronary Artery Disease (SCAD), 64 patients with Instable Coronary Artery Disease (ISCAD) and 104 CAD-free control subjects recruited from the health professional department while undergoing routine check-up. Informed consent was obtained from all of the participants, and the study was approved by the local ethics committee. The patients with CAD were included when presenting at least 50% stenosis in at least one of the major coronary arteries. Patients who suffered from inflammatory disease, heart failure, renal disease, rheumatoid arthritis or cancer were excluded. Selective coronary angiography was performed for all patients enrolled in this study. Cardiologists evaluated all of the coronary angiograms; the coronary angiogram established the lesion location and percentage of luminal stenosis among all coronary artery lesions. CAD defined as the presence of stenosis of at least 50% of the vessel diameter in any of the main coronary arteries, according to the American College of Cardiology/American Heart Association (ACC/AHA) lesion classification [19].

Measurement of plasma Gal-3 and stress oxidative parameters

Plasma samples were assayed for Galectine-3 by using an enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA), according to the manufacturer's instructions. Superoxide dismutase, glutathione peroxidase and malondialdehyde were assayed in erythrocytes by according methods Beyer and Fridovich, Flohé and Gunzler and Yoshioka and all respectively [20-23]. Other blood parameters were measured with standard hospital equipment.

Coronary angiography and assessment of coronary atherosclerosis severity

The Gensini scoring system used to identify the severity of CAD [24]. This method classifies and scores the degree and extent of the stenosis of the coronary arteries. This system scores 1 point for 1% to 25% stenosis, 2 points for 26% to 50%, 4 points for 51% to 75%, 8 points for 76% to 90%, 16 points for 91% to 99% stenosis, and 32 points for total occlusion. The score is then multiplied by a factor representing the importance of the lesion's location in the coronary arterial system. For the location, scores are multiplied by 5 for a left main lesion; 2.5 for the proximal Left Anterior Descending (LAD) or left Circumflex (LCX) artery; 1.5 for the mid-segment LAD and LCX; 1 for the distal segment of the LAD and LCX, first diagonal branch, first obtuse marginal branch, right coronary artery, posterior descending artery, and intermediate artery; and 0.5 for the second

diagonal and second obtuse marginal branches. According to their coronary angiograms, the patients with CAD were divided in two groups, patients with mild atherosclerosis (n=96; Gensini score <20 points) and severe atherosclerosis (n=156; Gensini score ≥ 20 points)).

Follow-up

The patients were contacted by telephone every 2 months. The primary endpoint was a composite of all-cause mortality, restenosis and rehospitalization due to CHD during 12-month follow-up.

Statistics

Continuous variables are expressed as median and interquartile range. Categorical variables were given as percentages. The normality of distribution for continuous variables was confirmed with the Kolmogorov-Smirnov test. According to the distribution pattern of the continuous variables, the independent-sample t-test or the Mann-Whitney U test was used for continuous variables, and the chi-square test was used for categorical variables. One-way Analysis of Variance (ANOVA) or Kruskal-Wallis test was used to compare 3 groups. Correlations were assessed using either Pearson's correlation test or Spearman's rank test according to the distribution pattern of the variable. Independent associations between Gensini score and independent variables were assessed by backward stepwise multiple linear regression analysis by including all parameters showing p value of less than 0.1 on univariate analysis. Receiver Operating Characteristic (ROC) curve analyses were performed to derive the optimal cut-off value of galectin-3 with the highest sensitivity and specificity associated with CAD.

Results

Baseline characteristics

The 361 subjects were divided into groups: the controls subjects are 104 healthy volunteers (age mean 50 ± 11 years) and 257 consecutive patients (age mean 61 ± 11 years) with angiographically documented CVD enrolled from the cardiovascular department of University Hospital Fattouma Bourguiba of Monastir, Tunisia. The clinical characteristic, including comorbidities and risk factors, are summarized in Table 1. The CHD population is grouped according to the stable and unstable CAD. As expected, the CHD population had a higher percentage of diabetes, hypertensive, hyperlipidemic and smokers' patients. In contrast, only diabetes mellitus was significantly higher in the stable CAD population. However, there are differences in medical treatment between the stable and unstable CAD, although the frequency of antidiabetic, insulin, calcium antagonist therapy exhibited a tendency to be higher in the stable CAD population. The capillary glycemia, HDL Cholesterol (HDL- C), total cholesterol, triglycerides, urea, sodium, creatinine, ASAT and ALAT were significantly higher in CHD patients compared to the control population, but only LDL-C was significantly higher in unstable CAD. In contrast, the BMI, LDL-C, potassium and hemoglobin 1Ac did not differ significantly between controls and HD patients (Table 1).

