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RESEARCH

The level of dementia biomarkers in type 2 diabetes mellitus

Tip 2 diyabet mellitusta demans biyobelirteçlerinin düzeyi

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Abstract

Purpose: Like Alzheimer's disease, a disease of the aging world, and metastasis in cancer, it is very important to elucidate the etiology of Type 2 diabetes, which causes tissue and organ damage by systematically spreading throughout. In this study, we aimed to examine whether markers used as biomarkers in Alzheimer's pathogenesis are effective in the pathogenesis of diabetes.
Materials and Methods: In our study, 30 type 2 diabetics, 30 type 2 diabetics individuals with the risk of dementia as a result of mini-mental test, and 28 healthy individuals aged 50-70 years were included, and brain-derived neurotrophic factor (BDNF), dual-specificity tyrosine-regulated kinase 1 (DYRK1A), Tau, fatty acid binding proteins 7 (FABP7) levels were measured from plasma samples.
Results: There was a significant difference between the diabetes group with a high risk of dementia (MMSE < 24) and the other groups in Tau, and FABP7 levels, but no significant differences were found in BDNF and DYRK1A levels.
Conclusion: These biomarkers might be used to diagnose Alzheimer's disease in patients with T2D and at risk of dementia before resorting to other more expensive and invasive diagnostic methods.
Keywords: Diabetes mellitus Type 2, Alzheimer's disease, brain-derived neurotrophic factor, tau protein, fatty acid-binding proteins 7, protein serin-threonine kinases

Öz

Amaç: Yaşlanan dünyanın bir hastalığı olan, Alzheimer hastalığı ve kanserdeki metastaz gibi, sistematik bir şekilde yayılarak doku ve organ hasarına neden olan Tip 2 diyabetin, etiyolojisinin aydınlatılması çok önemlidir. Bu çalışmada Alzheimer patogenezinde biyomarker olarak kullanılan belirteçlerin, diyabet patogenezinde etkili olup olmadığını incelemeyi amaçladık.
Gereç ve Yöntem: Çalışmamıza, 30 tip 2 diyabetli, minimental test sonucu demans riski taşıyan 30 tip 2 diyabetli birey ve 50-70 yaş arası 28 sağlıklı birey dahil edilerek plazma örneklerinden, beyin kaynaklı nörotrofik faktör (BDNF), dual-specificity tyrosine-regulated kinase 1 (DYRK1A), Tau, fatty acid binding proteins 7 (FABP7) düzeyleri ölçüldü.
Bulgular: Demans riski yüksek olan diyabet grubu (MMSE < 24) ile diğer gruplar arasında Tau ve FABP7 düzeylerinde anlamlı bir fark bulunurken DYRK1A ve BDNF düzeylerinde anlamlı bir fark bulunmamıştır.
Sonuç: Bu biyobelirteçler T2D'li ve demans riski taşıyan hastalarda daha pahalı ve invaziv tanı yöntemlerine başvurmadan önce Alzheimer hastalığını teşhis etmek için kullanılabilir.

Anahtar kelimeler: Diabetes mellitus Tip 2, alzheimer hastalığı, beyin kaynaklı nörotrofik faktör, tau proteini, yağ asidi bağlayıcı protein 7, protein serin-treonin kinazlar

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a disease with high prevalence in the population, and its rate is increasing significantly worldwide. Diabetes mellitus is a multifactorial disease, and many factors have been implicated in its pathogenesis. The majority of patients with diabetes mellitus exhibit amyloid deposits, a phenomenon also observed in Alzheimer's disease. Deposits found in T2DM contain amyloid, also known as islet amyloid polypeptide, similar to beta-amyloid involved in Alzheimer's pathology[1,2]. Due to the common pathogenic factors found in both T2DM and Alzheimer's disease, the levels of other markers used in Alzheimer's disease in T2DM are of interest.

