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Evaluation of galectin-3 levels in patients with type 2 diabetes mellitus and chronic kidney disease

Avaliação dos níveis de galectina-3 em pacientes com diabetes mellitus *tipo 2 e doença renal crônica*

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ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is the most common manifestation of diabetes, accounting for about 90% of diagnosed cases. The causes of T2DM are not fully understood, but its pathogenesis is possibly associated with increased adiposity and a chronic low-grade inflammatory response. The glycoprotein galectin-3 (Gal-3) is known to play an important role in the modulation of blood glucose, adiposity, and inflammation. **Objectives:** The aim of this study was to evaluate Gal-3 levels in patients with T2DM and chronic kidney disease (CKD), in addition to relating them with complications and comorbidities present in these patients, comparing them to a control group. **Materials and methods:** Gal-3 was evaluated in 84 selected individuals, of which 42 had clinical and laboratory diagnosis of T2DM and CKD (treated at Santa Casa Hospital in Belo Horizonte, Minas Gerais, Brazil), and 42 individuals from the local community, with no history of diabetes (control group). **Results and discussion:** Gal-3 levels were significantly higher ($p = 0.012$) in the T2DM group (15.17 ± 5.54 ng/ml) when compared to the control group (12.62 ± 3.2 ng/ml). There was a tendency for higher levels of Gal-3 in diabetic patients with hypertension (15.74 ± 5.61 ng/ml) when compared to patients without this complication (10.96 ± 2.49 ng/ml) ($p = 0.069$). **Conclusion:** The results suggest that Gal-3 may be involved in the pathophysiology of T2DM and still be a promising biomarker associated with hypertension in this group.

Key words: galectin-3; type 2 diabetes mellitus; inflammation; hypertension.

RESUMO

Introdução: *O diabetes* mellitus *tipo 2 (DM2) é a manifestação mais comum do diabetes; representa cerca de 90% dos casos diagnosticados. As causas do DM2 ainda não foram completamente estabelecidas, mas sua patogênese está, possivelmente, relacionada com o aumento da adiposidade e uma resposta inflamatória crônica de baixo grau. Sabe-se que a glicoproteína galectina-3 (Gal-3) possui papel importante na modulação de glicemia, adiposidade e inflamação.* ***Objetivos:*** *Avaliar os níveis de Gal-3 em pacientes com DM2 e doença renal crônica, além de relacioná-los com as demais complicações e comorbidades presentes nesses indivíduos, comparando-os com um grupo-controle.* ***Materiais e métodos:*** *A Gal-3 foi avaliada em 84 pacientes selecionados; destes, 42 possuíam o diagnóstico clínico e laboratorial de DM2 e doença renal crônica (atendidos no Hospital Santa Casa de Belo Horizonte, Minas Gerais, Brasil), e 42 eram da comunidade local, sem histórico de diabetes (grupo-controle).* ***Resultados e discussão:*** *Os níveis de Gal-3 foram significativamente mais elevados (*p *= 0,012) no grupo com DM2 (15,17 ± 5,54 ng/ml) quando comparados com os níveis do grupo-controle (12,62 ± 3,2 ng/ml). Houve tendência em maiores níveis de Gal-3 nos pacientes diabéticos com hipertensão (15,74 ± 5,61 ng/ml) em comparação com os pacientes sem essa complicação (10,96 ± 2,49 ng/ml) (*p *= 0,069).* ***Conclusão:*** *Os resultados obtidos sugerem que a Gal-3 pode estar envolvida na fisiopatologia do DM2 e ainda ser um promissor biomarcador associado à hipertensão nesse grupo.*

