Eskisehir Medical Journal
Eskişehir Tıp Dergisi

Eskisehir Med J. 2024; 5(1): 1-8.
doi: 10.48176/esmj.2024.151.

Original Article / Araştırma Makalesi

GALECTIN-3, AN INDICATOR OF INFLAMMATION AND OXIDATION, IS LINKED WITH THE SEVERITY OF ALZHEIMER'S DISEASE

ALZHEİMER HASTALIĞI'NDA İNFLAMASYON VE OKSİDATİF STRES GÖSTERGESİ OLARAK GALEKTİN-3'ÜN ROLÜ

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ABSTRACT

Introduction: Galectin-3 (Gal-3) is a multifunctional protein implicated in various biological processes, but the best-known role for galectin-3 is in acute and chronic inflammation. Inflammation plays an essential role in developing cognitive decline and dementia in old age. This study aims to investigate if galectin-3 can be an indicator of inflammation in Alzheimer's Disease (AD) pathogenesis and a feasible biomarker of the disease.

Methods: The study included 44 patients with Alzheimer's Disease and 44 patients with normal cognitive function. Patients with known acute or chronic infections, chronic inflammatory diseases, cancer patients, and patients with rheumatological diseases affecting galectin-3 levels were excluded. All patients underwent comprehensive geriatric assessment and cognitive assessment. Serum galectin-3 levels were measured.

Results: Analysis revealed that the galectin-3 level of the AD group was higher than the control group. However, it was not statistically significant. According to the Global Deterioration Scale (GDS), the galectin-3 levels of patients in the moderately severe AD group (GDS stage 6) were significantly higher than the patients in the mild-moderate AD group (GDS stage 4-5). There was a significant weak negative correlation between galectin-3 levels and the Digit Span Forward (r = - 0.216, P = 0.043) and Backward (r = - 0.233, P = 0.029) tests.

Conclusion: This study suggests that galectin-3 may play a role as an indicator of inflammation and oxidative stress in the pathogenesis of Alzheimer's Disease. It appears to be associated with the severity of AD. However, further and more extensive prospective studies are needed to clarify the association.

Keywords: Alzheimer Disease, Biomarker, Galectin-3, Inflammation, Oxidative stress

ÖZET

Giriş: Galektin-3 (Gal-3), birçok biyolojik süreçte rol alan multifonksiyonel bir protein olup en önemli rolü akut ve kronik inflamasyondadır. İnflamasyon, ileri yaşta kognitif gerilemenin ve demansın gelişmesinde önemli bir role sahiptir. Bu çalışmanın amacı galektin-3'ün Alzheimer Hastalığı (AH) patogenezindeki inflamasyonun bir göstergesi olup olmadığının ve bir biyobelirteç olarak kullanılabilirliğinin araştırılmasıdır.

Yöntemler: Çalışmaya, 44 Alzheimer hastası ve 44 normal kognitif fonksiyonlu olmak üzere toplam 88 hasta alınmıştır. Bilinen akut veya kronik infeksiyon tanısı olan, kronik inflamatuar hastalığı bulunan hastalar, kanser hastaları ve galektin-3 düzeyini etkileyen romatolojik hastalığı bulunan hastalar dışlanmıştır. Tüm hastalara kapsamlı geriatrik değerlendirme testleri ve nöropsikiyatrik testler uygulanmıştır. Serum galektin-3 düzeyleri ölçülmüştür.

Bulgular: Analizler sonucunda Alzheimer Hastalığı grubunun galektin-3 düzeyi, kontrol grubundan yüksek saptanmıştır. Ancak istatistiksel olarak anlamlı değildir. Global Detoriasyon Skalası (GDS) evrelemesine göre orta-şiddetli AH (GDS evre 6) grubundaki hastaların galektin-3 düzeyleri hafif-orta evredeki (GDS evre 4-5) hastalara göre istatistiksel olarak anlamlı şekilde yüksek saptanmıştır. Galektin-3 düzeyi ile sayı menzili ileri (r = -0,216 P = 0,043) ve geri (r = -0,233 P = 0,029) testi arasında ters yönde, düşük kuvvette, istatistiksel olarak anlamlı bir korelasyon saptanmıştır.

