The roles of galectin-3 and galectin-4 in the idiopatic Parkinson disease and its progression

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ABSTRACT

Objectives: : Idiopathic Parkinson's Disease is a neurodegenerative disease caused by the loss of cells that secrete dopamine in the basal ganglia. Galectins are multipotent, evolutionarily conserved, cell surface glycoconjugated and crosslinked carbohydrate-binding proteins. The roles of these proteins in the diagnosis of the disease have been investigated.

Patient and Methods: : Patients who were diagnosed with idiopathic Parkinson's disease were classified as early (stage 1–2) and advanced stage (stage 3–5) according to the Hoehn-Yahr classification. In addition, voluntary cases without parkinson disease constituted the control group. Serum samples of consecutive Parkinson patients and age and gender matched healthy controls were used to measure serum galectin-3 and serum galectin-4 levels. The levels were compared between Parkinson's patients and control groups and early and advanced stage Parkinson's groups.

Results: : Thirty age and gender-matched healthy controls and 60 parkinson patients were enrolled in the study. Serum galectin-3 levels were lower in controls compared with patients (892.9 (168.2–2416.3) *vs.* 2271.8 (375.9–9673.4), respectively, P < 0.01). Serum galectin-3 levels were related to Hoehn-Yahr stages and (r: 0.691, P < 0.001). The early stage group (20 patients) had lower serum galectin-4 levels compared with advanced stages (40 patients) (197.97 ± 46.42 *vs.* 334.263 ± 37, respectively, P < 0.01). Serum galectin-4 levels were also lower in controls compared with patients 185.1 (116.2–313.3) *vs.* 282.3 (156.9–984.8), respectively, P < 0.01. ROC analysis showed that serum galectin-3 and galectin-4 were statistically significant in the identification of Parkinson disease and advanced stages. The results were significant for galectin-3 (AUC: 0.89, SE: 0.034, P < 0.001 and CI: 0.823-0.958; P < 0.001) and for galectin-4 (AUC: 0.758, SE: 0.05, P < 0.001).

Conclusion: : Serum galectin-3 and galectin-4 may be potential noninvasive markers for the identification of Parkinson disease and advanced stages.

1. Introduction

Idiopathic Parkinson's Disease (IPD) is a neurodegenerative disease characterized by motor and non-motor symptoms caused by the degeneration and loss of cells that secrete dopamine in the nigra of the basal ganglia [1]. The diagnosis of the disease is based on whether there are some clinical features or not and the observations of the clinician's experience and individual skills. There is no imaging method that can definitively diagnose. One of the reasons in the difficulty of diagnosing the disease is that there is no clinical test or laboratory biomarkers [2]. In addition, it is not yet possible to detect this beforehand, even before

symptoms occur. It is not possible to explain the etiopathogenesis of all findings in a single mechanism in Parkinson's disease. While the main cause is the loss of dopaminergic cells in substantia nigra (SN) but there are genetic, cellular and metabolic reasons [3].

Galectins are multipotent, evolutionarily conserved, cell surface glycoconjugate-crosslinking carbohydrate-binding proteins. These proteins trigger the transmembrane signal cascade such as cell activation, cytokine release, migration and apoptosis [4,5]. Galectin-3 has been reported to be expressed in many cells. It is not only expressed in the cytosol and nucleus but also in extracellular area. They are detected in the central nervous system, peripheral nervous system, astrocytes,

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macrophages / microglial and endothelial cells. Galectin-3 is also involved in the physiology of almost all cells of the immune system [6]. Galectin-4 is excreted in hippocampal and cortical neurons and it enters the axonal membrane segments *via* microtubule and sulfatide pathways [7]. In addition, neuronal galectin-4 is a soluble regulatory candidate for oligodendrocyte differentiation and myelination [8]. However, there is no study of the role of galectin-4 in neurodegenerative diseases.

The aim of the present study is to investigate whether serum galectin-3 and serum galectin-4 levels are related to the diagnosis of Parkinson's disease and its stages.

2. Materials and methods

2.1. Patient selection

In this study, 60 consecutive patients over 30 years of age who were admitted to our neurology clinic with idiopathic Parkinson's disease according to the neurological examinations and UPDRS and 30 voluntary age and gender-matched control cases without parkinsonian diagnosis and bradykinesia were included. The demographic, epidemiological, and laboratory findings of the patients and control cases were recorded by the clinician on a computer system in a blinded manner.