Unitermos: *galectina-3; diabetes* mellitus *tipo 2; inflamação; hipertensão.*

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RESUMEN

Introducción: *La diabetes* mellitus *tipo 2 (DM2) es la forma más común de la diabetes; representa alrededor del 90% de los casos diagnosticados. Todavía no se conocen por completo las causas de la DM2, pero posiblemente su etiopatogénesis se relaciona con el aumento de adiposidad y una respuesta inflamatoria crónica de bajo grado. Se sabe que la glicoproteína galectina 3 (Gal-3) juega un papel importante en la modulación de glucemia, adiposidad e inflamación.* ***Objetivos:*** *Evaluar los niveles de Gal-3 en pacientes con DM2 y enfermedad renal crónica, además de relacionarlos con las otras complicaciones y comorbilidades presentes en eses individuos, comparándolos con un grupo control.* ***Materiales y métodos:*** *La Gal-3 fue evaluada en 84 pacientes elegidos; entre esos, 42 poseían el diagnóstico clínico y de laboratorio de DM2 y enfermedad renal crónica (atendidos en el Hospital Santa Casa de Belo Horizonte, Minas Gerais, Brasil) y 42 eran de la comunidad local, sin historial de diabetes (grupo control).* ***Resultados y discusión:*** *Los niveles de Gal-3 fueron más altos (*p = *0,012) en el grupo con DM2 (15,17 ± 5,54 ng/ml) que en el grupo control (12,62 ± 3,2 ng/ml). Hubo tendencia de mayores niveles de Gal-3 en los pacientes diabéticos con hipertensión (15,74 ± 5,61 ng/ml) que en aquellos sin esa complicación (10,96 ± 2,49 ng/ml) (*p = *0,069).* ***Conclusión:*** *Los resultados obtenidos apuntan que la Gal-3 puede estar involucrada en la etiología de la DM2 y aún ser un biomarcador prometedor de hipertensión en ese grupo.*

Palabras clave: *galectina 3; diabetes* mellitus *tipo 2; inflamación; hipertensión.*

INTRODUCTION

Diabetes mellitus (DM) is a chronic and complex disease that affects approximately 425 million individuals, according to data from the International Diabetes Federation (IDF), 2017. The most common is type 2 diabetes mellitus (T2DM), which represents about 90% of diagnosed cases and currently is one of the major public health problem worldwide[1, 2]. Studies indicate that the pathogenesis of T2DM and its micro and macrovascular complications are possibly linked to chronic low-grade inflammation with important influence of visceral adipose tissue in the activation of the immune response[3, 4].

Galectin-3 (Gal-3) is a chimeric glycoprotein from the lectin family that contains a carbohydrate recognition domain that binds to beta-galactosides[5]. Discovered about 20 years ago, this biomolecule can be found in the cytoplasm; it may be transported to the nucleus and be secreted to cell surface or extracellular space, forming multimeric structures[3, 5, 6]. Due to these characteristics, Gal-3 combines with several molecules and performs biological functions that vary according to its location[3, 7].

When on the cell surface, Gal-3 promotes interactions between cells, participates in the chemotaxis of monocytes and macrophages to tissues, induces the production of pro-inflammatory mediators and reactive oxygen species by mast cells, neutrophils, and macrophages and acts in intracellular processes, as a regulatory molecule for cell proliferation, differentiation and death[8,9]. This galectin is expressed ubiquitously in the body and can be found mainly in macrophages, fibroblasts, adipocytes and epithelial cells, as well as secreted in serum and urine[3, 5, 7, 9].

Gal-3 levels can gradually increase with age, and higher plasma levels have already been detected in women, when compared to men[10]. Altered concentrations of this lectin have been linked to the pathogenesis of several chronic diseases, including cancers, heart disease, and metabolic disorders, such as T2DM[11].

Currently, studies explain the mechanisms by which Gal-3 is altered in cardiometabolic diseases. In a cross-sectional analysis with 2,946 samples from individuals in the offspring cohort of the Framingham Heart Study, Gal-3 levels were shown to be associated with abdominal adiposity, dyslipidemia, and hypertension[12]. It is believed that the effects of this glycoprotein on the pathophysiology of T2DM and obesity would be related to its expression by adipocytes, induced by pro-inflammatory molecules, such as free fatty acids and interleukin-6 (IL-6), with consequent recruitment of macrophages to the site[11, 12]. Brazilian studies have already associated increased expression of this lectin with heart disease[13] and some types of cancer, such as breast and thyroid cancer[14-16], but there is still no assessment of this relationship in the Brazilian population with T2DM.

OBJECTIVES

The aim of the present study was to compare Gal-3 levels in patients with T2DM and chronic kidney disease with a

normoglycemic control group. Subsequently, Gal-3 levels were associated with the comorbidities presented by these patients.