Plasma Gal-3

By ELISA determined Galectin-3 in the plasma of controls and CHD patients, which have been divided into stable and unstable CAD (Tables 1-3). The median concentration of Gal-3 in the plasma was 14.46 [5.28- 72.85] ng/ml. Gal-3 was higher in CHD patients vs. controls (p<0.001) and in unstable CAD vs stable CAD patients

Table 1: Baseline clinical characteristics and risk factors in the patients with CHD, stable CAD and unstable CAD.

Control group n=104	CHD n=257	CAD stable n=193	CAD unstable n=64	p1	p 2
Gender (male) 45 (43.3)	117 (66.5)	128 (66.3)	43 (67.2)	<0.001	0.89
Age (year) 49.95 ± 11.03	61.49 ± 10.67	62.45 ± 10.64	58.58 ± 10.22	<0.001	0.011
BMI (kg/m²) 27.45 ± 4.41	27.95 ± 4.43	28.11 ± 4.54	27.48 ± 4.08	0.327	0.326
history of CAD (%) 0 (0)	135 (52.5)	107 (55.4)	28 (43.8)	-	0.105
Risk factors					
Hypertension (%) 1 (1)	145 (56.4)	104 (53.9)	41 (64.1)	<0.001*	0.155
Diabetes mellitus (%) 3 (2.9)	134 (52.1)	115 (59.6)	19 (29.7)	<0.001*	<0.001*
Hyperlipidemic (%) 0 (0)	63 (24.5)	43 (22.3)	20 (31.3)	<0.001*	0.148
Obesity (%) 29 (27.9)	92 (35.8)	73 (37.8)	19 (29.7)	0.149	0.239
Smokers (%) 6 (5.8)	136 (52.9)	107 (55.4)	29 (45.3)	<0.001*	0.194
IDM (%) 0 (0)	55 (21.4)	31 (16.1)	24 (37.5)	<0.001*	0.001*
Coronary revascularization (%) 0 (0)	32 (12.5)	26 (13.5)	6 (9.4)	<0.001*	0.383
Mortality 0 (0)	12 (4.7)	-	-	-	-
restenosis 0 (0)	13 (5.1)	-	-	-	-
Re-hospitalization 0 (0)	45 (17.5)	-	-	-	-
Laboratory data					
Capillary glycemia (mmol/l) 4.95 [2.1-16.84]	8.05 [2.91-35.3]	7.76 [2.91-35.3]	7.42 [3.72-20.9]	<0.001*	0.711
LDL-c (mmol/l) 2.4 [0.57-4.53]	2.8 [0.41-6.26]	2.74 [0.41-6.26]	3.14 [1.98-4.86]	0.135	<0.001*
HDL-c (mmol/l) 1.15 [0.64-2.32]	1.05 [0.27-4.27]	1.04 [0.27-4.27]	1.02 [0.94-1.32]	0.008*	0.232
Total Cholesterol (mmol/l) 4.16 ± 0.8	4.8 ± 1.15	4.76 ± 1.12	5.07 ± 1.28	<0.001*	0.15
Triglycerides (mmol/l) 1.14 [0.47-3.49]	1.59 [0.2-8.8]	1.59 [0.2-8.8]	1.28 [0.96-1.65]	<0.001*	0.339
Urea (mmol/l) 4.8 [2.1-9.4]	5.7 [2.2-18.8]	5.7 [2.2-18.8]	5.65 [2.4-17.2]	<0.001*	0.596
Sodium (mmol/l) 140.5 [131-147]	138 [30-411]	137 [30-149]	139 [127-411]	<0.001*	0.006*
Potassium (mmol/l) 4.19 [3.31-9.16]	4.18 [3.01-6.06]	4.2 [3.01-6.06]	4.15 [3.15-5.8]	0.526	0.177
Creatinine (µmol/l) 82 [57-135]	94 [43-274]	94 [52-274]	95 [43-205]	<0.001*	0.668
ASAT (U/l) 22 [15-55]	46.5 [16-77]	23 [10-86]	17.5 [8-422]	0.004*	0.095
ALAT (U/l) 18.5 [10-28]	41.5 [15-68]	24.5 [9-92]	22.5 [8-261]	<0.001*	0.379
Hemoglobin (%) 6 [6-9]	8 [6-10]	7.5 [5-15]	6.5 [6-7]	0.158	0.498
Galectin-3 (ng_ml) 9.87 [7.26-20.06]	14.46 [5.28-72.85]	13.85 [5.28-56.2]	18.19 [10.9-72.85]	<0.001*	<0.001*
GPx (U/mgP) 1.94 [0.4-5.45]	2.13 [0.08-14.56]	2.03 [0.35-5.45]	3.14 [0.08-14.56]	0.018*	<0.001*
MDA (U/mgP) 422.23 ± 169.48	2024.38 ± 747.8	2184.95 ± 756.58	1524.52 ± 437.35	<0.001*	<0.001*
SOD (U/mgP) 0.1 ± 0.003	0.0127 ± 0.10	0.012 ± 0.0034	0.011 ± 0.0028	<0.001*	0.042*
Drugs					
antihypertensive medication use 1 (1)	145 (56.4)	104 (53.9)	41 (64.1)	<0.001*	0.115
antibiabetes medication use 3 (2.9)	129 (50.2)	115 (59.6)	14 (21.9)	<0.001*	<0.001*
beta-blokers 0(0)	133 (51.8)	106 (54.9)	27 (42.2)	-	0.077
aldosterone/antagonistes 0(0)	15 (5.8)	12 (6.2)	3 (4.7)	-	0.651
diuretics 0(0)	61 (23.7)	47 (24.4)	14 (21.9)	-	0.686
antiplatelets 0(0)	163 (63.4)	118 (61.1)	45 (70.3)	-	0.187
calcium-antagonist 0(0)	118 (45.9)	96 (49.7)	22 (34.4)	-	0.033*
insulin 0(0)	37 (14.4)	35 (18.1)	2 (3.1)	-	0.003*
ACE inhibitor 0(0)	164 (63.8)	125 (64.8)	39 (60.9)	-	0.581
nitrates 0(0)	70 (27.2)	56 (29)	14 (21.9)	-	0.257
vitamine k antagonist 0(0)	201 (78.2)	154 (79.8)	47 (73.4)	-	0.253
statin 0(0)	62 (24.1)	41 (21.2)	21 (32.8)	-	0.064
HBPM 0(0)	108 (42)	76 (39.4)	32 (50)	-	0.144