Pregnancy and lactation, presence of secondary causes of parkinsonism, dementia, severe psychiatric symptoms (hallucinations, delusions, *etc.*), severe cardiac, hepatic or renal disease, peripheral vascular disease, uncontrolled systemic disease and the use of alcohol or addictive substances were defined as exclusion criteria.

All individuals signed consent forms in accordance with the decisions of our Medical Ethical Board. Whole blood was taken in ethylenediaminetetraacetic acid (EDTA)-containing tubes to prevent clotting and serum was obtained by centrifugation and stored at -20 °C until the day of study.

2.2. Research strategy

The staging of the disease in the IPH patient group was performed with the Hoehn-Yahr scale. The degree of clinical severity of the disease was assessed by the Unified Parkinson's Disease Rating Scale (UPDRS). Hoehn-Yahr scale examines the disease in 5 stages [9]. Stage 1–2 was considered early stage and stage 3–5 was classified as advanced stage. Serum galectin-3 and galectin-4 values, hemogram values, biochemical values of all patients and volunteer control group were studied under laboratory conditions. Hoehn-Yahr stages of the patient group were compared in terms of hemogram values and biochemical values. The association between Hoehn-Yahr stages and serum galectin levels were investigated.

2.3. Serum galectın-3 and galectın-4 levels

Hemogram, biochemistry, b12, folate, ferritin serum levels of patients and control group were studied between 08.30 and 10.00 in the morning. In addition, 5 cc of venous blood was taken from the patients and control group in the morning and centrifuged at $1500 \times g$ for 15 min, after which the serum was stored at -20 °C until the day of study.

Commercially available (active; human) ELISA kits containing the essential components required for the measurement of Galectin-3 and Galectin-4 from serum in a sandwich ELISA format was used. Human Galectin-3/ LGALS3 ELISA Kit Catalog no. EK0764, Lot no. 41511961023316 and Galectin-4 Catalog No. EK1275, Lot no. 8191250503 (Boster Immunoleader, Pleasanton, CA, USA) were used. The standard solution of Galectin-3 was prepared 2 h before the procedure. Two standard galectin-3 tubes were placed in each kit. The biotinylated anti-human galectin-3 and galectin-4 antibodies were also prepared 2 h before the procedure. Galectin-3 and galectin-4 concentrations were calculated as pg/ml. For galectin-3; range: 156 pg/

ml–10.000 pg/ml. sensitivity: < 10 pg/ml, specificity: Natural and recombinant human galectin-3. Intra-assay sensitivity: Mean 624-3138-6618, standard deviation: 27.46–163.2–271.3 CV was 4.4–5.2-4.1%. For galectin-4 Intra-assay precision: mean: 0.48–3.64–6.25, standard deviation: 0.02–0.14–0.35 and CV%: 4.2–3.8–5.6. Range: 156 pg/ml–10000 pg/ml, sensitivity: < 10 pg/ml, specificity: Natural and recombinant human Galectin-4.

2.4. Ethics

The study was carried out within the agreement of ethical recommendations of the 1975 Declaration of Helsinki that was modified in 2008 and also was approved by the Institutional Review Board.

2.5. Statistical analyses

Statistical analyzes were performed using SPSS (Chicago, IL, USA) version 22 and MedCalc, version 14 (MedCalc, Mariekerke, Belgium). The normal distribution of the variables was examined by using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyzes were done using median and min-max for non-normally distributed variables and frequency tables for ordinal variables. Because serum galectin-3 and serum galectin-4 levels were not normally distributed, these parameters and ordinal variables were compared between groups using the Mann-Whitney U test. Correlation coefficients and statistical significance were calculated using Spearman's test for at least one non-normal distributed variables or ordinal inter-variable relationships. Spearman correlation analysis was used because the Hoehn-Yahr stages were ordinal and serum galectin levels were not normally distributed. Diagnostic decision-making properties of serum galectin-3 and serum galectin-4 levels in Parkinson's disease were analyzed by Receiver Operating Characteristics (ROC) curve analysis. The level that had the best sensitivity and specificity in AUROCs (Youden Index) analysis was determined as the best cut off value. After observing the best cut off values for galectin-3 and galectin-4 in the identification of the Parkinson disease and early-late stages groups AUROCs, standard error (SE), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. In cases where the area under the curve is under 5% of the Type-1 error level, the diagnostic value of the test was interpreted as statistically significant. Statistically significant results were obtained when the P value was below 0.05.