Sonuç: Bu çalışma, galektin-3'ün Alzheimer Hastalığı'nın patogenezinde inflamasyon ve oksidatif stres göstergesi olarak önemli bir rol oynadığını göstermektedir. Bu ilişki Alzheimer Hastalığı'nın şiddeti ile ilişkili görünmektedir. Ancak, bu ilişkiyi netleştirmek için daha fazla ve daha kapsamlı prospektif çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Alzheimer Hastalığı, Biyobelirteç, Galektin-3, İnflamasyon, Oksidatif stres

INTRODUCTION

Alzheimer's Disease (AD), a progressive neurodegenerative disorder, accounts for 50-80% of dementia cases (1). The pathology of Alzheimer's Disease is characterized by brain atrophy, formation of neuritic plaques containing beta-amyloid peptide, and neurofibrillary tangles containing hyperphosphorylated tau protein (2, 3). Inflammation and oxidative stress play an essential role in pathogenesis. Accumulation of reactive oxygen species damages the major cell components, primarily mitochondria, in specific brain regions (4, 5). Inflammation has an essential role in developing cognitive decline and dementia in advanced age (6). A state of chronic brain inflammation exists in AD, characterized by activation of microglia and astrocytes, recruitment of peripheral immune cells, and excessive proinflammatory mediators. Cytokines

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Received date: 13.12.2023 **Accepted date:** 22.02.2024

Cite as: Güner G, Doğu BB, Kılıç MK, Kızılarslanoğlu MC, Varan HD, Sağır A, Halil M, Akbıyık F, Cankurtaran M. Galectin-3, An Indicator Of Inflammation And Oxidation, Is Linked With The Severity Of Alzheimer's Disease. Eskisehir Med J. 2024; 5(1): 1-8 doi: 10.48176/esmj.2024.151.

influence several different mechanisms that may induce or accelerate the development of neurodegeneration and the AD phenotype (6, 7). Persistent inflammation could play a role in the advancement of the disease and the decline of neurons (7).

Currently, the diagnosis of Alzheimer's Disease is made by clinical and cognitive assessment, neuropsychiatric tests, and excluding other causes of dementia. However, a definite diagnosis is possible by tracing characteristic pathological brain lesions, amyloid plaques, and neurofibrillary tangles in autopsy. Although the progression of the disease can be slowed down by early treatment, diagnosing the disease at an early stage is difficult. This clinical need has led to the search for biomarkers that can be used to diagnose Alzheimer's Disease in its early stages and to distinguish Alzheimer's Disease from other causes of dementia (8).

Galectins are a member of the animal lectin family with a beta-galactosidase affinity. Galectins interact with the cell surface and extracellular matrix glycoproteins through lectin-carbohydrate interaction. This interaction facilitates cell growth, increases cell survival, regulates cell adhesion, and induces cell migration (9-12). Galectin-3 (Gal-3) is a member of the Galectin family (13). Gal-3 is found in many cells and tissues. It is involved in many tasks, such as macrophage migration, fibroblast proliferation, and collagen synthesis (9, 12). However, the most crucial role of galectin-3 is in acute and chronic inflammation (14-17).

Galectin-3 has regulatory functions, especially in the hippocampus region of the brain. Due to its role in regulation and inflammation, we assume that gal-3 may have a role in cognitive functions and Alzheimer's Disease (10). This study evaluates the relationship between serum galectin-3 levels and Alzheimer's Disease.

MATERIALS AND METHODS

Study patients

This study was planned and conducted in a geriatric medicine outpatient clinic of a university hospital between December 2015 and May 2016. The study included 44 patients with Alzheimer's Disease and 44 patients with normal cognitive function. All patients underwent comprehensive geriatric assessment and cognitive assessment. The exclusion criteria were acute or chronic infections, chronic inflammatory diseases, cancer, and rheumatological diseases. Patients with coronary artery disease, diabetes mellitus, and hypertension were included in the study. But the patients who have these diseases were in a similar distribution both in Alzheimer's Disease and control group. There was no statistically significant difference in their distributions.