MATERIALS AND METHODS

Ethical guidelines

The research ethics committees of the Federal University of Minas Gerais (UFMG) – ETIC 0062.0.203.000-11 – and of the Hospital Santa Casa de Belo Horizonte – 059/2011 – approved this study, in accordance with the ethical guidelines of the Declaration of Helsinki. All participants signed the Free and Informed Consent Form (ICF).

Participants

Eighty-four individuals were selected for this case-control study. The case group consisted of 42 patients with clinical and laboratory diagnosis of T2DM, according to the criteria established by the American Diabetes Association (ADA)[17]; 42 non-diabetic individuals with body mass index (BMI), age, and sex paired with the T2DM group were included in the control group. BMI was calculated by dividing the weight in kilograms (kg) by the height in meters square (m^2). The abdominal circumference (AC) was measured between the lower ribs and the iliac crest, with the aid of a tape measure, with the individual standing, barefoot, and the waist free of clothes. The hip was measured on the gluteal region at the largest perimeter, over light clothes, also using a tape measure. The waist-to-hip ratio (WHR) was calculated by dividing the waist circumference in centimeters by the hip circumference in centimeters. Blood pressure measurement was performed with the patient seated, resting for 5 minutes, using a calibrated automatic sphygmomanometer.

Patients with T2DM were selected at the endocrinology clinic of Hospital Santa Casa in Belo Horizonte, Minas Gerais, Brazil, from June 2012 to September 2013. During this period, control patients were called in the local community for interviews and blood sample collection. The inclusion of patients with T2DM was performed according to their order of entry into the follow-up service at Hospital Santa Casa, circumstantially, since the inclusion criteria were met.

The exclusion criteria were: age over 70 years, diagnosis of cancer, pregnancy, autoimmune diseases, and recent history, in the last five years, of infarction, stroke and/or thrombosis; in addition to infections and/or inflammatory processes, current or recent, one month before the interviews. The confirmation of these data was performed by analyzing the medical records, in the case of patients with T2DM and self-reported by each participant in the control group. The latter was composed of individuals with normal fasting glucose levels (60-99 mg/dl) who did not use hypoglycemic agents.

Clinical and laboratory data

Clinical data [sex, age, BMI, abdominal circumference (AC), WHR, and hypertension] and laboratory data [fasting glucose and glycated hemoglobin A1c (HbA1c)] data were obtained for all patients with T2DM through the analysis of medical records and interviews, which was carried out by only one researcher.

Diabetic retinopathy was diagnosed by ophthalmological examination using fundoscopy and microscopic slit lamp examination. The presence of diabetic nephropathy (DNP) was defined as urinary albumin excretion (UAE) > 30 mg/24 h in at least two of three collections at different times in the three-month period, as well as the absence of kidney disease caused by diseases other than diabetes. DNP was not considered when EUA < 30 mg/24 h, was detected in at least two of the three urine collections. Neuropathy was defined according to the criteria of the Diabetes Control and Complication Trial (DCCT), through a physical examination by a neurologist, who detects the presence of signs and symptoms, such as dysesthesias, paraesthesias, hypersensitivity or burning pain to touch, or absence of deep tendon reflexes.

The criteria used to determine systemic arterial hypertension were: systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 80 mmHg, or use of antihypertensive drugs[17]. To classify individuals as dyslipidemic, the following criteria were adopted: low density lipoprotein cholesterol (LDL-C) ≥ 100 mg/dl, and/or high density lipoprotein cholesterol (HDL-C) ≤ 50 mg/dl, and/or triglycerides ≥ 150 mg/dl, and/or use of antilipemics[17].

Clinical data (sex, age, BMI, AC, and WHR) for the controls were also obtained through interviews and direct measurements. Fasting blood glucose in this group was measured in samples collected at the end of the interview.

Venous blood samples were collected from each participant in tubes containing sodium heparin and tubes without anticoagulant; then, they were left to rest for 30 minutes and centrifuged at 1100× g for 20 minutes at 25°C. The fractions obtained in the supernatant (plasma and serum) were aliquoted in microtubes and stored at -80°C until biochemical analysis.