Neurotrophins are responsible for the survival of neurons and their resistance to damage. Brain-derived neurotrophic factor (BDNF), an important neurotrophin, is one of the factors involved in the pathogenesis of Alzheimer's disease. Decreased levels of BDNF in Alzheimer's disease contribute to the development of the disease [3]. In addition to its effects on neurons, BDNF also has essential effects on energy balance, glycemic control, and lipid metabolism in humans. Some changes in BDNF levels have been reported in diabetic patients, but it is unclear whether these changes play a role in disease pathogenesis [4].

The most studied subtype of Dual-Specificity Tyrosine-Regulated Kinase (DYRK), a member of the Serine/Threonine Kinase family, is DYRK1A. DYRK1A is ubiquitously present throughout the body, with the central nervous system being one of its most prevalent locations. DYRK1A plays a role in many tasks, including cell survival and neuronal development. Due to these effects, the contribution of DYRK1A to neurodegenerative diseases has been a subject of interest. DYRK1A is involved in the phosphorylation of substrates such as tau and the amyloid precursor protein, suggesting that it plays a vital role in the pathogenesis of Alzheimer's disease. Beta cell proliferation is essential for providing endogenous insulin in diabetes mellitus, and studies have shown that inhibition of DYRK1A induces human beta cell proliferation [5].

Free radicals formed in functioning cells cause oxidative damage and are responsible for the pathogenesis of various diseases. The Tau protein, a microtubule-binding protein, stabilizes neuronal microtubules, but phosphorylation-mediated alteration of this protein leads to the formation of abnormal aggregates that are toxic to cells. This is demonstrated in Alzheimer's disease. It has also been reported that tau phosphorylation in the brain is increased in animal models of T2DM [6].

Fatty acid binding proteins (FABPs) are effective factors in the uptake and intracellular distribution of fatty acids and thus play influential roles in metabolism. FABP7 binds to n-3 polyunsaturated fatty acids and is crucial for the development of neurons. FABP7 is reported to play a critical role, especially in astrocyte profiling. FABP7, by facilitating the formation of lipid droplets, has demonstrated a protective effect on cells against oxidative stress, implying its potential involvement in the development of neurodegenerative diseases like Alzheimer's disease [7] It has also been reported that FABP7 may play an active role in maintaining systemic energy balance by sensing neuronal leptin in hypothalamic astrocytes [8].

Diabetes and Alzheimer's disease share many common points in their pathogenesis. Moreover, the frequency of Alzheimer's disease increases in patients with diabetes, and diabetes is more common in individuals with Alzheimer's disease. In our study, we aimed to examine the levels of markers that are effective in the pathogenesis of Alzheimer's disease and are used as markers in diabetic patients. We also aimed to examine whether these markers are effective in the pathogenesis of diabetes. These biomarkers might be used to diagnose Alzheimer's disease in patients with T2D and at risk of dementia before applying to other more expensive and invasive diagnostic methods. We show for the first time that these biomolecules were screened in this disease pattern in humans.

MATERIALS AND METHODS

Study population

In our prospective case-control study, we included 30 individuals aged 50-70 years with type 2 diabetes mellitus (case group 1), 30 individuals with type 2 diabetes mellitus and at risk of dementia as determined by a mini-mental test (case group 2), and 28 healthy individuals (control group) who were admitted to the Endocrinology and Metabolic Diseases Clinic at Kahramanmaraş Sütçü İmam University Faculty of Medicine between June 2020 and May 2022. The blood samples required for the study were collected by specialist nurses from the

Endocrinology and Metabolic Diseases Clinic. The preservation of the blood and the experimental protocol were carried out in the Medical Biology Laboratory of Kahramanmaraş Sütçü İmam University. Patients with any central nervous system disease or suspected disease, any hemorrhagic stroke, a previous clinical diagnosis of dementia, or any e impairment based on neuro-radiological findings were excluded. We obtained an ethics committee certificate for this study from the KSU Faculty of Medicine Clinical Research Ethics Committee with the meeting decision dated 27.05.2020 and numbered 09. Written informed consent was obtained from each patient before inclusion in the study. The protocol was conducted according to the Declaration of Helsinki. We recorded information on age, gender, diabetes process, treatment modalities, and coronary and hypertension history of the individuals included in our study.