The required approval for conducting the study was obtained from the Ethics Committee of the Faculty of Medicine, Hacettepe University (Date 26.12.2014/ Number GO 14/649). The study protocol was in adherence with the principles in the Declaration of Helsinki. Informed consent was obtained from all participants.

Comprehensive geriatric assessment and cognitive assessment

The demographic characteristics, comorbidities, and medications of the patients included in the study were recorded in the form prepared. To assess patients' activities of daily living objectively, KATZ Activities of Daily Living (ADL) (18), Lawton-Brody Instrumental Activities of Daily Living (IADL) (19), and Disability Assessment for Dementia (DAD) (20) scales were performed. Among instruments used to assess basic activities of daily living (BADLs), Katz ADL is the most widely used one in clinical studies. The Katz ADL measures self-care tasks including; bathing, dressing, toileting, transferring to and from a chair, maintaining continence, and feeding.

The assessment is based on the patient's ability to perform tasks either independently or with assistance. The resulting score reflects the level of independence, with lower scores indicating a higher degree of dependence in basic ADLs (18). Lawton-Brody IADL scale assesses the more complex ADLs necessary for living in the community. The eight domains of function measured with the Lawton IADL scale are the ability to use the telephone, shopping, food preparation, housekeeping, laundry, mode of transportation, responsibility for medications, and ability to handle finances. The acquired score reflects the level of independence, with increased scores indicating higher levels of individual capabilities (19). The DAD scale is a widely recognized assessment tool for evaluating the functional abilities of individuals with AD. This scale assesses the performance of BADL and IADL over the preceding two weeks. Comprising 10 domains and 40 items, the scale relies on caregiver interviews for information gathering. Six instrumental activities (meal preparation, telephoning, going on an outing, finance and correspondence, taking medications, leisure activities, and housework) and four basic self-care daily activities (hygiene, dressing, continence, and eating) were evaluated. The total score is between 0 and 100. Higher scores correspond to reduced functional disabilities, whereas lower scores are indicative of increased dysfunction (20).

Cognitive function assessment scales and objective diagnostic criteria were used for cognitive assessment. Mini-Mental State Examination (MMSE) (21), Clock Drawing Test (22), Montreal Cognitive Assessment Scale (MOCA) (23), Trail Making Test A and Trail Making Test B (24), Forward and Backward Digit Span Test (25), and Category Fluency Test (25) were performed. MMSE and clock drawing test were used as screening tests. MMSE is the most commonly used test to screen for dementia. It consists of 11 questions and is evaluated over 30 points. A score of <24 is the generally an accepted cutoff indicating the presence of cognitive impairment. It tests orientation, memory, attention,

calculation, recall, language, motor function, perception, and visuospatial abilities (21). The Clock drawing test is considered one of the first tests to fail in the early stages of dementia. The patient is asked to draw a clock, insert the numbers into it, and mark the said time. It is evaluated out of six points and <4 points is consistent with impaired cognitive function (22). MOCA is a rapid screening instrument developed specifically to evaluate the early stages of cognitive impairment. It assesses different cognitive domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation. The maximum achievable score is 30 points, and a score of 21 or higher is deemed within the normal range (23). The Trail Making Test is a neuropsychological assessment comprising parts A and B designed to accurately gauge executive functions such as intricate visual-motor conceptual screening, planning, organization, abstract thinking, and response inhibition. Each segment of the Trail Making Test involves 25 circles arranged on a sheet of paper. In Part A, the circles are sequentially numbered from 1 to 25, and the patient is required to connect the numbers with lines in ascending order. For Part B, the circles encompass both numbers (1 – 13) and letters (A – L). Similar to Part A, the patient connects the circles in ascending order, but in this case, there's an additional challenge of alternating between numbers and letters (e.g., 1-A-2-B-3-C, and so on) (24). Digit span test is particularly used to determine attention range, the ability to keep a certain amount of information in mind at a given time. It can be used in two formats, Forward Digit Span and Reverse Digit Span. Participants are presented with a random series of digits, and are asked to repeat them in either the order offered (forward span) or in reverse order (backward span) (25). The Category Fluency Test, a brief and easily administered assessment, has demonstrated its utility in AD diagnosis. The prevalent version often focuses on the semantic category of animals. The participant is asked to name as many animal names as possible in one minute. The number of animal names that a participant says in one minute is recorded as the categorical fluency score (25).