(p<0.001). By contrast, we did not find any difference in the level of Gal-3 according to our number of vessel stenosis between any of four CAD groups. Systemic Gal-3 showed a positive correlation with HDL-cholesterol, Triglyceride, and Urea but did not correlate with BMI, LDL-cholesterol, and SOD activity. Table 4 of note, there was a negative correlation between the plasma levels of galectin-3 and MDA activity (r= -0.203; p=0.02) (Figure 1A), but there was a moderate positive correlation between galectin-3 and GPx (r=0.218; p=0.01) (Figure 1B).

Diagnostic value of galectin-3 for CAD

ROC curves were plotted according to the data of CHD patient and control patients; galectin-3 had the greatest Area Under the Curve (AUC) of 0.801 (P<0.001), with an optimal cutoff of 11.73 ng/ml, wielding a sensitivity of 72.6% and a specificity of 82.3% (Figure 1C). During 12-month follow-up period, 12 patients died 13 were restenosis and 45 were re-hospitalized due to worsened CHD.

Stress oxidative parameters

The parameters that show oxidant and antioxidant status are presented in Table 1. As observed, the CHD patients exhibited elevated states of oxidative stress (indicated by both the GPx concentration and the level of MDA) when compared to the control population. In contrast, the activity of erythrocyte SOD in CHD patients was lower when compared to the control population. According to the stability