Mini-Mental State Examination (MMSE)

The Mini-Mental State Examination (MMSE) is a test used to evaluate cognitive functions in a clinical setting. It was first developed by Folstein et al. and is considered a valid and reliable tool in the diagnosis of mild dementia. The test assesses the perception of place and time, memory, speed of thought, attention, calculation, recall, and language. The maximum score on the test is 30; individuals who score 24 points or lower are further examined for signs of dementia [9,10].

Enzyme-linked immunosorbent assay (ELISA)

Peripheral blood was collected from both control and patient groups. The blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 1.800 × g for 10 minutes at 4°C. The resulting serum was collected, mixed by inverting, aliquoted into 0.5 mL Eppendorf tubes, and stored at −80°C until further processing. To assess serum levels of BDNF, FABP7, Tau, and DYRK1A (FineTest, EH0043, EH3033, P0455, EH4723, Wuhan Fine Biotech Co., Ltd, Wuhan, China), a quantitative sandwich ELISA was performed in according with the manufacturer's instructions. The absorbance of the reaction product was measured at 450 nm using a microplate reader.

Statistical analysis

Power calculations for testing the sample size were performed using the PASS software package program (NCSS, LLC, UT, version 11.0 for Windows; desired study power 80% α error= 0.005, two-tailed. As a result, the minimum required sample size was calculated as 27 for each group. In the data evaluation process, the Shapiro-Wilk test was used to examine the normal distribution of variables. For comparisons between two groups, the independent samples t-test was utilized, and for comparisons between three or more groups, one-way analysis of variance (ANOVA) was employed. Among the post hoc pairwise comparison tests, the Tukey HSD test and Dunnett test were applied. In cases where variables did not follow a normal distribution, the Kruskal-Wallis test was used for comparisons between three or more groups, and Dunn's test and Bonferroni correction were applied for pairwise comparisons. For qualitative variables, differences in frequency distribution between groups were analyzed using the Chi-Square test or the Exact test. Statistical significance was considered as $p<0.05$. Data evaluation was performed using IBM SPSS version 22 (IBM SPSS for Windows version 22, IBM Corporation, Armonk, New York, United States) and R.3.3.2 software. Statistical analyses for the ELISA experiment were performed using the one-way ANOVA test (Prism 8, GraphPad Software Inc., San Diego, CA).

RESULTS

The clinical and biochemical data of T2DM cases with and without dementia risk, as well as the control group included in our study, are listed in Table 1. There is a statistically significant difference between the groups in terms of gender distribution ($p < 0.01$), but there is no significant difference in terms of age distribution (Table 1). A statistically significant difference was found between all groups in terms of the results of the mini-mental state memory test, which differentiates between the two case groups in our study and determines the risk of dementia ($p < 0.001$). Age, gender, education, and the MMSE score were no significant differences among the 3 groups. Also, patients with T2D had the lowest MMSE score, while patients with T2D second group and non-demented controls had the intermediate and highest MMSE scores, respectively (Table 1). A significant difference was also found between all groups in terms of blood glucose levels ($p < 0.001$). When evaluated in terms of biochemical data, a statistically significant difference was found between the groups in HbA1c,

which provides information about the management of the diabetes process ($p < 0.001$). There was also a significant difference between the groups in terms of diabetes treatment type ($p < 0.001$).

A statistically no significant difference was found between the diabetes group with a high risk of dementia (MMSE < 24) and the other groups in terms of serum BDNF levels, which plays an important role in brain functions and is considered an important biomarker in Alzheimer's development ($p = 0.031$, Figure 1A). In individuals with Alzheimer's disease, no significant difference was observed between our study groups in the level of DYRK1A, which is positively correlated with the disease ($p > 0.01$, Figure 1B). The tau level, which is considered to be a potential biomarker to identify neurodegeneration, was statistically significant in the comparison between the groups in our study ($p < 0.01$, Figure 1C). The level of FABP7, which is expressed in brain tissue and has an important role in nervous system development, was also found to be statistically significant in our study groups ($p < 0.01$, Figure 1D).