The diagnosis of AD is based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (26) and the National Institute of Neurological Disorders and Stroke–Alzheimer Disease and Related Disorders (NINCDS–ADRDA) working group (27). All patients were evaluated with the Global Deterioration Scale (GDS) (28). GDS comprises seven stages, spanning from normal functioning to extremely advanced cognitive decline. These stages are stage 1 – No cognitive decline, stage 2 - Age-associated memory impairment, stage 3 – Mild cognitive decline, stage 4 – Mild Alzheimer's Disease, stage 5 – Moderate Alzheimer's Disease, stage 6 – Moderately severe Alzheimer's Disease, stage 7 – Severe Alzheimer's Disease. Patients diagnosed with Alzheimer's Disease have a GDS score of 4 or higher (28). When we divided the patients according to GDS staging, the number of patients per group was small. So we have to reunite the patients as normal (GDS 1-2), mild-moderate AD (GDS 4-5), and moderately severe AD (GDS 6) to perform the statistical analysis. For patients to be admitted to the normal cognitive functioning group, the conditions of not meeting the criteria of DSM-IV and NINCDS-ADRDA for dementia and not meeting the criteria of Petersen for mild cognitive impairment (MCI) (29) were sought after undergoing comprehensive geriatric assessment. The GDS score of the control group was 1 or 2. Mini Nutritional Assessment Short Form (MNA-SF) was used for malnutrition screening (30), and the Yesavage Geriatric Depression Scale short form was performed to screen for presence of depressive mood (31). The MNA-SF is extensively employed for evaluating the nutritional aspect in the comprehensive geriatric assessment. The instrument consists of six items that assess decline in food intake, weight loss, mobility, psychological stress, neuropsychological problems, and the body mass index (30). The Yesavage Geriatric Depression Scale short form is one of the most commonly used self-rated depression scales in the elderly. It is a very quick, easy-to-administer screening test. A score above five points may be compatible with depression, and should be evaluated with the patient's clinic (31). KATZ ADL test, Lawton-Brody IADL test, MNA-short form test, Clock drawing test, MMSE test, Three-word memory test, Attention and calculation test, Yesavage Geriatric Depression Scale-short form test, GDS, and DAD tests were administered by the same practitioner to all patients. On the other hand, the MOCA test, Trail test A and B, Digit span test, and Categoric fluency test were conducted by another practitioner for all patients.

Forty-four patients diagnosed with AD based on DSM-IV, NINCDS-ADRDA criteria, and neuroimaging methods, and who did not meet any of the exclusion criteria, were included in the patient group after cognitive evaluation. Forty-four individuals with normal cognitive function and no exclusion criteria were included in the control group. MRI was performed on all Alzheimer's Disease patients in our study. MRI has been used in the clinical diagnosis of AD and in distinguishing AD patients from other reasons of dementia, such as vascular dementia, and reversible cognitive dysfunction causes (subdural hematoma, normal pressure hydrocephalus, intracranial tumor, etc.). Dementia patients without any AD-specific findings in neuroimaging methods were excluded.

Laboratory measurements

Complete blood count, erythrocyte sedimentation rate, C reactive protein (CRP), renal function tests, fasting blood glucose, HbA1c, TSH, vitamin B12 level, vitamin D level, lipid profile, and galectin-3 level were requested as laboratory tests.

For galectin-3 measurement, blood samples were drawn

from the patients and centrifuged at 4000 × g for 10 minutes. All serum samples were kept at - 80°C until assayed. Serum galectin-3 levels were analyzed by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (R & D Systems, Minneapolis, USA). Measurements were carried out using an ELISA plate reader (BioTek Instruments Inc, Winooski, VT, USA), and results are presented as ng/mL.