3. Results

The parkinson and control groups were similar in age and sex. The median age of the control group was 72 and min-max (61–88), while that of the patient group was 72.5 (49–88), and the difference was statistically insignificant (P > 0.05). When we compared serum Galectin-3 levels between control group and Parkinson's patients they were 892.9 pg/ml (168.2–2416.3) *vs.* 2271.8 pg/ml (375.9–9673.4) respectively, P < 0.001. When serum galectin-4 levels were compared between the groups 185.1 pg/ml (116.2–313.3) *vs.* 282.3 pg/ml (156.9–984.8) and P < 0.001.

Patients with Parkinson's disease were diagnosed for 24 months (12 months-72 months) on average. The median age of the women in the patient group was 74 (51–88), while in males was 72 (49–84). Forty percent of the patients were female. Comparisons of the demographic and laboratory values of the control group and the patient group are shown in Table 1.
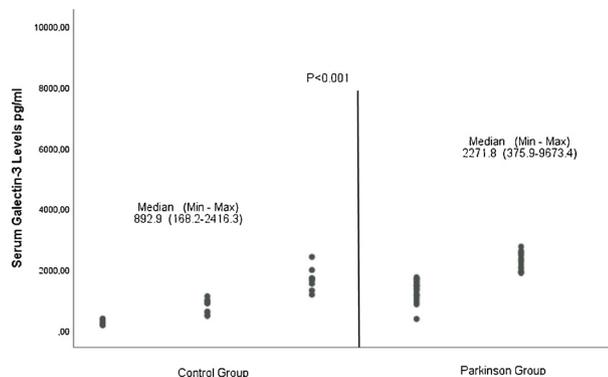
The comparison of serum levels of Galectin-3 between the control group and Parkinson group is shown as a scatter plot of individuals in Fig. 1 and P < 0.001. Scatter plot of individuals serum levels of Galectin-4 between control group and Parkinson group is represented in Fig. 2 and P < 0.001.

The patients were staged according to the Hoehn-Yahr

Table 1
Comparisons of the demographic and laboratory values of the control group and the patient group.

Factors	Control group (n:30)	Parkinson patients (n:60)	P Value
Galectin-3 pg/ml	892.9 (168.2–2416.3)	2271.8 (375.9–9673.4)	**< 0.001**
Galectin-4 pg/ml	185.1 (116.2–313.3)	282.3 (156.9–984.8)	**< 0.001**
Age, years, median (min–max)	72 (61–87)	72.5 (49–88)	0.94
Gender, F (%)	12 (40)	24 (40)	1
Time to diagnosis (Month)	–	24 (12–72)	–
Hemoglobin, g/dL	12.63 ± 0.89	13.7 ± 1.78	0.12
Platelets, 10^3/mm3	241 ± 26.65	272.79 ± 135.5	0.57
WBC, 10^3/mm3	5.76 ± 0.7	8.13 ± 4.2	0.88
Neutrophils (%)	54.76 ± 3.45	68.88 ± 11.49	0.32
Lymphocytes (%)	34.63 ± 3.20	21.96 ± 8.9	0.46

SD: Standart Deviation, F: Female, WBC: White blood cells.

Fig. 1. The comparison of serum levels of Galectin-3 between the control group and Parkinson group and the results were statistically significant.
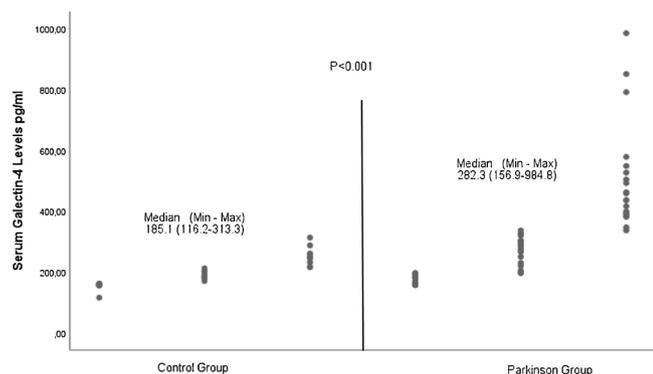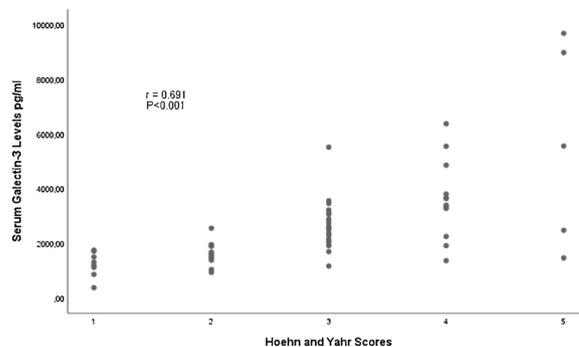

Fig. 2. The comparison of serum levels of Galectin-4 between the control group and Parkinson group and the results were statistically significant.