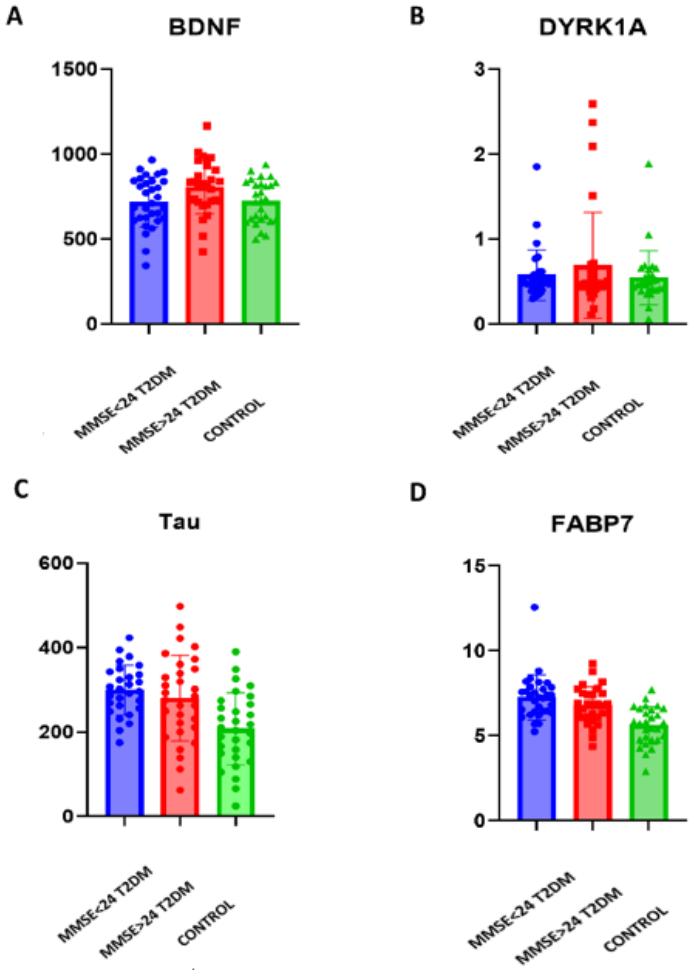

Figure 1. Q-Q plot for plasma molecules status in the cohort and control groups: A) BDNF, B) DYRK1A, C)Tau, and D) FABP7 were measured by Elisa in individuals per group.

The plasma level of molecules was calculated and groups were compared by one-way analysis of variance (ANOVA) with GraphPad Prism software (version 8.0.2).

Table 1. Biochemical and clinical characteristics of all participants in the study.