The levels of high-sensitive C-reactive protein (hsCRP) and HbA1c were measured in serum samples using the immunoturbidimetric method, following the manufacturer's

instructions, in Vitros Chemistry 5.1 FS System (Ortho Clinical Diagnostics, USA). All samples were analyzed at the same time.

The fasting glucose levels in the control group were measured in serum samples collected after 8 hours of fasting. The tests were performed by the enzymatic-colorimetric method using Glicose-PP kit (Gold Analisa, Brazil) and BTR 811 spectrophotometer (Biotron, Brazil) – following the manufacturer's instructions –, which found intra- and inter-assay coefficients of variation (CV) of 0.9%-1.2% and 1.9%-2.7%, respectively.

Gal-3 levels were measured in serum samples by the enzyme-linked fluorescence assay (ELFA) technique, with mini VIDAS (BioMerieux®, France) equipment, following the manufacturer's recommendations. The intraassay CV described by the manufacturer was equal to 1.25% and the interassay, equal to 5.5%. The concentrations obtained from this glycoprotein were expressed in ng/ml.

Statistical analysis

Data were analyzed using the Statistical Package of the Social Sciences (SPSS) version 17.0. They were tested for normality by the Shapiro-Wilk test. Parametric data were presented as mean ± standard deviation (SD) and non-parametric data as median (interquartile range).

Student's t test was used for parametric variables; Mann-Whitney test for non-parametric variables; and chi-square test (χ^2) for categorical variables. Correlation analyzes were assessed using Spearman's correlation test. For all analyzes, $p < 0.05$ was considered significant.

RESULTS

All patients selected with T2DM had chronic kidney disease. The clinical and laboratory characteristics of the T2DM and control groups are shown in **Table 1**. As expected, significant differences were not observed between the groups regarding age and BMI values. The number of women was equal in both groups ($n = 36$) as well as the number of men ($n = 6$), totaling 42 participants in each group.

The values of fasting glucose and WHR were higher in the T2DM group comparing to the control group ($p < 0.001$ and $p = 0.001$, respectively). For the variables AC and hs-CRP, no significant differences were observed between the groups studied ($p = 0.425$ and $p = 0.693$, respectively). Gal-3 levels were significantly higher in the T2DM group when compared to the control group ($p = 0.012$).

TABLE 1 – Clinical and laboratory parameters in the T2DM with kidney disease group and the control group

Variable	T2DM ($n = 42$)	Control ($n = 42$)	p
Age (years)	58.5 (55-62)	53.5 (48.25-60.75)	0.09
Female (%)	85	85	1
Male (%)	15	15	1
BMI (kg/m²)	28.11 (24.32-34.04)	28.12 (24.26-33.21)	0.897
AC (cm)	100.88 ± 15.25	98.1 ± 16.56	0.425
WHR	0.942 ± 0.0728	0.885 ± 0.0763	0.001*
Fasting blood glucose level (mg/dl)	153.43 ± 61.34	86.19 ± 8.31	< 0.001*
HbA1c (%)	8.98 ± 1.56	-	-
hsCRP (mg/l)	2.85 (1.03-9.8)	2.7 (1.43-4.7)	0.693
Gal-3 (ng/ml)	15.17 ± 5.54	12.62 ± 3.2	0.012*

Data were expressed as mean ± standard deviation for parametric and median variables and (interquartile range) for non-parametric variables; analyzes were performed using the Student's t-test or Mann-Whitney test, respectively. For the categorical variable (sex), the data were expressed as a percentage of the total, and the analysis of the difference in this distribution was performed using the chi-square test (χ2).
*T2DM: type 2 diabetes mellitus; BMI: body mass index; AC: abdominal circumference; WHR: waist-hip ratio; HbA1c: glycated hemoglobin A1c; hsCRP: high-sensitivity C-reactive protein; Gal-3: galectin-3; *p < 0.05 was considered significant.*

There was no significant relationship between the levels of Gal-3 and the clinical and laboratory parameters studied in the T2DM group ($p > 0.05$) (**Table 2**). Furthermore, when correlating Gal-3 with the parameters age, BMI, AC, fasting blood glucose and hsCRP for individuals in the control group, a positive correlation was observed between BMI and AC ($p = 0.001$ and $p = 0.009$, respectively).