Fig. 3. Correlation analysis between serum galectin-3 levels and Hoehn and Yahr scores of Parkinson group.

Fig. 4. Correlation between serum galectin-4 levels and Hoehn and Yahr scores of Parkinson groups.

classification. Twelve (13%) of the patients had stage 1, 12 (69%) stage 2, 23 (38.3%) stage 3, 12 (20%) stage 4 and 5 (8.3%).
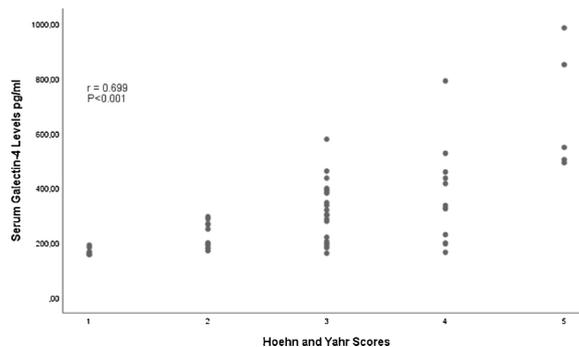
We performed Spearman test and calculated the correlation analysis between serum galectin-3 levels and Hoehn and Yahr scores of Parkinson groups and r: 0.691 and P < 0.001 and this association is shown in Fig. 3.

A statistically significant positive correlation was found between Hoehn-Yahr classification of Parkinson's patient group and serum ga-lectin-4 levels. Positive correlation was found in the correlation analysis (r: 0.699 and P < 0.001). Statistically significant correlation (r : 0.699, P < 0.001) between serum galectin-4 levels and Hoehn and Yahr scores of Parkinson groups is shown in Fig. 4.

ROC analysis was performed with Parkinson's patient group and control group's serum galectin-3 levels. The best sensitivity at 1720.06 pg/ml level had 68.33% sensitivity and 93.33% specificity. The area under the curve (AUC) was 0.89, SE: 0.034, P < 0.001 and confidence interval CI: 0.823-0.958. With these results, serum galectin-

3 level was determined as a good predictor between Parkinson's disease and control group. The ROC analysis results of the patient and control groups for galectin-3 are shown in Fig. 5.

ROC analysis showed good predicting results among the patient group and control group's serum galectin-4 levels. At the cutt-off of 260.61 pg /mL level there were best sensitivity (55%) and specificity (93.34%). AUC was 0.758, SE: 0.05, CI: 0.666-0.886, P < 0.001. Serum galectin-4 level was a good predictor between Parkinson's disease and control group. The ROC analysis results of the patient and control groups for galectin-4 are shown in Fig. 6.