	MMSE score <24 T2DM	MMSE score >24 T2DM	Control	P value
Age (years)	60.23±7.07	58.87±4.78	56.68±5.01	0.121
Famale/Male (n%)	26/4 (86.7/13.3)	16/14 (53.3/46.7)	23/5 (82.1/17.9)	**P<0.001***
MMSE Score	23.00 (22.00-23.00)	29,00 (28.00-29.00)	29.00 (27.50-30.00)	**P<0.001***
Glucose (mg/dl)	145.00 (96.00-182.00)	115.00 (100.00-149.00)	94,00 (89.00-103.00)	**P<0.001***
Diabetes duration (year)	11.89±6.99	9.25±6.43		0.226
HbA1c	7.60 (6.60-8.60)	6.75 (5.90-8.20)	5.60 (5.50-5.90)	**P<0.001***
TSH	1.57 (1.03-3.62)	1.72 (1.18-2.81)	1.02 (0.62-2.66)	0.152
LDL (mmol/L)	100.90 (85.00-125.00)	131.50 (87.50-165.00)	113.00 (99.00-151.00)	0.251
Trigliserid (mmol/L)	124.00 (100.00-194.00)	150.00 (107.50-222.50)	124.00 (114.00-152.00)	0.437
HDL (mmol/L)	46.40±8.48	48.79±11.63	52.07±10.74	0.162
Free Tiroid (mmol/L)	1,21 (1.10-1.40)	1.30 (1.20-1.40)	1.30 (1.20-1.60)	0.432
BDNF (pg/mL)	714.00±149.02	804.17±152.82	721.96±125.82	0.031
Tau (pg/mL)	316.13 (255.60-338.53)	274.67 (206.40-342.93)	185.33 (137.60-287.73)	**P<0.001***
DYRK1A (pg/mL)	0.49 (0.45-0.58)	0.47 (0.43-0.60)	0.48 (0.41-0.60)	0.792
FABP7 (pg/mL)	7.24±1.34	6.79±1.09	5.59±1.11	**P<0.001***
Coronary Artery Disease yes/no (%)	7/23 (23.3/76.7)	5/24 (17.2/82.8)	1/26 (3.7/96.3)	0.122
Treatment modality (%)				
Oral hypoglicemic agents	14 (46.7)	13 (44.8)	2 (7.4)	**p<0.001***
Insulin therapy	1 (3.3)	1 (3.4)	0 (0.0)	
Both oral and insulin	11 (36.7)	8 (27.6)	0 (0.0)	
No treatment	4 (13.3)	7 (24.1)	25 (92.6)	
Hypertension yes/no (%)	17/13 (56.7/43.3)	9/20 (31/69)	8/19 (29.6/70.4)	0.059

The data are mean±SD values or n (%). *p<0.001. MMSE-Mini-Mental State Examination, BDNF-brain-derived neurotrophic factor, FABP7-fatty acid- binding proteins 7, DYRK1A-dual-specificity Tyrosine-Regulated Kinase 1A, TSH-Thyroid stimulating hormone, HbA1c-Glycated hemoglobin, LDL-Low density Lipoprotein, HDL-High density lipoprotein.

DISCUSSION

In our study, we compared the serum levels of BDNF, Tau, FABP7, and DYRK1A, which are found to be associated with Alzheimer's disease, between healthy individuals and those diagnosed with T2DM. We also assessed the risk of dementia in individuals diagnosed with T2DM by administering the Mini-Mental State Examination. This is the first study to investigate these biomarkers together in a patient group, and among humans. In a study investigating the levels of DYRK1A, BDNF, and homocysteine in the blood, which are evaluated together as diagnostic markers for Alzheimer's disease, it was determined that they have the potential to be used for diagnosis[11].

BDNF, which is found to be decreased in both hippocampal and plasma levels in patients with Alzheimer's disease, has also been found to be

decreased in patients with T2DM. This decrease has been associated with impaired glucose metabolism [12,13]. Decreased levels of BDNF have been observed in brain cells of animals with hypoglycemia induced by dietary restriction, and BDNF has been reported to have beneficial effects on metabolic regulation[4,14].

Both T2DM and AD are diseases whose incidence and prevalence increase with advanced age. Early detection of AD is likely to occur in patients with T2DM and is important for approaches to improve the prognosis of patients with diabetes[15,16].

Increased expression of DYRK1A, which contributes to tau hyperphosphorylation, one of the pathological findings of Alzheimer's disease, through glycogen synthase kinase 3β (GSK3β), which is included in the insulin signaling pathway, is found to be associated with cognitive dysfunctions in Alzheimer's disease[17]. These results are consistent with our findings. Although the DYRK1A level is higher in the group with a high risk of dementia (0.49 pg/ml) compared to other groups, the figures for both the T2DM group without a dementia risk and the control group (0.48 and 0.49 pg/ml) are strikingly similar. The lack of sufficient sample size in our study could be the cause of this finding. However, another study showed that decreased DYRK1A induces diabetes with decreased pancreatic beta cell mass[18].