Statistics

Statistical analysis was performed using the SPSS software version 22. First of all, the variables were analyzed by visual (histogram, probability plots) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilks test) to determine whether or not they are normally distributed. Descriptive statistics are presented using mean and standard deviation (mean ± SD) for normally distributed variables, median (minimum-maximum, interquartile range) for skew distributed variables. Categorical variables are reported as numbers and frequencies. Comparisons between groups were performed by t-test, ANOVA, Mann Whitney U, or Kruskal Wallis tests according to normal distribution and number of groups for numerical variables, and chi-square test for categorical variables. The relationship between numerical variables was calculated by Pearson or Spearman correlation analysis. A value of $P < 0.05$ was considered to indicate a statistical significance.

RESULTS

The median (min-max) age of the participants was 78 (66 - 85), and 45 (51.1%) were female. Forty-four (50%) of the study participants were in the AD group, and 44 (50%) were in the control group with normal cognitive function. General characteristics are shown in Table 1. Accompanying diseases and laboratory results were similar between groups (Table 1). In the AD group, 20 (45.5%) patients were diagnosed with mild AD, 17 (38.6%) patients with moderate AD, and 7 (15.9%) patients with moderately severe AD, according to GDS. The cognitive and comprehensive geriatric assessment test scores are given in Table 2.

The median (min-max) galectin-3 level in the AD group was 7.52 (2.22-16.19) ng/ml, whereas the median (min-max) galectin-3 level in the control group was 7.02 (1.87-20) ng/ml. Although the median galectin-3 level was numerically higher in the AD group compared to the control group, the difference did not reach statistical significance ($P = 0.443$). The relationship between galectin-3 level and the stage of AD was evaluated (Figure 1). The median (min-max) galectin-3 level in moderately severe AD [GDS stage 6, 10.42 (6.29 - 13.59) ng/mL] was significantly higher compared to the mild-moderate stage [GDS stage 4-5, 7.09 (2.22 - 16.19) ng/mL] ($P = 0.007$), and the normal stage [GDS stage 1-2, 7.02 (1.87 – 20) ng/mL] ($P = 0.017$).

Correlations between cognitive assessment test scores and galectin-3 levels were performed, and Digit Span Test, which measures attention, was significantly correlated with galectin-3 levels. There was a statistically significant negative and weak correlation observed between galectin-3 levels and the scores on the Digit Span Forward ($r = -0.216$, $P = 0.043$) and Digit Span Backward ($r = -0.233$, $P = 0.029$) tests.

DISCUSSION

This study showed that galectin-3 levels were similar between AD and control groups. Although the galectin-3 levels in patients with Alzheimer's Disease were slightly higher than in control group, this difference was not statistically significant. In the AD group, the galectin-3 level was significantly higher in GDS stage 6 (moderately severe AD) patients than in other stages. Furthermore, a weak but significant correlation was found between the galectin-3 levels and the Digit Span Test scores, suggesting that the increase in galectin-3 may be associated with attention.

In the literature, Wang et al. demonstrated the association of AD with galectin-3. The relationship between galectin-3 levels and three different groups - Alzheimer's Disease, mild cognitive impairment, and normal cognitive status - was examined. The galectin-3 level in the AD group was significantly higher than in the normal group (13). They found no significant difference between AD-MCI or MCI-normal groups. The authors do not mention the stage of the disease in the AD group. In another study, Yazar et al. found that serum galectin-3 levels were higher in patients with AD compared with control group, in particular with increasing disease stage (32).

The results of our study revealed that severe stage had increased levels of galectin-3.

In our study group, patients in the severe stage were only 15.9%, and most of the patients were in mild and moderate stages. This may be the reason for the nonsignificant difference between AD and the control group. It may be possible that in an AD group with more severe stage patients, galectin-3 levels may be significantly higher than in normal group. Further studies with more significant numbers, including different stages, are required to clarify this aspect. To the best of our knowledge, in the previous studies, acute or chronic infections, chronic inflammatory diseases, cancer, and rheumatological diseases were not excluded, but these diseases can influence levels of galectin-3. One of the strengths of our study is excluding the possible factors that may be associated with galectin-3 levels. Acute or chronic infections, chronic inflammatory diseases, cancer, and rheumatological diseases were excluded. Therefore, we tried to examine the association between Alzheimer's Disease and galectin-3 independent of possible other factors. Another advantage of our study was the cognitive assessment tests performed. A comprehensive assessment was performed, including MMSE, GDS, MOCA, Trail Making Test, Digit Span Test, and Category Fluency Test.