**Table 2**: Comparison of demographic and biochemical parameters.

	SG<20 n=96	SG>20 n=156	p value
Gender (male)	54 (56.3)	115 (51.3)	0.004
Age (year)	61.7 ± 10.33	61.51 ± 10.88	0.894
BMI (kg/m²)	28.29 ± 4.83	27.8 ± 4.2	0.395
history of CAD	54 (56.3)	80 (51.3)	0.443
Risk factors			
Hypertension	61 (63.5)	81 (51.9)	0.071
Diabetes mellitus	47 (49)	85 (54.5)	0.393
Hyperlipidemic	16 (16.7)	45 (28.8)	0.028
Obesity	40 (41.7)	51 (32.7)	0.15
Smokers	45 (46.9)	90 (57.7)	0.112
IDM	14 (14.6)	40 (25.6)	0.055
Coronary revascularization	8 (8.3)	24 (15.4)	0.09
Laboratory data			
Capillary glycemia	7.2 [3-35]	7.88 [2.91-27.67]	0.221
LDLc (mmol/l)	3.02 [0.8-4.67]	2.57 [0.41-6.26]	0.408
HDLc (mmol/l)	1.03 [0.27-3.84]	1.03 [0.48-4.27]	0.57
TC (mmol/l)			
TG (mmol/l)	1.61 [0.5-5]	1.52 [0.2-8.8]	0.6
Urea	5.8 [2.4-17.2]	5.6 [2.2-18.8]	0.344
sodium	137 [70-149]	138 [30-411]	0.668
potassium	4.16 [3.01-5.41]	4.17 [3.11-6.06]	0.648
creatinine	93.5 [43-274]	94.5 [57-255]	0.266
ASAT	26 [8-390]	27 [9-422]	0.56
ALAT	22.5 [11-132]	25 [8-261]	0.814
Hemoglobin 1ac	8 [9-14]	6 [5-15]	0.197
Galectin-3 (ng/ml)	15.39 [5.28-56.13]	13.96 [5.99-72.85]	0.711
GPx (U/mgP)	2 [0.08-10.52]	2.15 [0.67-14.56]	0.068

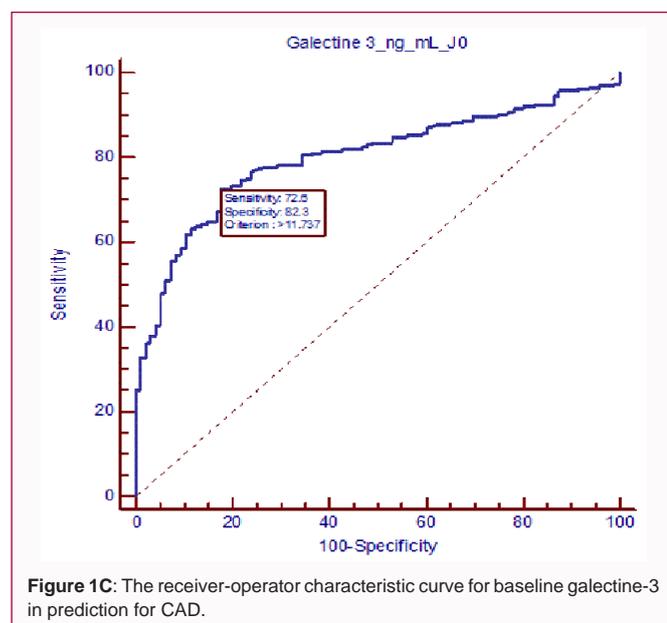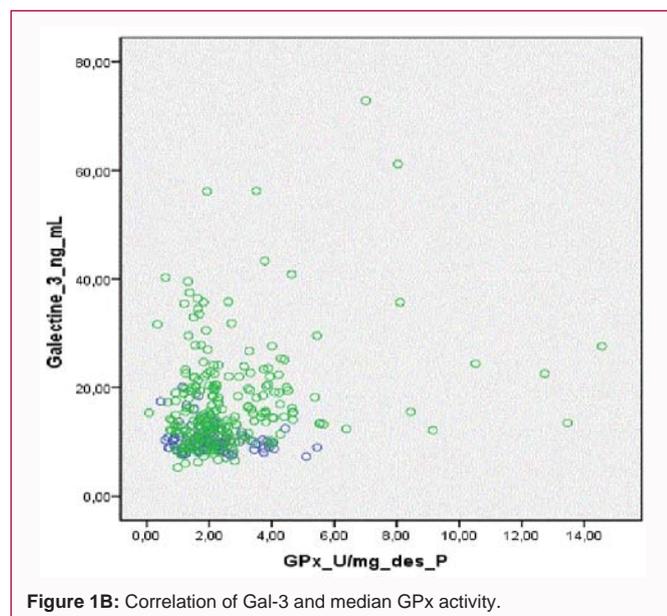**Table 3**: Multivariate binary logistic regression analysis.

	p value	OR	95% CI
Gensini score	0.012*	1.007	1.001-1.012
Diabetes - mellitus	<0.001*	0.171	0.08-0.367
hypertension	0.038*	2.257	1.046-4.871
dyslipidemia	0.139	1.846	0.819-4.16
obesity	0.412	0.73	0.343-1.55
CAD history	0.134	0.599	0.306-1.172
smoking	0.044	0.428	0.187-0.977

Table 4: Correlation of systemic galectin-3 levels with risk factors in CAD patients.