Patients with Parkinson's disease were graded according to Hoehn-Yahr classification as early stage (stage 1–2) and advanced stage (stage 3–5). When the predictability of early and advanced stage patients was evaluated in terms of serum galectin-3 levels, the best predictability was at the 1909.81 pg/ml level with sensitivity: 87.50%, specificity: 90% and AUC: 0.923, SE: 0.035, 95%CI: 0.855–0.991, P < 0.001. Galectin-3 was a good predictor for predicting advanced stage disease. ROC analysis of serum galectin-3 levels in early and late stage Parkinson is shown in Fig. 7.
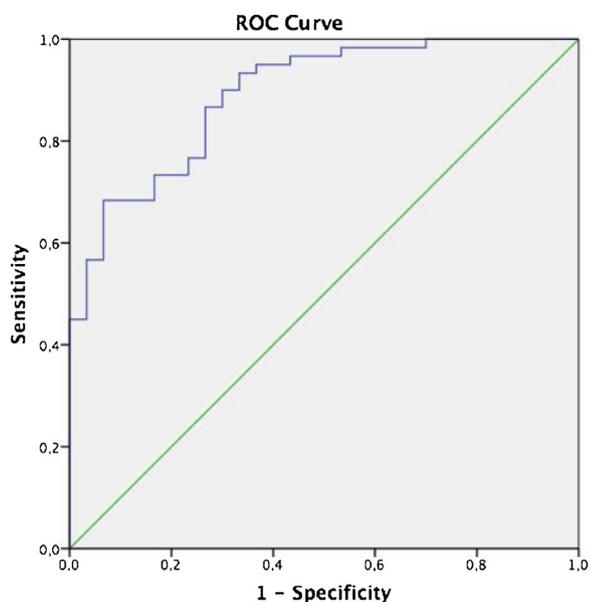
Fig. 5. The ROC analysis results of Galectin-3 for the patient and control group.
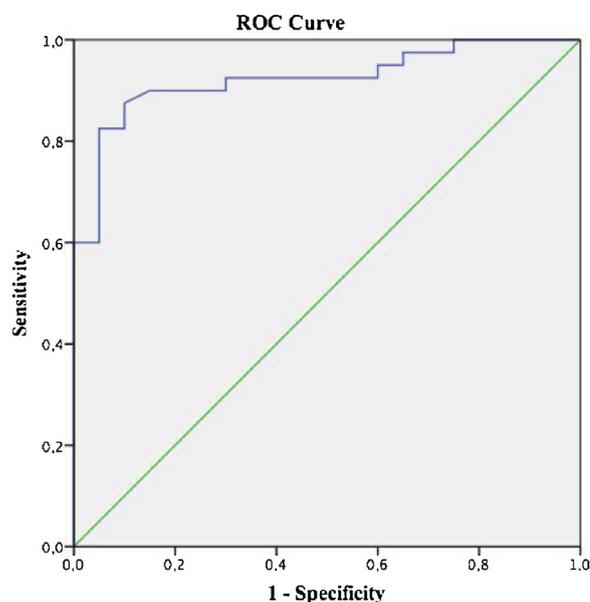

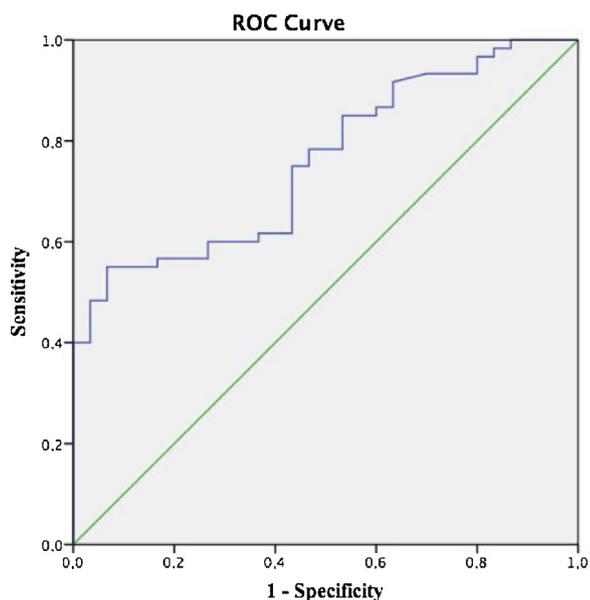**Fig. 7.** ROC analysis of serum Galectin-3 levels in early and late stage Parkinson.

Fig. 6. The ROC analysis results of Galectin-4 for the patient and control groups.
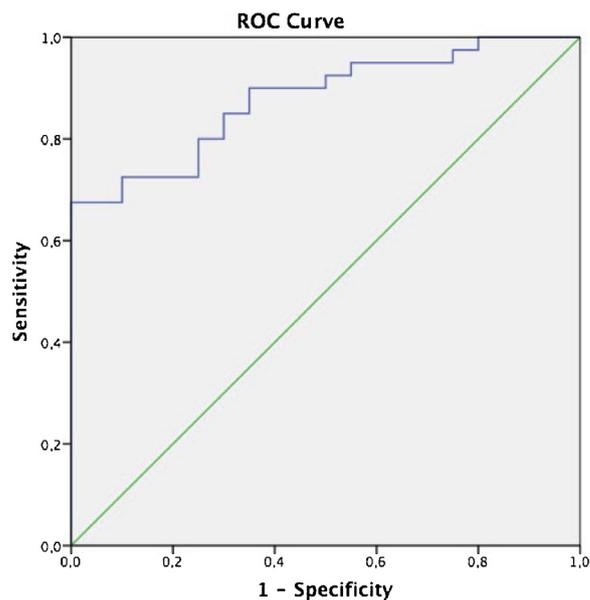

Fig. 8. ROC analysis of serum Galectin-4 levels in early and late stage Parkinson.

