Original Research Article

Association of galectin-3 with high cardiovascular risk in patients with type 2 diabetes attending a multi-specialty hospital in South India

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ABSTRACT

Background: Diabetes mellitus is a chronic hyperglycemic condition that continues to be associated with high mortality and morbidity. Galectin-3 is a β-galactoside binding lectin and is a potential marker for inflammation, immunity and fibrosis. The aim of the study was to evaluate the relationship between Galectin-3 and baseline characteristics and to find the association between Galectin-3 and high cardiovascular risk in a group of Type-2 diabetes patients.

Methods: A total of 100 patients with type-2 diabetes mellitus were included in the study after obtaining informed consent. The study participants were divided into two groups; those with diabetes for duration of less than 5 years as Group-1 and diabetes greater than 5 years as Group-2. Patients were also divided into three tertiles based on the Inter heart risk score (IHR). Five ml of blood was withdrawn from the ante-cubital vein and the serum obtained was subjected to analysis for estimating concentration of Galectin-3.

Results: There was no significant difference between the Group-1 and Group-2 except gender variation with a significantly higher preponderance of males in Group 2. There was a significant correlation between Galectin-3 and parameters such as systolic blood pressure (r=0.033, p=0.03), creatinine (r=0.20, p=0.03), IHR (r=0.69, p=0.0001). Patients in the high risk category had greater levels of Galectin-3 compared to those with low and intermediate IHR.

Conclusions: Galectin-3 concentration is elevated in diabetes patients and correlates with the IHR-Score. Thus Galectin-3 could be a useful biomarker for prediction of cardiovascular risk in diabetic subjects.

Keywords: Cardiovascular risk, Diabetes, Galectin-3, Heart disease, Interheart risk score

INTRODUCTION

Diabetes mellitus (DM) is a chronic hyperglycemic condition that eventually results in complications such as retinopathy, nephropathy; peripheral neuropathy, cardiac vascular disease and sexual dysfunction.[1] According to the Diabetes Atlas published by the International Diabetes Federation (IDF), there are an estimated 40 million persons with diabetes in India in 2007 and this number is predicted to rise to almost 70 million people by 2025 by which time every fifth diabetic subject in the world would be an Indian.[2]

The alarming rate of diabetes mellitus in Indian population leads to significant mortality and morbidity, enhancing its impact on overall health population. Some of the reasons that contribute to the mortality include infections, chronic renal failure, coronary artery disease, cerebrovascular disease, hypoglycaemia, diabetic ketoacidosis and hyperosmolar coma.[3]

Galectin-3 is a 31 kDa member of β-galactoside binding soluble protein belongs to lectin family, is a multifunctional protein involved in multiple biological processes.[4,5] Galectin-3 is extensively found among different types of cells, tissues and is evenly distributed in the intracellular compartment of nucleus, cytoplasm and in the extracellular compartment.[6] The unique chimeric structure of galectin-3 enables it to interact with intracellular and extracellular proteins influencing various biological processes such as cell growth, adhesion, differentiation, inflammation, angiogenesis.[7] However the exact role of galectin-3 is not understood clearly.[8]

The level of Galectin-3 were higher in patients with T2DM and hypertension, also was found to be correlated with glycosylated hemoglobin (HbA1c) and N-terminal fragment B-type natriuretic peptide.[9] In a cross-sectional study, elevated levels of Galectin-3 concentration were associated with insulin resistance in patients with T2DM.[10] The serum Galectin-3 was significantly increased in diabetic and pre-diabetic patients and strongly correlated with CRP.[11] Similarly Galectin-3 was elevated in obesity and negatively correlated with glycated haemoglobin in T2DM.[12] Though Galectin-3 is a promising biomarker, further studies need to be demonstrated to explain the role of galectin-3 in predicting the cardiovascular risk.

Interheart risk score (IHR) is a simple web based score that assesses patients who have risk factors for heart disease such as diabetes, hypertension, obesity, unhealthy dietary habits, smoking. Based on the score obtained patients could be classified into low, intermediate and high risk. Similar to the IHR, there are other tools to assess future cardiovascular risk in diabetic subjects. Although galectin-3 is a biomarker that has been widely studied in patients with heart failure for it prognostic ability, it is not known if galectin-3 levels reflect the degree of cardiovascular risk among subjects with risk factors for IHR, but who do not have established disease. So the aim of the study was to assess the utility of galectin-3 in predicting the cardiovascular risk among type 2 diabetes subjects.

