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Dose Hypothyroidism Inhibit the Synthesis of Galectin-16? A New Trial to Find a Decisive Diagnostic Marker for Hypothyroidism

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Abstract. The objective of this study was to determine the levels of galectin-16 in serum samples from patients with thyroid disease (specifically hypothyroidism and hyperthyroidism) and healthy individuals as controls. Interestingly, galectin-16 was not detected in serum samples from patients with hypothyroidism. However, the study found that the protein was also present in samples from the other two groups. When comparing galectin 16 levels between hypothyroid and hyperthyroid patient groups and controls, a statistically significant difference was observed (p=0.000). Notably, galectin-16 levels were not statistically significant between hyperthyroid patients and control subjects (p = 0.689). The results also showed that galectin-16 levels were significantly elevated in male patients with hypothyroidism and hyperthyroidism compared with healthy males (p = 0.000). Similar results were observed when comparing female patients with healthy individuals. However, the study did not find any statistical differences between men and women in patients or controls. It is worth mentioning that galectin-16 has 100% sensitivity and specificity for hypothyroidism. Galectin-16 has a sensitivity of 13% and a specificity of 93.33% for detecting hyperthyroidism. From these findings it can be concluded that galectin-16 is an important diagnostic indicator of hypothyroidism, exceeding its effectiveness in distinguishing between normal and pathological conditions.

1 Introduction

Thyroid disease is a prevalent condition with various causes, such as hyperthyroidism and hypothyroidism. Hypothyroidism is a disorder that hinders the synthesis of thyroid hormones (T3, T4, and low levels of FT4), either due to external or internal factors, often genetic. This results in an overproduction of TSH, leading to the formation of goiters and stimulating the entire process of hormone production [1]. Subclinical hypothyroidism is characterized by elevated TSH levels while FT4 levels remain normal [2]. Hypothyroidism affects approximately 5% of the global population [3]. Several factors, including age, gender, and geographical location, influence its prevalence [4]. It is more common in women, increases with age [4], and is more prevalent in white individuals compared to those with darker skin [5]. Hyperthyroidism, also known as thyrotoxicosis [6, 7], is a

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pathological condition characterized by decreased TSH levels and increased levels of thyroid hormones (T4 and T3). In subclinical hyperthyroidism, TSH levels are reduced while FT4 levels remain normal [8]. Hyperthyroidism can impact multiple organ systems, including the cardiovascular system, nervous system, gastrointestinal tract, and liver [9].

The LGALS16 gene encodes Galectin-16, a monomeric protein consisting of 142 amino acids and weighing 16.6 kDa [10]. Despite being discovered a decade ago, the exact function of this gene remains a mystery. Galectin-16 is involved in cell differentiation and apoptosis [10], and it is present in both the brain and placenta. Elevated levels of Galectin-16 have been linked to the development of preeclampsia and hypertension [11].

2 Materials and Methods

Limitations of the study: The current study precluded the inclusion of the following cases: all participants with chronic disease (i.e., patients with breast cancer or controls), people with chronic disease. H.; participants in this study included patients with liver, kidney, cardiovascular disease, diabetes, high blood pressure and obesity, all of these diseases are associated with smoking, patients who have undergone surgery that is invasive in nature, patients who are treated for thyroid disease, and patients who have undergone total or partial thyroidectomy. Those who took supplements for protein became pregnant.

Sample collecting: Get 5 ml of blood from patients and healthy individuals using gel tubes. Separate the serum by centrifuging at 5,000 xg for 5 minutes. Preserve serum samples in Eppendorf containers at -20°C until needed.

The evaluation of Galectin-16: The concentrations of Galectin-16 in the serum samples of the participants were determined using sandwich ELISA technology, a technology that is produced by China's PARS Biochem Company.