In a proteomic study of post-mortem AD brains, elevated levels of FABP7 were observed in the brains of symptomatic AD patients[19]. In another study, a 29% increase in serum FABP7 levels was observed in patients with Alzheimer's disease. These findings are consistent with our studies. Additionally, there are studies demonstrating that APOE (Apolipoprotein E), which poses a high risk for the development of Alzheimer's disease, may alter the functional expression of FABP7[20]. The fact that APOE alleles and lipid metabolism disorders are involved in the common molecular pathology of Alzheimer's disease and diabetes suggests that FABP7 may also be involved in this pathway.

Previously, a study in Goto-Kakizaki rats, a spontaneous model of Type 2 DM (which is the closest model of Type 2 DM to humans), found a strong association between cystathionine beta-synthase (CBS)-mediated insulin and homocysteine metabolism. In this study, DYRK1A, a serine-threonine kinase-regulated protein, as well as BDNF and Tau phosphorylation, which are both correlated with CBS activity, were shown to be biomarkers in both T2DM and Alzheimer's disease. As a result of the examination of plasma levels of Alzheimer's biomarkers (DYRK1A, BDNF, and Tau), which are thought to be potentially associated with T2DM, a decrease in BDNF levels was observed in T2DM model rats compared to control group rats. An increase in DYRK1A levels was observed, as well as an increase in plasma tau levels[21]. In another study with Alzheimer's, Parkinson's, and dementia patients, serum FABP (brain-type fatty acid binding protein) levels were found to be higher than in control groups[22]. Consistent with these studies, our study found that serum BDNF levels were lower in the T2DM group with dementia risk compared to the T2DM group without dementia risk and the control group but the difference was not statistically significant. Additionally, an increase in serum DYRK1A and Tau levels was observed in the T2DM group with dementia risk compared to the other groups. Furthermore, consistent with the study by Teunissen et al., FABP levels were found to be higher in the case groups compared to the control group in our study, and the result was statistically significant.

In conducted studies, when evaluated in terms of gene expression, and genetic and hormonal factors, it has been determined that the incidence of Alzheimer's disease is significantly higher in women [23]. In our study, it is thought that the reason for the difference in the HbA1c data we obtained, being higher in the group with a high risk of dementia (7.60) compared to the group without (6.75), originates from the gender differences in these two different type 2 diabetic groups.

The serious increase in cases of T2DM and Alzheimer's indicates a rising socio-economic burden from these two diseases in the coming years[24]. Our study, which aims to identify the molecules involved and their relationships in mechanisms triggered by the disruption of insulin signaling in the brain, will contribute to the development of new pharmacological agents or alternative treatment methods. In the literature, there is an animal study in which the biomolecules included in our study were investigated, but this is the first study in which these biomolecules were screened in this disease pattern in humans[21].

According to these results, the higher levels in Tau and FABP7 levels in patients with a high risk of dementia (MMSE < 24) may indicate that these biomarkers can be used to diagnose Alzheimer's disease in T2DM patients. Our sample size for the

study is limited, and we were unable to investigate other molecules involved in the same pathways as the biomarkers we examined. As also the sample size was limited, the subjects in the MMSE<24 groups could not be subdivided according to the test score. The biochemical data obtained in this study should be supported by studies at the molecular level. Therefore, further studies with a larger study population are needed to clarify this subject. In upcoming studies, the examination of more biomarkers, including the phosphorylated tau protein, is planned.

It is not yet clear whether T2DM causes Alzheimer's or Alzheimer's causes T2DM, like an egg-and-chicken dilemma with common molecular mechanisms. Although cerebrospinal fluid (CSF) is widely used in clinical practice for the diagnosis of Alzheimer's disease, its use is extremely limited due to its invasive nature. Positron emission tomography (PET), which measures Aβ deposition in the brain, is a costly method. At this point, it is essential to develop less expensive and more accessible methods using non-invasive materials. The primary target for this is plasma biomarkers. The sensitivity and specificity of blood biomarkers have the potential to help make significant strides in the future of disease diagnosis and treatment. Therefore, more clinical applications and treatments are needed.

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