When the predictability of early and advanced stage patients was evaluated in terms of serum galectin-4 levels, the best predictability was found to be at the 295.3 pg/ml level with sensitivity 67.5%, specificity 99.9% and a AUC: 0.879, SE: 0.043, CI:0.795-0.962, P < 0.001. Galectin-4 level was a good predictor marker for predicting advanced stage disease. ROC analysis of serum galectin-4 levels in early and late stage Parkinson is shown in Fig. 8.

4. Discussion

Consecutive patients with the diagnosis of IPD and age and sex matched control group were compared in terms of demographic, clinical, and laboratory values. Patients in the Parkinson group were evaluated as early and advanced stages according to the Hoehn-Yahr stage. In this study, the level of serum galectin-3 and galectin-4 in diagnosis of Parkinson's disease was compared between patients and control groups. We also investigated whether serum galectin-3 and

galectin-4 levels predicted early stage in the diagnosis of idiopathic Parkinson's disease.

The mean age of the patients in the Parkinson group was 72.5 (49–88) and the value was found to be consistent with the literature. Parkinson's disease usually begins between the ages of 40 and 75, and as you get older, there is an increased risk of coming to the rink, such that after age 65, the frequency is about 1–2%. In one study, over 60% of the disease was seen in 2% of cases, but in 80% of cases it increased to 4%. It may also occur in young adults, although it usually occurs at older ages. Approximately 10% of all patients are seen under 40 years of age [10]. The possibility of being ill for life is reported to be 1.3% in women and 2% in men [11]. However, our study did not have patients under 40 years old. The reason for this is probably due to the short duration of our work.

Forty percentage of our patients in our study were women, which seems to be consistent with the literature. The relatively small number

of diseases in women may be due to the positive effects of hormones, and the existence of such a relationship with large cohort studies on this subject should be investigated. Van Den Eeden and his colleagues found that 90% more parkinson was found in men than in women after 60 years of age. In addition, studies have shown that gender is effective on the nigrostriatal dopaminergic pathway that causes degenerative process leading to parkinsonism. 17B estradiol has been reported to be the most estrogenic and neuroprotective effect in nonpregnant women. More prevalence of Parkinson disease has been shown in women who underwent premenopausal oophorectomy. In addition, parkinson was less common in patients receiving estrogen-dependent hormone replacement therapy and worsening of Parkinson symptoms when the hormone was discontinued [12].

Since the onset of Parkinson's disease is sneezing, it has long been reported that neurodegenerative change begins before the disease is symptomatic. In our study, the mean detection of the disease was approximately 24 months (12–72) [13].

In our study, serum galectin-3 levels were statistically significantly higher in the parkinson group than in the control group. A strong positive correlation was found between the patient's serum galectin-3 levels and the Hoehn-Yahr staging of the patients. In further analyzes, patients were classified as early and advanced patient groups according to the Hoehn-Yahr classification and then analyzed. Serum galectin-3 levels were significantly higher in the advanced stage group than in the early stage. As a result, serum galectin-3 levels were detected as an important serum marker both in the separation of patients from non-patients and in the detection of advanced disease. This result suggests that increased proapoptotic activation of galectin-3 may play a role in inflammation and neurodegeneration. Galectins are multipotent, carbohydrate-binding proteins that are cross-linked with cell surface glycoconjugate, over time protected in structure. These proteins trigger the transmembrane signal cascade such as cell activation, cytokine release, migration and apoptosis [4]. Despite their strong role in the immune system, it is not known whether monocytes and macrophages interact with these lectins and how they interact. Although all members of the galectin family are bound to the residues of galactose B, each galectin has its own specific complex-containing oligosaccharides, and as a result, the members of the different galectin family can bind the galectins to different glycoconjugate receptors [14].

Galectins are not only found in the gastrointestinal immunity system, but also potentially control epithelial and T cell functions [15]. Increased soluble or cellular levels of galectin-3 have been found to be associated with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and Behcet's disease. Furthermore, galectin-3 was found to be related to disease activity [16].