Statistical analysis: The results of this study were evaluated using the Statistical Package for the Social Sciences (SPSS) version 26, which is a software application that is used for statistical analysis, and Excel (Statistical Package), which is a Microsoft Excel add-on. Variables are characterized by the mean plus or minus the standard error, the minimum value, the maximum value, the frequency, the percentage and the cumulative percentage. Rock graphs are employed to demonstrate the degree to which the evaluated parameters are affected by one another. Inferential data analysis involved: One-way analysis of variance (ANOVA) tests were employed to assess the potential differences between the chemical compounds evaluated. The probability of deviation from the intended path was considered significant if the p-value was less than 0.05. Sensitivity and specificity percentages were derived from medical statistics.

3 Results and Discussion

The study group's samples were analyzed to determine the levels of galectin-16. The findings of this research are incredibly encouraging, as galectin-16 was not found in the serum samples of patients with hypothyroidism. However, the presence of this protein was detected in samples from two other groups, as illustrated in figure 1.

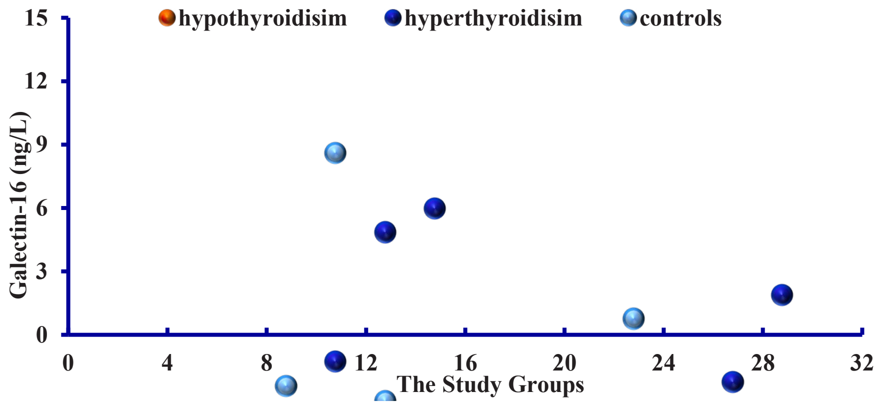

Fig. 1. Distribution of Galectin-16 in The Study Groups

Upon conducting a statistical analysis using the ANOVA test, it was determined that a statistically significant difference (p=0.000) existed when comparing the hypothyroidism patient group and the hyperthyroidism patient group with the control group members (p=0.000). However, when examining the galectin 16 levels, there was no statistically significant difference found between the hyperthyroidism group and the control group (p=0.689), as indicated in Table 1.

Table 1. Levels of Galectin-16 (ng/L) in the Sera of Study Samples

	Subjects (N) Mean ± S.E. Minimum-Maximum			*p-value*
	***Hypothyroidism* 30**	***Hyperthyroidism* 30**	***Controls* 30**	
Galectin-16 (ng/L)	0.000±0.000 0.000-0.000	4.140±0.574 0.503-10.832	4.396±0.5271 1.047-12.111	**0.000 For G1 *vs* G2** **0.000 For G1 *vs* C** 0.689 **For G2 *vs* C**

G1: Group of Hypothyroidism, G2: Group of Hyperthyroidism, C: Group of Healthy Controls, the difference is considered significant at p<0.05

Upon conducting a statistical analysis, it was determined that there were no noteworthy variances between genders within a particular group, regardless of the health conditions of the individuals within that group. Similarly, the study revealed no significant distinctions when comparing galectin-16 levels in a group of men and women with hyperthyroidism to those in a control group consisting solely of men. However, research indicates a remarkably significant differentiation between genders in patients with hypothyroidism in comparison to patients with hyperthyroidism or individuals of the same gender in good health (p=0.000).