However, although no statistical difference was found ($p = 0.069$), higher levels of Gal-3 were observed in diabetic patients with hypertension when compared to those without such complication. For the variables gender, time of diagnosis, and presence of other complications or comorbidities, no significant difference was observed in relation to Gal-3 levels ($p > 0.05$) (**Table 3**).

TABLE 2 – Correlations between Gal3 levels and clinical or laboratory variables for the T2DM with kidney disease group and the control group

Correlated variables	T2DM		Control	
	r	p	r	p
Age (years)	0.134	0.397	0.263	0.092
BMI (kg/m²)	0.032	0.839	0.485	0.001*
AC (cm)	0.216	0.169	0.397	0.009*
WHR	0.137	0.385	0.13	0.411
Fasting blood glucose level (mg/dl)	-0.026	0.869	-0.108	0.501
HbA1c (%)	0.107	0.501	-	-
hsCRP (mg/dl)	0.285	0.075	0.037	0.817

Spearman correlation.
*Gal-3: galectin-3; T2DM: type 2 diabetes mellitus; BMI: body mass index; AC: abdominal circumference; WHR: waist-hip ratio; HbA1c: glycated hemoglobin A1c; hsCRP: high-sensitivity C-reactive protein. *p < 0.05 was considered significant.*

TABLE 3 – Gal-3 levels in relation to the descriptive variables in the T2DM with kidney disease group

Variables (n = 42)	Gal-3 (ng/ml)	p
Sex		
Male (n = 6)	15.55 ± 9.47	0.86
Female (n = 36)	15.11 ± 4.81	
Hypertension		
No (n = 5)	10.96 ± 2.49	0.069
Yes (n = 37)	15.74 ± 5.61	
Retinopathy		
No (n = 12)	13.79 ± 3.74	0.312
Yes (n = 30)	15.73 ± 6.08	
Neuropathy		
No (n = 21)	15.49 ± 6.23	0.716
Yes (n = 21)	14.86 ± 4.88	
Dyslipidemia		
No (n = 2)	12 ± 4.52	0.413
*Yes (n = 40)	15.33 ± 5.58	
Diagnostic time		
≤ 10 years (n = 17)	14.01 ± 4.34	0.265
> 10 years (n = 25)	15.97 ± 6.18	

Data were expressed as mean ± standard deviation. The p *values were obtained using the chi-square test ($\chi 2$).*

**the presence of dyslipidemia was categorized as HDL< 50 mg/dl and/or LDL > 100 mg/dl and/or triglycerides > 150 mg/dl; T2DM: type 2 diabetes mellitus; HDL: high-density lipoprotein; LDL; low-density lipoprotein; Gal-3: galectin-3.*

DISCUSSION

In this study, we observed that Gal-3 levels were increased in patients with T2DM and kidney disease when compared to the control group without diabetes. The data further suggest that Gal-3 levels need to be investigated in a larger sample to evaluate its correlation with hypertension.

Studies have described an important role of Gal-3 in the modulation of adiposity and insulin resistance[18]. This glycoprotein also showed a positive correlation with parameters such as age and inflammatory markers, and direct association with the presence of obesity, diabetes, and hypertension in analyzes carried out in the general population[11].

In a study developed by Yilmaz *et al*. (2014)[18] with groups of normoglycemic, pre-diabetic and diabetic individuals, after performing the oral glucose tolerance test (OGTT), Gal-3 levels were higher in the last group ($p < 0.001$) when compared with the others. This lectin was also shown to be more increased in pre-diabetic individuals compared to those who did not have the disease. Moreover, Gal-3 show significant and positive correlation with fasting glucose levels, postprandial glucose, C-reactive protein (CRP) and homeostasis model assessment of insulin resistance (HOMA-IR)[18]. In the study conducted by Holmager *et al*. (2017)[19], higher levels of Gal-3 were also found in patients who had diabetes, as well as in individuals who had only reduced glucose tolerance, when compared to healthy controls.