Correlation of galectin-3 with	Pearson correlation	P value
HDLc (mmol/l)	-0.117	0.01
Triglyceride (mmol/l)	0.226	0.001
Urea	0.171	0.008
creatinine	0.253	<0.001
Homocysteine (µM)	0.243	0.001
GPx (U/mgP)	0.218	0.001
MDA (U/mgP)	-0.203	0.002

**Figure 1A**: Correlation of Gal-3 and median MDA levels.
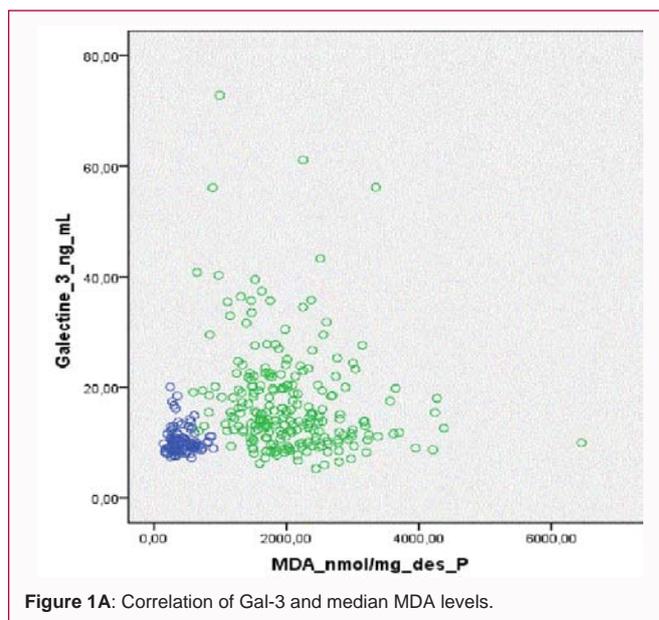
of CAD, we subdivided the CHD population into 2 groups (stable CAD and unstable CAD): The levels of erythrocyte-derived SOD and GPx as well as MDA, a lipid oxidation marker in CHD patients had significantly different when compared CHD patients vs. controls and CAD stable vs. CAD unstable patients. Among patients with unstable CAD, the variation of stress parameters after 48 h showed that were a significant difference in MDA levels (p=0.008) and SOD levels SOD levels (0.041) but no variation in GPx levels. By contrast, we did not find any difference in the level of many oxidative stress status parameters according to the stenosis degree or according to our number of vessel stenosis between any of four CAD groups. There was also no difference in CHD patient populations that were subdivided into four groups (0: stenosis less than 50%, 1: Stenosis of one vessel, 2: Stenosis of two vessels and 3: Stenosis of three vessels) (data not shown). There was also, no difference between the groups with high and low Gensini scores Table 2. Gensini scores were available in

Figure 1B: Correlation of Gal-3 and median GPx activity.

Figure 1C: The receiver-operator characteristic curve for baseline galectine-3 in prediction for CAD.

all patients. Comparison of demographic, clinical and laboratory characteristics of the subjects according to Gensini score is expressed in Table 2. Hyperlipidemic patients were higher in the group with a higher Gensini score (p=0.028). There were no differences in terms of presence of diabetes, hypertension and smokers of CAD between the groups with high and low Gensini scores. We did not find any difference in the level of stress status parameter according to the stenosis degree, between the groups with high and low Gensini scores Table 2. In the multivariate binary logistic regression analysis, Gensini score was significant independent predictor for CAD. Diabetes mellitus and hypertensive were other significant predictors for CAD in subjects with CHD Table 3. According to multivariate logistic regression analysis with previous coronary risk factors, such as Gensini score, diabetes mellitus, HTA and smoking, all of these were independently associated with severity of CAD Table 3.