Table 2. Levels of Galectin-16 (ng/L) in the Sample of the Study Individuals

Subjects (n)	*Sex (n)*	*Gal-16 (ng/L)Mean ± S.E.*	*Minimum-Maximum*	*p-value*
Hypothyroidism (30)	**Male 10**	0.000±0.000	0.000-0.000	1.000 **For 1 vs 2** 0.807 **For 3 vs 4**

	Female 20	0.000±0.000	0.000-0.000	0.302 **For 5 vs 6** **0.000 For 1 vs 3** **0.000 For 1 vs 5** 0.511 **For 3 vs 5** **0.000 For 2 vs 4** **0.000 For 2 vs 6** 0.975**For 4 vs 6**
Hyperthyroidism (30)	**Male 10**	4.298±1.194	0.509-10.800	
	Female 20	4.061±.6445	0.503-10.832	
Controls (30)	**Male 11**	5.016±0.943	1.047-12.111	
	Female 19	4.037±0.634	1.195-12.010	

1: Males wit Hypothyroidism, 2: Females with Hypothyroidism, 3: Males with Hyperthyroidism, 4: Females with Hyperthyroidism,5: Healthy Males, 6: Healthy Female. The difference is Considered Significant at $p<0.05$.

The atomic structure of Gal-16, along with its two mutants, has yet to be fully understood. To shed light on this, X-ray crystallography techniques were employed. In addition, the oligomeric state of galectin-16 was examined using gel filtration, while its hemagglutination activity and lactose-binding ability were determined through ITC. The interaction between galectin-16 and c-Rel was thoroughly investigated using pull-down studies, microthermophoresis, and immunofluorescence. Unlike other galectin family members, galectin-16 exists solely as a monomer and lacks the capability to bind β-galactosyl lactose. Moreover, it does not induce red blood cell agglutination. Through the use of enhanced green fluorescent protein (EGFP)-tagged galectin-16, it was observed that this protein predominantly localizes in the nucleus across various cell types and can interact with c-Rel. Notably, galectin-16 plays a regulatory role in signaling pathways involving the c-Rel axis in B cells or T cells at the maternal-fetal interface [12].

The outcomes of this study indicated that individuals with hyperthyroidism exhibit comparable levels of galectin-16 as those in the control group. This implies that the production of galectin-16 remains unaffected by the alterations associated with excessive thyroid hormone production. In contrast, the results point towards a drastic upheaval in co-production of Galectin-16 among hypothyroid individuals; this disruption may be fueled by structural modifications favoring excessive storage of Galectin-16 which in turn suppresses production pathways leading to it. The compromised synthesis of Galectin-16 could exacerbate disease manifestations that impede energy resource provision and promote fat mass: a result induced due to hampering energy expenditure and constraining it to minimal levels, with an added dimension through which Galectin-16 promotes programmed cell death pathways as well as CD3+ T cell apoptosis. This supports further evidence against normal synthesis for galectin 16 under such conditions.

The initiation of this pioneering research on galectin-16 levels among thyroid disorder patients marks the first of its kind. The results hold great promise — which implies that galectin-16 might be used as a diagnostic tool to identify hypothyroidism not caused by other thyroid diseases. A parallel independent study is delving into galectin-16 levels in women suffering from polycystic ovary syndrome: surprisingly, no disparities worth noting were unearthed between those that had the syndrome and healthy women. This finding further adds weight to using galectin-16 as a diagnostic marker for hypothyroidism and underscores the need for more studies on this protein under different pathological and non-pathological conditions, thus driving home the point on its potential utility.

The ROC-AUC curve was used to show how effective Galectin-16 is in diagnosing thyroid diseases. The data shown in Table 3 reveal that Galectin-16 has a sensitivity of 100% it was able to catch all 30 cases of hypothyroidism. Furthermore, the levels of Galectin-16 surpassed the average values observed in healthy individuals, resulting in a specificity of 100% for the identification of hypothyroidism.