Gal-3 was also elevated in diabetic individuals compared to the control group in the cohort study carried out by Flores-Ramirez *et al*. (2017)[20] with 121 participants. Patients were divided into two groups – diabetics with mild depressed ejection fraction and diabetics with preserved ejection fraction, to check for possible variations in Gal-3 levels in the context of preclinical heart failure (HF) and establish a relationship with global longitudinal strain (GLS). However, no differences were found between groups ($p > 0.05$)[20]. It is important to note that the values found for this glycoprotein in diabetic patients, in the study mentioned above, were lower than those obtained in patients participating in our study (3.46 ± 1.36 ng/ml *versus* 15.17 ± 5.54 ng/ml, respectively). This difference between results could be associated with the methodology used, as well as the variation of anthropometric and clinical characteristics of the studied participants. The measurement of Gal-3 was performed on plasma samples using the enzyme immunoabsorbent assay (ELISA) technique, unlike our work, in which we used serum samples analyzed by the enzyme-linked fluorescence assay (ELFA). In addition to the differences in the type of sample, which can lead to different concentrations of the analyte, these kits also have different calibrator, which prevents their comparison.

To explain the mechanisms by which Gal-3 is involved in the pathogenesis of DM, some authors[5, 9, 21] used animal models and cell cultures. In an obesity study developed with animal model, an increase in the concentration of Gal-3 in adipose tissue was detected when a diet rich in fats was provided[21]. In addition, knockout mice (KO) for the gene encoding Gal-3 (*LGALS3*), when fed with a high-fat diet, showed less weight gain than those that contained the wild-type gene[22, 23]. Baek *et al*. (2015)[23] found higher concentrations of the adipocyte triglyceride lipase (ATGL) enzyme in the white adipose tissue of the epididymis of these deficient mice in Gal-3. Therefore, the decrease in adiposity in KO mice was associated with the increase in ATGL expression, although the mechanisms by which this lectin regulates the enzyme expression are not yet clear[23].

In the analyzes performed with cells of the human subcutaneous adipose tissue, this glycoprotein was found mostly expressed in preadipocytes[21]. When administering recombinant human Gal-3 in the culture of these cells, it was noted that this lectin was able to induce cell proliferation and also increased the synthesis of deoxyribonucleic acid (DNA), through activation of the peroxisome proliferator-activated receptor γ (PPAR-γ),

possibly by a lectin-carbohydrate interaction mechanism[11, 12, 21, 23]. It was also observed that the expression of Gal-3 in adipocytes can be induced by pro-inflammatory mediators, such as free fatty acids and IL-6, indicating that this glycoprotein may be involved in the pathogenesis of obesity and, consequently, T2DM[12, 21].

Another population of KO mice for Gal-3 showed a less intense inflammatory response, atherosclerotic lesions, and susceptibility to type 1 diabetes mellitus (T1DM), induced by streptozotocin doses, and improved insulin sensitivity in mice already obese and resistant to this hormone[5, 22, 24]. When Gal-3 was administered directly to these KO animals, this lectin improved macrophage chemotaxis, decreased plasma glucose uptake, and led to insulin resistance[5, 22].

In the work carried out by Li *et al*. (2016)[5], *in vitro* treatment with Gal-3 resulted in reduced insulin sensitivity in myocytes, hepatocytes, and adipocytes. Experimental evidence has shown that *in vivo* treatment, using selective inhibitors of Gal-3, is able to resume insulin sensitivity in myocytes and adipocytes that was previously decreased[5, 22].

Gal-3 can interact directly with the insulin receptor and inhibit subsequent cell signaling that would lead to peripheral glucose uptake. Thus, it was proposed that this glycoprotein would be one of the etiological factors that contribute to obesity-induced insulin resistance and chronic tissue inflammation[5].

In contrast to the works already mentioned, Darrow and Shohet (2015)[25] described that low levels of Gal-3 could lead to metabolic imbalance and endothelial dysfunction in T2DM and impair tissue glucose uptake by decreasing the expression of glucose transporter 4 (GLUT4).

Ohkhura *et al*. (2014)[26] also reported that reduced concentrations of Gal-3 would be linked to insulin resistance, after negative correlation was observed between levels of this glycoprotein and parameters such as fasting insulin and HOMA-IR index, in the insulin tolerance test and in the hyperglycemic and euglycemic clamp, performed in a group of 20 patients with T2DM. There was also a positive correlation between Gal-3 and the clearance rate of glucose, the insulin sensitivity index (ISI) and the serum level of adiponectins[26]. Thus, increased levels of this lectin would be exerting a protective effect in the context of diabetes. However, the study mentioned above is limitated by the low number of patients participating in the analyiss.

