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Evaluation of Galectin-3 in Graves' Disease With and Without Ophthalmopathy

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ABSTRACT

Objective: There is a link between thyroid-associated ophthalmopathy and Graves' disease; however, the exact pathophysiological mechanism remains unclear. Galectin-3 expressed by immune and inflammatory cells plays a role in various vital cellular functions as well as supports angiogenesis and fibroblastic activity. The role of Galectin-3 in thyroid-associated ophthalmopathy has not been studied yet, so we aimed to evaluate the alteration of Galectin-3 level in thyroid-associated ophthalmopathy.

Methods: This cross-sectional descriptive study was conducted from May 2018 to April 2020. This study consisted of 63 participants, who were divided into 3 groups: group 1 was composed of 21 patients with Graves' disease with thyroid-associated ophthalmopathy, group 2 consisted of 21 patients with Graves' disease without orbitopathy, and group 3 consisted of 21 healthy individuals.

Results: Although the mean Galectin-3 levels in group 1 (8.6 ± 4.1 ng/mL) and group 2 (7.1 ± 5.4 ng/mL) were higher than group 3 (3.7 ± 2.8 ng/mL), there was no significant difference in Galectin-3 levels between groups 1 and 2 ($P = .001$ and $P = .030$, respectively). In Pearson's correlation analyses, there was a significantly positive correlation between Galectin-3 and thyroid-stimulating hormone ($r = 0.452$, $P = .003$) and a negative correlation between Galectin-3 and thyrotropin receptor autoantibody ($r = -.318$, $P = .040$).

Conclusion: In this study, it is shown for the first time that Galectin-3 levels are higher in patients with Graves' disease than in healthy individuals. Increasing Galectin-3 levels may trigger autoimmunity or may lead to the development of thyroid hyperplasia. Although Galectin-3 level was found to be high in patients with Graves, we did not find any relationship between Galectin-3 and thyroid-associated ophthalmopathy.

Keywords: Galectin-3, Graves' disease, ophthalmopathy

Introduction

Graves' ophthalmopathy or thyroid-associated ophthalmopathy (TAO) is closely related to Graves' disease, both of which are autoimmune diseases, and approximately 25%-50% of patients with Graves' disease have TAO.[1] Although the pathophysiological mechanisms of TAO are not fully understood, the infiltration of lymphocytes and macrophages may be the key element that causes the inflammation of the extraocular muscles and the connective tissues surrounding the eye.[2] Infiltrating immune cells and their products activate the orbital fibroblasts. A proliferation and differentiation process results in the orbital tissue remodeling and expansion. Furthermore, inflammatory mediators and growth factors secreted by activated orbital fibroblasts contribute to uncontrolled inflammation.[3,4]

Galectin-3 (Gal-3) is a β-galactoside-binding lectin, and as the most common type of galectin, expressed in many immune and inflammatory cells which has multiple functions including adhesion, signaling, proliferation, differentiation of cell, and apoptosis and angiogenesis.[5,6] It is known that Gal-3 supports fibroblast proliferation and transformation by activating various profibrotic factors and mediates collagen production.[7] There are various data showing that it can be used as a marker or as a treatment target in fibrotic diseases such as heart failure, kidney failure, and cirrhosis.[8]

To our knowledge the role of Gal-3 in the TAO process has not been studied before. The purpose of this study is to examine whether plasma Gal-3 levels are related to orbitopathy in patients with Graves' disease.

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Material and Methods

We conducted a cross-sectional descriptive study with a total of 63 participants including Graves' disease patients with TAO (group 1, n = 21), Graves' disease patients without orbitopathy (group 2, n = 21), and a healthy control group that was matched for age and sex (group 3, n = 21).

Participants were included in the order of admission to the outpatient clinic. All the patients were still being treated with anti-thyroid drugs. Patients with pregnancy, rheumatological disease, organ failure, malignancy, diabetes, hypertension, and dyslipidemia were excluded. Graves' patients with and without orbitopathy were divided into study groups taking into account age and gender. The Ethics Committee of Harran University approved the study protocol according to the Helsinki Declaration (Date: December 3, 2019, Decision No: 19240). All subjects approved the written consent.

Power analysis was done with the G-power program. For an effect size of 0.3, an alpha error of 0.5%, and a power of 80%, the sample size to represent the population was determined to be 64.

Disodium Ethylenediaminetetra-acetic acid (EDTA) tubes were used to collect venous blood samples from the ulnar region. Afterward, the samples were centrifuged at 4000 rpm for 15 minutes and stored at –80°C. The level of Gal-3 was measured using the enzyme-linked immunosorbent assay (ELISA) method with a kit from Elabscience (catalog number: E-EL-H1470) according to the directions provided by the manufacturer. Values above 1.75 IU/L were considered positive for the plasma thyrotropin receptor autoantibody (TRAb) concentration as measured by the radioreceptor assay. The plasma levels of thyroid-stimulating hormone (TSH), free T3 (fT3), and free T4 (fT4) were measured using chemiluminescence immunoassay (SIEMENS, Atellica). The reference ranges were as follows: Gal-3: 0.16-10 ng/mL, TSH: 0.35-5.5 mIU/L, fT3:2.3-4.2 pg/mL, fT4: 0.89-1.76 ng/dL.