Table 1. Demographic properties and general characteristics according to groups

	AD (n = 44)	Control (n = 44)	P value
Age (years); median (min-max)	78 (68-85)	78 (66-85)	0.33**
Gender Female; n (%)	26 (59.1)	19 (43.2)	0.135#
Educational level Illiterate; n (%) Primary and secondary school; n (%) High school and university; n (%)	 15 (34.1) 25 (56.8) 4 (9.1)	 9 (20.5) 17 (38.6) 18 (40.9)	0.003#
Smoker*; n (%)	2 (4.5)	1 (2.3)	
Family history of AD; n (%)	15 (34.1)	1 (2.3)	<0.001#
Hypertension; n (%)	25 (56.8)	32 (72.7)	0.12#
Coronary artery disease; n (%)	7 (15.9)	13 (29.5)	0.13#
Diabetes mellitus; n (%)	17 (38.6)	16 (36.4)	0.83#
Hypothyroidism; n (%)	6 (13.6)	6 (13.6)	1#
Hyperthyroidism; n (%)	1 (2.3)	2 (4.5)	1#
Dyslipidemia; n (%)	26 (59.1)	27 (61.4)	0.83#
Hb (g/dL); mean ± SD	13.4 ± 1.5	13.8 ± 1.5	0.17##
Leukocyte (x10^3/µL); median (min-max)	7200 (4400-16000)	7200 (4400-10400)	0.65**
ESR (mm/h); median (min-max)	12.5 (2-45)	10.5 (2-60)	0.37**
CRP (mg/dL); median (min-max)	0.38 (0.1-3.1)	0.4 (0.2-2.9)	0.71**
Creatinine (mg/dL); mean ± SD	0.91 ± 0.25	0.95 ± 0.24	0.51##
Albumin (g/dL); mean ± SD	4.2 ± 0.3	4.3 ± 0.3	0.23##
ALT (U/L); median (min-max)	13 (5-39)	15.5 (8-50)	0.001**
AST (U/L); mean ± SD	19.4 ± 4.9	23.1 ± 4.4	0.01##
FPG (mg/dL); median (min-max)	97 (97-214)	102 (77-212)	0.52**
HbA1c (%); median (min-max)	6.4 (5.5-10.3)	6.4 (5.3-8.6)	0.57**
TSH (µIU/mL); median (min-max)	1.3 (0.08-9.07)	1.75 (0.16-55)	0.19**
Vitamin B12 (pg/mL); median (min-max)	329 (67-1501)	282.5 (105-1160)	0.48**
Vitamin D (µg/L); median (min-max)	24.3 (5-92.5)	20.5 (5-65.8)	0.89**
LDL-C (mg/dL); median (min-max)	160 (80-358)	143 (80-239)	0.18**

Data are given as n (%), mean ± SD or median (min-max). AD: Alzheimer's Disease, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CRP: C reactive protein, ESR: Erythrocyte sedimentation rate, FPG: Fasting plasma glucose, Hb: Hemoglobin, HbA1c: Hemoglobin A1c, LDL-C: Low density lipoprotein cholesterol, N: Number, SD: Standard deviation, TSH: Thyroid stimulating hormone

*: Statistical analysis was not performed because the number of patients in the groups was small. **Mann Whitney U test, #Chi-Square test, ##Student's t-test