Regarding the anthropometric indices of diabetic patients analyzed in this study, Gal-3 showed no correlation with AC and BMI. On the other hand, a significant correlation was observed between these same parameters and Gal-3 in the control group. These results suggest that in diabetic patients, the disease is predominant in relation to these changes, that is, such observations did not have an important impact in this group. However, in the general population without diabetes, changes in Gal-3 levels could significantly impact anthropometric parameters and still be related to the amount of adipose tissue present in these individuals.

On the other hand, in 2010, Weigert *et al*.[24] observed increased serum levels of Gal-3 in overweight non-diabetic individuals, as well as in T2DM patients who were also overweight, when compared to a control group. It is important to highlight that this analysis was performed using only male individuals. This glycoprotein also demonstrated a positive correlation with BMI, age, and IL-6 and leptin levels[24].

It has been reported that Gal-3 concentrations would be increased in the serum of patients with high CRP levels[26]. According to Menini *et al*. (2016)[11], in samples from diabetic patients, Gal-3 levels correlated inversely with HbA1c and were higher in individuals with hsCRP values greater than 5 mg/l. In the analyzes carried out by Holmager *et al*. (2017)[19], a positive correlation between HbA1c and an increase in plasma Gal-3 levels was observed in patients diagnosed with DM and HF. In the present study, there was no significant correlation between the hsCRP and HbA1c levels with Gal-3 levels in the T2DM group.

Some authors have reported that the increased plasma concentrations of Gal-3 are related to the micro and macrovascular complications of T2DM[19]. Clinical and epidemiological data suggest that the presence of T2DM is strongly associated with mortality and morbidity of cardiovascular diseases[1]. In our study, although no significance was observed, there was a trend towards higher levels of Gal-3 in hypertensive diabetic patients (15.74 ± 5.61 ng/ml) when compared with those who did not have this clinical condition (10.96 ± 2.49 ng/ml).

According to Bobronnikova (2017)[27], higher concentrations of Gal-3 were observed in patients with hypertension and T2DM, compared to a group of patients who had only hypertension (group 1); prediabetic, and hypertensive patients (group 2); and the control group. A positive correlation between this lectin and insulin resistance indices and vascular and cardiac remodeling was also described in this study population[27].

In the analyzes developed by González *et al*. (2016)[9], using KO mice for Gal-3, after inducing hypertension by angiotensin II (AG II), a lower macrophage infiltrate was observed in the myocardium (51 ± 10 cells/mm^3) compared to the C57BL/6J that contained the healthy gene for that glycoprotein (118 ± 6 cells/mm^3). It was also found that significant myocardial fibrosis occurred in C57BL/6J mice (9.3 ± 1.3%), and lower effect in KO

mice for Gal-3 (3.4 ± 0.8%) after the infusion of AG II. In addition to these data, lower plasma levels of IL-6, increased number of splenic regulatory T lymphocytes ($CD4^+/CD25^+/FOXP3^+$) and reduced cardiac expression of intercellular adhesion molecule-1 (ICAM-1) in animals with Gal-3 deficiency were found, which could confirm the effects of Gal-3 as a modulator of inflammation, fibrosis and dysfunction in hypertension[9].

Berezin *et al*. (2017)[1] suggested that the measurement of Gal-3 levels in association with other cardiac markers, such as troponins and natriuretic peptides (NPs), could help to stratify the risk of cardiac events in diabetic patients[1].

When analyzing the plasma of 3,450 individuals from Framingham Offspring Cohort, Ho *et al*. (2012)[28] found higher levels of Gal-3 in women [14.3 (12 to 16.8 ng/ml)] than in men [13.1 (11.1 to 15.4 ng/ml)][28]. De Boer *et al*. (2012)[6] also observed higher values of this glycoprotein in women [11 (9.1 to 13.4 ng/ml)] when compared to men [10.7 (8.9 to 12. 8 ng/ml)], in the cohort study Prevention of Renal and Vascular ENd-stage Disease (PREVEND), with 7,968 participants. This difference may be related to the influence of sex hormones on the modulation of Gal-3 levels. However, the mechanisms underlying this observation are still unclear[6]. However, no significant differences ($p = 0.860$) in Gal-3 values between women and men (15.11 ± 4.81 ng/ml versus 15.55 ± 9.47 ng/ml) were observed. It is important to note that the number of women was greater than the number of men selected, representing 85.7% ($n = 36$) of the participants in each group. Although the selection was made of convenience, without selecting the patient by gender, the difference between the groups stems from the characteristic of the service that provides care to these patients.