METHODS

The study protocol was approved by the Institute Ethics Committee, and all study procedures were performed in patients in accordance with the principles of the Declaration of Helsinki (2013). Written informed consent was obtained from all study patients. We included subjects with a diagnosis of diabetes of either gender above 18 years of age who were willing to provide informed consent and follow study procedures. We excluded diabetes subjects who had concomitant established coronary artery disease, cancer, autoimmune states and other inflammatory conditions. The baseline characteristics such as age, gender, BMI, waist circumference, hip circumference, Hypertension, stress

and depression were obtained from hospital records. The IHR score was calculated based on a standard web based questionnaire. 5 ml of blood was withdrawn from the ante-cubital vein of forearm using aseptic precaution. The same was centrifuged at 2000G for ten minutes and the separated serum was stored in eppendorf tubes at -40 degree Celsius for further analysis. Galectin-3 concentration was estimated using sandwich ELISA technique (Bio-Assay).

Statistical analysis

Data were expressed as mean ± standard deviation or median with interquartile range. The baseline characteristics between the study groups were compared using t-test or chi-square test. The correlation of Galectin-3 with other clinical and laboratory variables was performed using Spearman rank correlation test. A p value ≤0.05 was considered statistically significant. SPSS 16.0 version was used for statistical analysis.

RESULTS

A total of 100 patients participated in this study, of which 37% had diabetes for a period greater than 5 years. The baseline characteristics among patients with diabetes for a period less than 5 years (Group-1) was compared with those patients with diabetes for a duration greater than 5 years (Group-2) (Table 1).
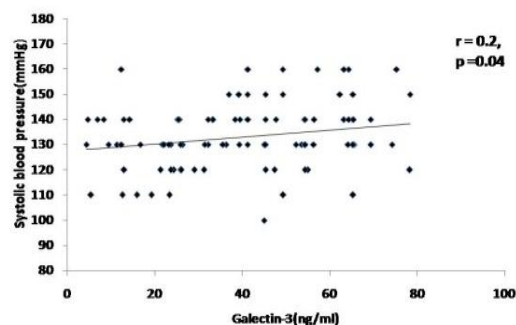

Figure 1: Correlation of galectin-3 (ng/ml) vs systolic blood pressure in patients with diabetes.
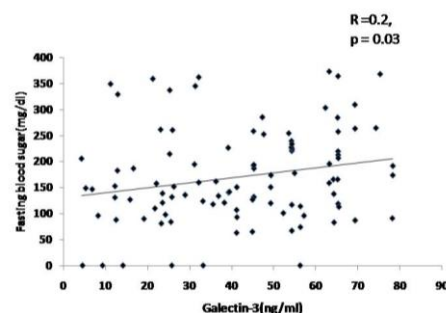

Figure 2: Correlation of galectin-3 (ng/ml) vs fasting blood sugars in patients with diabetes.
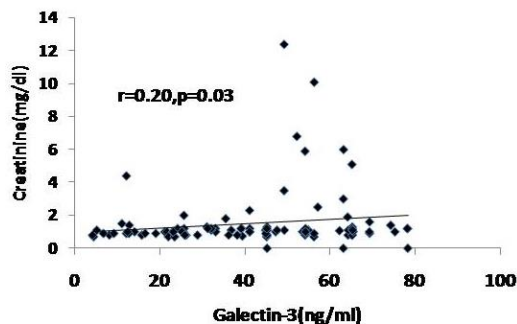
Figure 3: Correlation of galectin-3 (ng/ml) vs serum creatinine in patients with diabetes.
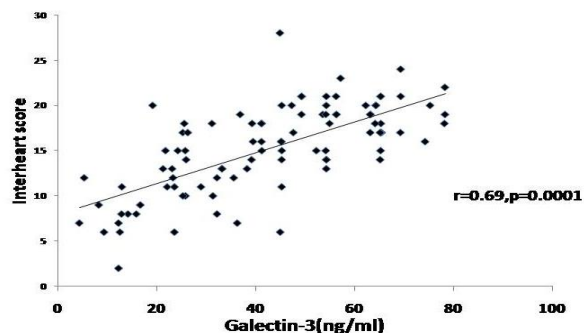

Figure 4: Correlation of galectin-3 (ng/ml) vs interheart risk score in patients with diabetes.

Table 1: Baseline characteristics of study patients.

| Characteristics	Diabetes < 5years (N=63)	Diabetes > 5years (N=37)	P value
Age (years)	55.46±13.03	59.91±13.77	0.10
Gender (male %)	28 (44.4)	25(67.6)	0.03
BMI (kg/m2)	30.80±26.54	24.66±9.56	0.17
Waist circumference (cm)	83.88±23.07	91.40±24.41	0.12
Hip circumference (cm)	90.06±25.40	99.75±26.90	0.07
Hypertension (%)	28 (44.4)	23(62.2)	0.10
Stress (%)	41 (75.1)	17(14.9)	0.008
Depression (%)	5 (7.9)	7(18.9)	0.12
SBP (mmHg)	133.31±12.04	133.24±14.15	0.09
DBP (mmHg)	82.85±13.25	81.62±9.28	0.61
PR (bpm)	81.41±4.91	81.81±7.73	0.75
RR (mg/dl)	23.65±7.40	21.02±3.38	0.04
FBS (mg/dl)	164.35±89.54	181.57±92.77	0.36
PPBS (mg/dl)	205.86±143.71	242.28±147.41	0.23
RBS (mg/dl)	148.17±137.63	128.54±144.26	0.50
HbA1c (%)	5.81±3.811	6.54±3.48	0.34
Creatinine (mg/dl)	1.56±1.83	1.43±1.75	0.72
eGFR (ml/min)	67.12±28.71	63.16±28.30	0.51
T.CHOL (mg/dl)	63.07±97.04	49.67±93.34	0.50
TGL (mg/dl)	51.20±82.37	49.35±106.72	0.92
HDL (mg/dl)	12.14±19.24	9.28±17.23	0.45
LDL (mg/dl)	42.23±69.00	30.59±59.72	0.39
VLDL (mg/dl)	12.21±34.46	7.24±15.66	0.41
IHR (mg/dl)	14.65±5.30	16.33±3.75	0.09
Galectin-3 (ng/ml)	39.99±19.90	45.20±20.43	0.21