Currently, there is no definitive biomarker for diagnosing Alzheimer's Disease. However, this is a hot topic, and ongoing studies demonstrate many promising biomarkers (33). Inferences can be made about the role of galectin-3 in the pathogenesis of AD when its known properties and functions are considered. Galectin-3 has been identified in the central nervous system and peripheral nervous system in macrophages/microglia, astrocytes, endothelial cells, and Schwann cells (34, 35). Activation of microglia and endothelial cells is associated with the pathogenesis of AD (13). Galectin-3 plays a role in the functioning of immune cells as well. Thus, upregulation of galectin-3 expression may be associated with immunological activation and regulation. However, the studies and information on this subject are scarce (13). It has been shown in mouse models that galectin-3 regulates functions in the brain, especially in the hippocampus region. In a study by Trompet et al., although observed differences were minor, they found that carriers of variant alleles within the lectin galactoside-binding soluble-3 gene (LGALS3) performed worse on the four neuropsychological performance tests compared with the carriers of the wild-type allele (10). In this study, it was discovered that the three LGALS3 polymorphisms were linked to elevated CRP levels, suggesting that a heightened proinflammatory profile might contribute to diminished cognitive performance in advanced age. However, even after accounting for CRP levels, the relationship between LGALS3 polymorphisms and cognitive function remained largely

Table 2. Results of comprehensive geriatric assessment and cognitive assessment test scores

	AD (n = 44)	Control (n = 44)	P value
KATZ ADL; median (IQR)	5 (4 - 6)	6 (6 - 6)	< 0.001**
Lawton-Brody IADL; median (IQR)	5 (1 - 11)	17 (15 - 17)	< 0.001**
MNA-short form; median (IQR)	11 (10 - 12)	14 (13 - 14)	< 0.001**
Clock drawing test; median (IQR)	0 (0 - 4)	6 (6 - 6)	< 0.001**
MMSE; median (IQR)	18 (12 - 22)	29 (27 - 30)	< 0.001**
Three-word memory test; median (IQR)	0 (0 - 1)	3 (3 - 3)	< 0.001**
Attention and calculation; median (IQR)	0 (0 - 1)	5 (5 - 5)	< 0.001**
Yesavage geriatric depression scale-short form; median (IQR)	2 (0 - 5)	0 (0 - 2)	< 0.001**
GDS 2 (Age-Associated Memory Impairment); n (%) 4 (Mild AD); n (%) 5 (Moderate AD); n (%) 6 (Moderately Severe AD); n (%)	 - 20 (45.5) 17 (38.6) 7 (15.9)	 44 (100) - - -	
DAD; median (IQR)	44 (23 - 70)	100 (100 -100)	< 0.001**
MOCA; median (IQR)	6 (3 - 11)	19 (16 - 21)	< 0.001**
Trail test A; n (%) Test score (sec); mean ± SD	4 (9.1) 71 ± 17.1	21 (47.7) 62.2 ± 13.95	< 0.001# 0.27##
Digit span test Forward; median (IQR) Backward; median (IQR)	 2 (1 - 4) 2 (0 - 2)	 4 (3 - 6) 3 (2 - 4)	 < 0.001** < 0.001**
Categoric fluency test; mean ± SD	8.3 ± 4.7	16.6 ± 4.5	< 0.001##

Data are given as n (%), mean ± SD or median (IQR). AD: Alzheimer's Disease, DAD: Disability assessment for dementia, GDS: Global deterioration scale, IQR: Interquartile range, KATZ ADL: KATZ activities of daily living, Lawton-Brody IADL: Lawton-Brody instrumental activities of daily living, MMSE: Mini-mental status examination, MNA: Mini nutritional assessment, MOCA: Montreal cognitive assessment, N: Number, SD: Standard deviation
**Mann Whitney U test, #Chi-Square test, ##Student's t-test