Statistical Analysis

An analysis of the data was carried out using the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows. To check the normality of the data, we used the Kolmogorov–Smirnov test. To describe normally distributed data, mean and standard deviation were calculated, while median (minimum–maximum) was used to describe nonnormally distributed data. Categorical variables were compared using the chi-square test and presented in the form of number and percentage. Three or more groups were compared using one-way analysis of variance (ANOVA) if the data were normally distributed. In post hoc analyses, Tukey's test was used if homogeneity of variance was assumed. Pearson's correlation coefficient was used to measure the correlations between the parameters in the study. A P value of $<.05$ was considered significant.

MAIN POINTS

- Galectin-3 influences a wide range of biological and pathological processes, such as autoimmunity, inflammation, fibrosis, cell adhesion, proliferation, differentiation, and tumor invasion.
- Thyroid-associated ophthalmopathy is characterized by the activation of orbital fibroblasts by immune cells and their products.
- It was found that patients with Graves' disease had elevated Galectin-3 levels compared with healthy controls. However, there were no differences between Graves' patients with thyroid-associated ophthalmopathy and those without.

Results

In terms of age, gender, and body mass index, there was no difference between the groups. Mean TSH levels were significantly lower in group 1 (0.17 ± 0.48 mIU/L) and group 2 (0.54 ± 1.11 mIU/L) when compared to group 3 (1.98 ± 1.03 mIU/L) ($P < .001$ for both). Mean free T4 levels were significantly higher in group 1 (2.4 ± 2.2 ng/dL) and group 2 (2.6 ± 1.8 ng/dL) when compared to group 3 (1.1 ± 0.2 ng/dL) ($P = .040$ between group 1 and 3, $P = .010$ between group 2 and 3). Mean TRAb levels of group 1 (22.2 ± 17.5 IU/L) were significantly higher than group 2 (13 ± 11.8 IU/L, $P = .040$) and group 3 (0.8 ± 0.2 IU/L, $P < .001$). Mean TRAb levels of group 2 were also significantly higher than group 3 ($P < .001$). Mean Gal-3 levels were significantly higher in group 1 (8.6 ± 4.1 ng/mL) and group 2 (7.1 ± 5.4 ng/mL) when compared to group 3 (3.7 ± 2.8 ng/mL) ($P = .001$ and $P = .030$, respectively). There was not a significant difference between groups 1 and 2 in terms of Gal-3 levels (Table 1).

In Pearson's correlation analyses, Gal-3 levels were significantly and positively correlated with TSH ($r = 0.452$, $P = .003$) and significantly and negatively correlated with TRAb ($r = –.318$, $P = .04$) (Table 2).

Discussion

According to this study, plasma Gal-3 concentrations in patients with Graves' disease were significantly higher than in those without Graves' disease. On the other hand, plasma Gal-3 concentrations in Graves disease with and without TAO did not differ between groups. The variation in Gal-3 levels in TAO had not been studied previously. The results obtained from this study suggest that neither the presence nor absence of ophthalmopathy in Graves' patients alters plasma Gal-3 levels.

Gal-3 is pivotal in numerous cell functions including differentiation, transformation, growing, pre-mRNA splicing, and apoptosis, as well as in other biological activities including inflammation, host defense, angiogenesis, and fibrosis.[8] In Graves' disease, immunotolerance to thyroid structures is disrupted by a number of factors, including both endogenous and environmental factors. This results in the induction of TRAb production by B cells.[9]

Among thyroid diseases, Gal-3 has been mostly studied in thyroid nodules and thyroid malignancies. High levels of Gal-3 have been associated with thyroid cancer, especially papillary subtypes, and may play a role in the pathogenesis of well-differentiated thyroid carcinomas.[10] Various studies have shown that increased Gal-3 expression in thyroid tissue can differentiate malignant and benign adenomas as an immunohistochemical marker.[11-13] The study conducted by Savin et al[14] revealed that Gal-3 is not present in thyroid epithelial cells in fetal development, which suggests that Gal-3 is predominantly expressed during malignant transformation of thyroid epithelial cells. It is unknown whether Gal-3 plays a direct role in cancer development; however, increased expression of Gal-3 has been shown to alter the adhesion and motility of tumor cells, increasing the possibility of metastasis.[15] A study by Dikker et al[16] found that Gal-3 levels were increased in hypothyroid patients, which may contribute to thyroid gland hyperplasia. There is no previous research examining whether Gal-3 levels are altered in Graves'