The presence of the other diabetic complications analyzed – retinopathy, neuropathy, and nephropathy – also showed no association with Gal-3 concentrations in patients with T2DM. When analyzing those who had dyslipidemia concomitant with DM, the mean values of this glycoprotein were not different when compared with patients who did not have this comorbidity.

It is worth mentioning that all patients in the present study with T2DM already had DNP, due to the characteristic of the clinical service responsible for the care of these individuals. The role of Gal-3 in the context of DNP is not very evident, although a strong relationship has already been observed between increased Gal-3 expression and renal fibrosis[29]. Some authors have described that increased levels of this lectin are possibly associated with the action of advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) in the kidneys and, thus, would cause endothelial dysfunction and interfere with glomerular filtration[11]. However, there are studies that state that the deficiency of this lectin would accelerate the deposition of these products[30], since Gal-3 would be able to bind to them, facilitating their clearance, and would have a protective effect on the DNP condition[29, 31].

A three-year prospective study by Hodeib *et al*. (2019)[29] with 300 patients with DNP showed that the concentration of Gal-3 was higher in those who already had longer duration of T2DM and showed a significant correlation ($p < 0.05$) with serum creatinine levels, creatinine/albumin ratio, systolic and diastolic blood pressure, in addition to other parameters previously mentioned, such as age and BMI. The highlight of this work was the fact that the Gal-3 average levels were higher in patients with macroalbuminuria when compared to those who had microalbuminúria, and were higher in the latter than in those with normoalbuminuria. Thus, this glycoprotein could assist in monitoring these patients in order to infer about the progression of renal disease in diabetes[29]. In contrast, in the present study, patients who had a longer time of diagnosis for T2DM did not show higher levels of Gal-3 compared to those with a time of diagnosis less than 10 years ($p = 0.265$). Furthermore, there was no correlation between age ($r = 0.134$, $p = 0.397$) and Gal-3 levels in our study. However, it has been reported that elderly patients with cardiovascular risk factors, such as hypertension, diabetes, previous coronary heart disease, high BMI, and lower estimated glomerular filtration rate (eGFR) presented higher Gal-3 values ($p < 0.0001$)[28].

Our study has important limitations, such as sample size, especially with regard to the proportion of individuals between the groups of diabetic patients with and without dyslipidemia, which limits the statistical comparison between the two groups. Another issue is related to the fact that the control group is not identical to the T2DM group, since the latter showed statistically higher values for WHR, in addition to kidney disease. Knowing that Gal-3 is related to adipose tissue hypertrophy, according to studies previously cited the WHR parameter may be a confounding factor to be considered. Moreover, there are no data on Gal-3 levels in diabetic patients at different times – before, after diagnosis, and initiation of treatment – to evaluate the variation in plasma levels of this glycoprotein over time. As highlighted by De Boer *et al*. (2012)[6], circulating levels of this glycoprotein may not reflect its activation by a single organ or tissue. Therefore, caution should be exercised when inferring about altered Gal-3 concentrations.

CONCLUSION

Our data suggest that Gal-3 may be involved in the pathophysiology of T2DM, since elevated levels of this

glycoprotein have been observed in diabetic patients. Increased circulating levels of this glycoprotein point to it as a future biomarker or pharmacological target of the disease, since increased concentrations of Gal-3 may favor insulin resistance, hyperglycemia, obesity, and heart disease. Despite not showing any significance, Gal-3 seems to be related to arterial hypertension in diabetic patients, which was already observed by other studies. Therefore, further studies designs allowing comparability of cases and controls are needed, especially of diabetic patients with and without complications and comorbidities of the disease, in order to assess the influence of other parameters over time, such as medication use, gender, and age, at the levels of that glycoprotein.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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