There was gender variation between the groups with a significantly higher preponderance of males in group 2. There was no other significant difference with respect to baseline characteristics between the two study groups. There was a significant correlation between Galectin-3 and parameters such as systolic blood pressure (r=0.033, p=0.03), creatinine(r=0.20, p=0.03), IHR (r=0.69, p=0.0001). (Figure 1-4) Patients were divided into three tertiles based on IHR score. Patients in the high risk category had greater levels of Galectin-3 compared to those with low and intermediate IHR (Figure 5).

DISCUSSION

The main findings of present study were that Galectin-3 levels were significantly correlated with IHR. The

PREVEND study (Prevention of renal and vascular end stage study) that was performed in 5958 subjects from the general population showed that subjects with persistently elevated Galectin-3 had a higher risk of heart failure.[13] In a cross-sectional analysis of 2946 participants of the Framingham study, elevated Galectin-3 was associated with abdominal adiposity, dyslipidemia and hypertension.[14] Similarly in a study of 158 subjects with T2DM undergoing elective coronary computed tomography angiography, it was observed that Galectin-3 levels were higher in the CAD group as compared to non CAD group. The authors of this study have hypothesized that identity of high Galectin-3 levels could identify those DM patients who need early coronary intervention due to coronary atherosclerosis.[15]
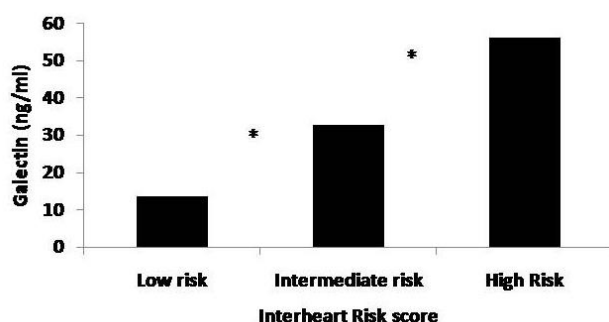
*P<0.0001 among all study groups.

Figure 5: Galectin concentration with respect to interheart risk score in patients with diabetes.

Although galectin-3 was higher among subjects with long standing diabetes compared to those with shorter duration of the illness, this was not statistically significant. Similarly Galectin-3 did not show any correlation with HbA1c or diabetes duration in other studies.[16] The concentration of Galectin-3 was found to influence insulin resistance but had no effect on the blood glucose level. However in present study we did not measure the fasting insulin level and so insulin resistance could not be assessed.[10] It has been hypothesized that harmful signals that precede frank CVD could initiate subclinical changes associated with galectin-3 activation and increase in its secretion.[17]

Present study also showed that Galectin-3 levels correlated with systolic blood pressure. These findings are in resonance with earlier studies. In a study performed in European population, high Galectin-3 levels were observed in patients with hypertension and diabetes. Since Galectin-3 is involved in the fibrotic response to aldosterone in vascular smooth muscles, it could play a significant role in the pathophysiology of hypertension.[18] There was also a positive correlation between Galectin-3 and serum creatinine among our study subjects. In a study performed among dialysis patients with T2DM, Galectin-3 concentration were observed to be increased in patients with progressive renal impairment.[19] In a study that

included Framingham offspring participants, elevated Galectin-3 levels were associated with increased risk of worsening eGFR.[20] Thus it appears that galectin-3 does have a role in the progression of renal disease.

The main limitations for study were its cross-sectional nature that precluded our ability to follow up the subjects for progression of heart disease. Serial measurement of galectin-3 to understand how changes in biomarker levels correlate with disease progression were not performed. A larger sample would enable us to define the relationship between galectin-3 and the other clinical variables. Future studies should also explore the relationship between galectin-3 expression and insulin resistance.

CONCLUSION

Galectin-3 is a novel biomarker that is elevated in patients with diabetes and has a significant correlation with Inter heart risk score and thus could be useful as a potential biomarker for prediction of cardiovascular disease in this population.

ACKNOWLEDGEMENTS

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