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Table 3. Receiver Operating Characteristic Analysis of the Galectin-16 as Diagnostic Markers for Hypothyroidism

Galectin-16	*AUC*	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
	0.000	**0.000**	**0.000**	**13.111**	**100**	**100**	**0.000-0.000**

AUC: Area Under Curve, SE: Standard Error

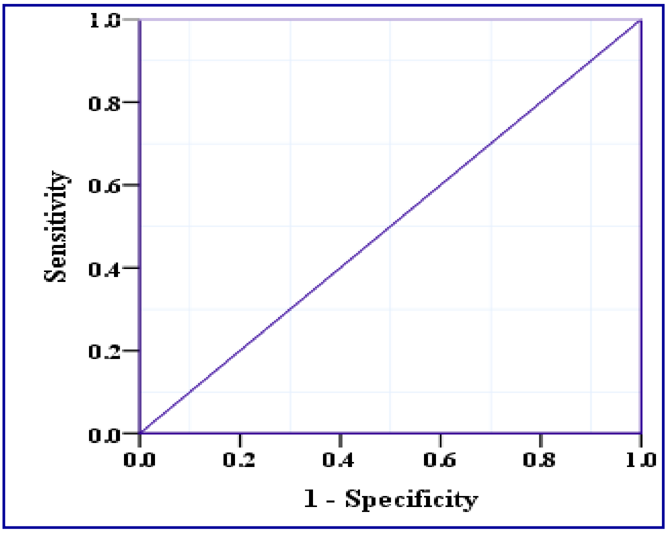

Fig. 2. Receiver Operating Characteristic Curve of Galectin-16 in the Hypothyroidism Cases

In Table 4, it is demonstrated that the sensitivity of galectin-16 was 13% when 28 cases of hyperthyroidism exhibited galectin-16 levels surpassing the average of these measurements observed in the healthy control group. Conversely, the specificity of galectin-16 was 93.33% when 28 hyperthyroidism cases displayed galectin-16 levels exceeding the predetermined threshold.

Table 4. Receiver Operating Characteristic Analysis of Galectin-16 as Diagnostic Markers for Hyperthyroidism

Parameters	*AUC*	*SE*	*p-value*	*Cutoff value*	*Sensitivity%*	*Specificity%*	*CI (95%)*
Galectin-16	**0.449**	**0.076**	**0.501**	**8.492**	**13**	**93.33**	**0.301-0.598**

AUC: Area Under Curve, SE: Standard Error

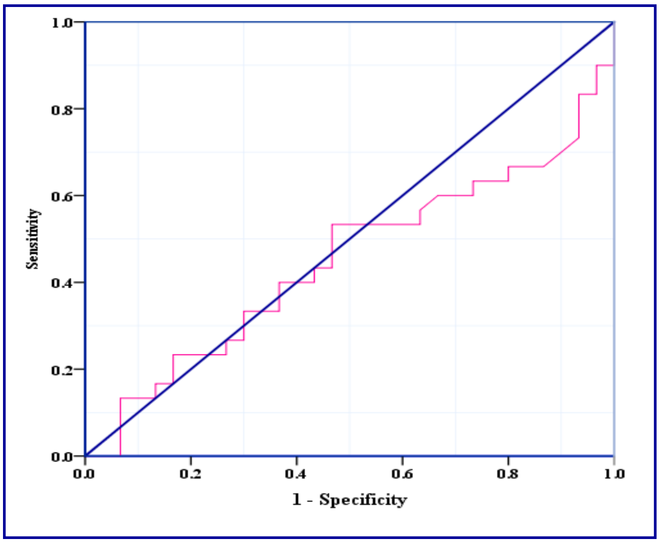

Fig. 3. Receiver Operating Characteristic Curve of Galectin-16in the Hyperthyroidism Diseases

4 Conclusion

The study did not find any statistical differences between men and women in patients or controls. It is worth mentioning that galectin-16 has 100% sensitivity and specificity for hypothyroidism. Galectin-16 has a sensitivity of 13% and a specificity of 93.33% for detecting hyperthyroidism. From these findings it can be concluded that galectin-16 is an important diagnostic indicator of hypothyroidism, exceeding its effectiveness in distinguishing between normal and pathological conditions.

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