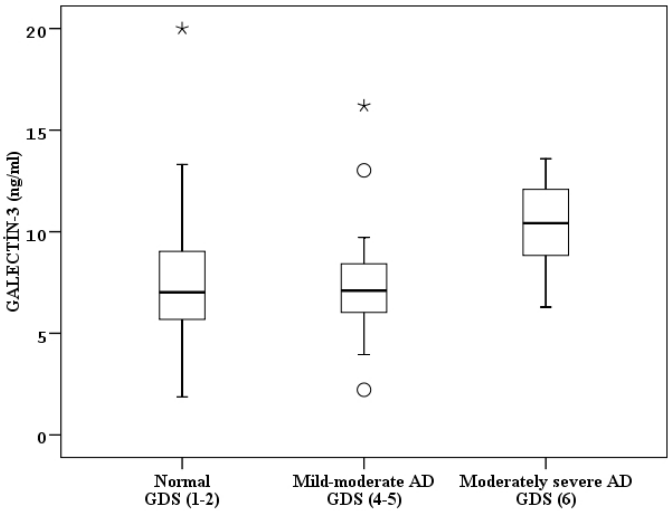

Figure 1. Galectin-3 levels according to GDS (Global Deterioration Scale) stage.
Galectin-3 levels were significantly higher in the moderately severe AD (GDS stage 6) group (GDS Stage 6: 10.42 [6.29-13.59] ng/mL, GDS Stage 4-5: 7.09 [2.22-16.19] ng/mL, GDS Stage 1-2: 7.02 [1.87-20] ng/mL; P = 0.032). The P values for the comparisons of moderately severe AD vs. normal GDS and moderately severe AD vs. mild-moderate AD were 0.017 and 0.007, respectively.

unchanged (10). Individuals exhibiting a proinflammatory profile face a heightened risk of cognitive decline compared to those with an anti-inflammatory profile (36, 37). In the geriatric age group, inflammation is a significant factor in the development of cognitive decline and dementia (6). Galectin-3, given its regulatory function in inflammation, may impact cognitive function in older individuals through its involvement in the inflammatory process (10). Therefore, the shared characteristics suggest the possibility that galectin-3 could serve as a potential biomarker for Alzheimer's Disease. Additionally, measuring galectin-3 is noninvasive, reproducible, inexpensive, and easy to implement. These all suggest that galectin-3 may be one of these promising biomarkers. The demonstration of elevated levels of galectin-3 at the severe stage of AD in our study supports that it may be a promising biomarker. Long-term follow-up studies with more patients will show this relationship more clearly.

There may be some possible limitations in this study. The first one is its cross-sectional design. While there is a notable increase in galectin-3 levels during the advanced stage, we can not say there is a cause-and-effect relationship. We can not tell whether the galectin-3 level increases due to the increase in inflammatory and oxidative stress load as the disease progresses or whether patients with high levels of galectin-3 at the early stages get to the advanced stage easily due to the excess of this burden. The second limitation concerns the patient number. Our patient number may not be enough for supporting galectin-3 as a biomarker for AD. Prospective studies with long-term follow-ups are needed to clarify this relationship.

CONCLUSION

Different hypotheses have been proposed to reveal the relationship between galectin-3 and AD. Our study found that serum levels of galectin-3 were higher in AD than in control subjects, though not significant. In addition, we found that galectin-3 levels in patients with moderately severe AD (GDS stage 6) group were significantly higher than in the earlier stages. These findings may support that galectin-3 is linked to AD, especially its severity. Galectin-3, a marker for inflammation and oxidation, may be a promising biomarker for AD, and prospective cohort studies should support this.

Additional information: Presented in at the 12th International Congress of the European Union Geriatric Medicine Society, 5-7 October 2016 Lisbon, Portugal. This study has been conducted as Dr. Gürkan Güner's specialization thesis.

Ethics Committee Approval: The required approval for conducting the study was obtained from the Ethics Committee of the Faculty of Medicine, Hacettepe University (Date 26.12.2014/ Number GO 14/649). The study protocol was in adherence with the principles in the Declaration of Helsinki.

Informed Consent: Informed consent was obtained from all participants.

Authorship Contributions: Idea/Concept: BBD, MH, MC, Design: BBD, MH, MC, Supervision: BBD, MH, MC, Data collection and/or processing: GG, MKK, MCK, HDV, AS, FA, Analysis and/or interpretation: GG, BBD, Literature search: GG, BBD, Writing: GG, BBD, Critical review: BBD, MH, MC, References and fundings: GG, Materials: -.

Conflicts of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research was supported by a grant from Hacettepe University Scientific Research Projects Coordination Unit (THD-2015-5092). The funding organization had no role in any of the following: design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

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