Discussion

Multiple processes of inflammation influence the initiation,

Figure 1D: The comparison of galectin-3 levels in patients with unstable CAD and controls.
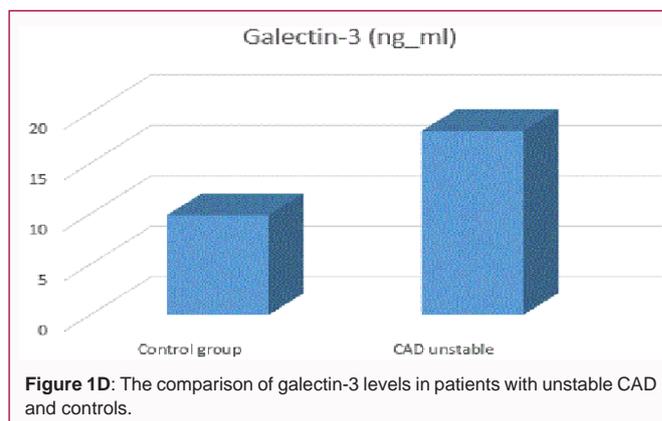
progression and propagation of atherosclerosis in coronary arterial disease. Previous studies that Gal-3 has been shown to be an important contributor to inflammation and markers of systemic inflammation through macrophages activation promotion and monocytes attraction predicts CVD [25-30]. Analysis of group of symptomatic subjects with CAD showed that Gal-3 levels were higher in patients with unstable angina as compared to stable angina. This suggested the involvement of Gal-3 in promoting macrophages activation and monocytes attraction [31]. This finding indicate that Gal-3 play an important role in many phases of acute and chronic inflammatory response. In a study by Nachtigal [32], Gal-3 levels were found to be higher in atherosclerotic arteries atheromatous plaques was lower and galectin-3 was strongly expressed in the foam cells of the atheromatous plaques. All of these observations suggest that Gal-3 may involve in the active phase of the vulnerable atherosclerotic plaques. We found elevated galectin-3 levels in patients with unstable CAD as compared to control group (Figure 1D). Additionally, we found that galectine-3 is higher in patients with CHD as compared to control and unstable CAD group. These patients with CHD had higher baseline Gal-3 concentration (median 14.46 ng/ml [5.28-72.85] compared the controls and the optimal cut-off for prediction of CAD was 11.737 ng/ml. Many observations have indicated that Gal-3 measured in the acute phase of myocardial infarcts was higher and that the cut-off value was 18.1 ng/ml [33]. The patients with three-vessel disease tended to have higher levels of Gal-3, by contrast, we did not find any difference in the level of Gal-3 according to our number of vessel stenosis between any of four CAD groups. Tunón evaluated the prognostic value of plasma biomarkers in patients with chronic coronary artery disease. They showed that development of mortality wase independently associated with increased levels of galectin-3. De Boer evaluated the relationship between galectin-3 and survival in the general population [34,35]. Compared with previous study in the literature, we founded lower Gal-3 levels in unstable patients in our cohort. In a study by Falcone galectin-3 levels were 27.75 ng/mL in unstable patients [36]. In our study, during 12-month follow-up period, 12 (4.7%) patients died, 13 (5.1%) were restenosis and 45 (17.5%) were re-hospitalized due to worsened CHD. The association between Gensini scores and galectin-3 suggested that galectin-3 may predict the severity of the coronary artery disease in clinical practice, and may become an additional tool in identifying high-risk patients. The median Gensini score of the patients was 20, and according to this value, patients were separated in two groups: Less intensive CAD and intensive CAD. There was no significant difference with regard about the Gensini score. Inflammation and oxidative stress play a key role in all stages of atherosclerosis and vascular remodeling, from initiation,

progression of athermanous plaques and luminal narrowing, finally leading to acute coronary syndromes as a consequence of plaque rupture. In addition, we found that Gal-3 was positively correlated with GPx activity but had a negative correlation with MDA levels. We have not found any relation between oxidative stress markers and the complexity of CAD, determined according to Gensini score.

Conclusion

Our study indicates that Gal-3 measured in CAD and unstable CAD could provide additional information about the pathobiology and prognosis in this group of patients, but further studies are needed. According to our findings, galectin-3 may become a major biomarker and provide complementary information for assessment of early risk stratification in patients with ACS.

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