In a recent study by Wang X et al., Increased levels of galectin-3 have been shown to potentially play a role in inflammation and impaired neurodegeneration in the proapoptotic activation of Alzheimer's disease. In addition, serum galectin-3 levels were correlated with cognitive status of all patients and controls. It has been shown to be a potential biomarker in Alzheimer's because of its ability to be expressed in secreted nature and serum [17]. This result suggests that increased proapoptotic activation of galectin-3 may play a role in inflammation and neurodegeneration. In one study, lower perivascular inflammatory infiltration was observed in double knock-out mice in the proinflammatory role-play of galectin-3 [18]. Galectin-3 affects the differentiation and growth of different immune cells. Induces apoptosis in T cells and neutrophils, namely, different mast cells activate lymphoid and myeloid cells, including neutrophils, monocytes and Th cells, causing mediator release, superoxide causes anion production [19]. Galectin-3 is a multifunctional protein that functions in many biological events such as fibrosis, angiogenesis, apoptosis and immunological activation [20].

One of the main findings of this study was that serum galectin-3 levels were higher in patients with Parkinson's disease than controls. It was also found that there was a significant positive correlation between

Hoehn-Yahr score and serum galectin-3 levels. This result demonstrates that serum galectin-3 can be used as a good marker for idiopathic Parkinson's disease. According to our knowledge, these findings are the first in the literature to show elevated serum galectin-3 levels in Parkinson's disease. The functions of galectin-3 in the central nervous system have been reported in the study. It has been reported that the induction of galectin-3 occurs in some diseases such as ischemic brain diseases, ALS and prion disease [21]. In our study, we think that high galectin-3 levels are associated with immunological activation, regulation and inflammation in the Parkinson disease. However, there is limited information about the regulatory mechanism of galectin-3 [22]. Galectin-3 has been reported to be expressed in many cells. The central nervous system has also been detected in astrocytes, macrophages / microglial, endothelial cells and Schwann cells in the peripheral nervous system. Galectin-3 is also involved in the physiology of almost all cells of the immune system [6].

Galectin-3 has been reported to play a role in the inflammatory pathway. The increased inflammatory response of galectin-3 is associated with a reduction in the production of antiinflammatory interleukin (IL-10). IL-10 released from microglia has a neuroprotective effect. In some studies galectin-3 has been implicated in increasing central nervous system damage by inhibiting peripheral nerve regeneration after axotomy. It also functions as galectin-3 receptor for advanced glycation end products (AGEs) in physiopathology of aging [23]. This may take place in Parkinson's disease.

In our study, serum galectin-4 levels were higher in patients with Parkinson's disease than in healthy controls and when patients were classified as early and advanced Parkinson's disease according to Hoehn-Yahr classification, serum galectin-4 levels were higher in advanced disease. According to these results, serum galectin-4 levels have been shown to be a good serum marker for diagnosing both Parkinson's disease and advanced-stage Parkinson's disease.

Galectin-4 is excreted in hippocampal and cortical neurons and enters the segments of the axonal membrane *via* microtubular and sulphated pathways. In addition, neuronal galectin-4 is a soluble regulatory candidate for oligodendrocyte differentiation and myelination. However, the role of galectin-4 in neurodegenerative diseases has been less investigated. Further work is needed. Axons which are open channels of neurons, are involved in rapid neuronal transmission. Galectin-4 is necessary for axon growth. The growth of axons increases the size and cluster numbers of neural cell adhesion molecules. Synaptic glycoproteins play a regulatory role in axonal transmission. Neural cell adhesion molecule (NCAM) L1 allows branching in neural growth, neural conduction. Galectin-4 has a vital prescription for L1 function and proper organization [17,24–26].

4.1. Limitations

There were some limitations in our study. First, our research study was single- center. Second, only spot galectin-3 and galectin-4 levels were used. Using temporal trend and multiple levels would be better clarify the association. Comparison of other ther inflammatory cytokins would give more valuable results in clarifying the causality and association in the diagnosis and progresssion of IPD.

5. Conclusions

Serum galectin-3 and serum galectin-4 levels were significantly higher in patients with Idiopathic Parkinson's disease than controls without this disease. In addition, in Parkinson's patients they showed strong correlation with Hoehn-Yahr scores and were identified as powerful predictors of differentiation of early and advanced stages of the disease.

By suppression of these molecules, it may be possible to prevent both the onset and progression of the disease. It may be a guide to progressive work for its use as a treatment option.

Conflicting interest

The authors report no conflicts of interest.

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