Table 1. Comparison of Clinical and Biochemical Parameters between Groups

Parameters	Group 1 (n = 21)	Group 2 (n = 21)	Group 3(n = 21)	*P*
Age (year)	35.1 ± 9.5	33.7 ± 11.3	32.0 ± 10.8	.847
Gender (M/F) (n(%))	8/13 (38)	10/11 (48)	7/14 (33)	.638
BMI (kg/m^2)	24.6 ± 2.7	23.4 ± 2.5	23.1 ± 3.3	.187
Glucose (mg/dL)	96.0 (78.0-121.0)	91.0 (72.0-131.0)	90.0 (72.0-126.0)	.235
TSH (mIU/L)	0.001 (0.001-2.0)[a]***	0.001 (0.001-3.5)[b]***	1.8 (0.6-4.7)	<.001
fT4 (ng/dL)	1.6 (0.6-10.5)[a]*	2.0 (0.9-6.5)[b]**	1.1 (0.8-1.4)	.008
TRAb (IU/L)	22.2 ± 17.5[a*,c]***	13 ± 11.8[b]***	0.8 ± 0.2	<.001
Galectin-3 (ng/mL)	8.6 ± 4.1[a]*	7.1 ± 5.4[b]*	3.7 ± 2.8	.001

Data are expressed as the mean and standard deviation or median (min-max) or the number (%) of patients. *P* value < .05 was considered significant.
BMI, body mass index; fT4, free T4; TRAb, thyrotropin receptor autoantibody; TSH, thyroid-stimulating hormone.
[a]Between group 1 and 3, [b]between group 2 and 3, [c]between group 1 and 2.
*P < .05, **P < .01, ***P<.001.

Table 2. Correlation of Galectin-3 with Other Parameters in Patients with Graves Disease (n = 42)

		Age	BMI	Glucose	TSH	fT4	TRAb
Galectin-3	*r*	0.144	0.171	−0.077	0.452	−0.161	−0.318
	P	.363	.279	.630	0.003	.309	0.040

BMI, body mass index; fT4, free T4; TRAb, thyrotropin receptor autoantibody; TSH, thyroid-stimulating hormone.
P value < .05 was considered significant.

disease or thyrotoxicosis. As compared with healthy controls, Gal-3 levels were significantly higher in patients with Graves' disease, which suggests autoimmune reactions may be triggered by Gal-3. It is well known that the pathological process of Graves' disease leads to hyperplasia and overstimulation of the thyroid epithelium.[17] Increased level of Gal-3 in our study may be related with thyroid gland hyperplasia.

Thyroid-associated ophthalmopathy is the most common extrathyroidal complication of Graves' disease and is estimated to affect approximately 25%-50% of patients.[18] Thyroid-associated ophthalmopathy is generally accepted as an autoimmune condition, but its exact mechanism remains unknown. The development of TAO is influenced by numerous environmental, immune, and genetic factors that can lead to inflammation, adipogenesis, edema, and fibrosis.[19] It is believed that TAO results from the formation of autoantibodies against an autoantigen present in thyroid and retrobulbar tissues.[2] Thyroid-stimulating hormone receptors, which are expressed on fibroblasts and orbital preadipocytes, might be the common antigen.[20] First, immune cells infiltrate extraocular muscles and connective tissues, followed by the release of cytokines, activating orbital fibroblasts to synthesize glycosaminoglycans.[21] This process results in the accumulation of adipose tissue, swelling of muscle, and fibrosis.[21] Basically, we expected that Gal-3 may be related with TAO. However, Gal-3 levels were not different among groups with and without TAO, Thus no relationship was found between Gal-3 and TAO. These results suggested that Gal-3 may be important in the inflammatory process of Graves' disease, but did not contribute to the fibrosis of orbital tissue.

We were unable to stage TAO based on severity since there were not enough participants in our study. This is the most important limitation of our study. However, fibrosis might not have developed since our patients consisted of those who started treatment at an early stage. Fibrosis of the eye muscles is generally observed during the final stages of TAO and Gal-3 may be altered at this final stage.[17] In addition, a study conducted on animals has shown that inhibition of the Gal-3 pathway can reduce the fibrosis of orbital cells.[22] If Gal-3 can be proven to be the cause of TAO fibrosis at an advanced stage, Gal-3-directed therapy may become an option.

The presence of high levels of TRAb has been associated with clinical activity and severity of TAO.[23] The positive correlation of Gal-3 with TSH and the negative correlation with TRAb in this study led us to believe that Gal-3 was not associated with TAO.

In conclusion, for the first time in the literature, we found elevated Gal-3 levels in patients with Graves' disease compared with healthy controls. This increment may be one of the pathological trigger of autoimmunity or may be related with thyroid gland hyperplasia. Further studies are needed to show the relation between advanced TAO and Gal-3.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Harran University, (Date: December 3, 2019, Decision No: 19240).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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Declaration of Interests: The authors have no conflicts of interest to declare.

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