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Is there a role of insulin degrading enzyme in Parkinson's disease?

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Abstract

Objectives: Alpha-synucleins are the basic structural components of Lewy bodies, which are the pathognomonic feature of Parkinson's disease (PD). It is propounded that alpha-synucleins interact with the insulin-degrading enzyme (IDE). In this study, it was aimed to evaluate the relationship between the insulin-degrading enzyme (IDE) level and the disease and the symptomatology of the disease in patients with PD. *Methods:* A total of 98 individuals, including 57 patients with PD and 41 healthy controls, were enrolled in this cross-sectional, case-control study. The clinical characteristics of the patients with PD, modified Hoehn and Yahr (mHY) stages, and Unified Parkinson's Disease Rating Scale (UPDRS) scores were recorded. The mini-mental state examination (MMSE) was applied to all participants. Serum samples were collected for the level of IDE. *Results:* No significant difference was found in terms of IDE between PD patients and the control group with similar sociodemographic characteristics ($p>0.05$). No statistically significant difference was revealed between the level of IDE and the age of onset of disease, initial symptom, disease subtype, mHY stage, LED dose, MMSE score, UPDRS part II, III and IV. In PD patients, a weak negative correlation was found between IDE, duration of disease, and UPDRS part I ($p=0.049$, $r=-0.299$ and $p=0.003$, $r=-0.431$, respectively). *Conclusion:* No significant difference was determined in the level of IDE between PD patients and the controls. There was no relationship between IDE level and onset symptom, disease subtype, disease stage. It was suggested that the serum level of IDE was related to a longer duration of disease and the affected mentation, behavior and mood. The confirmation of these results with larger patient series will contribute to clarifying the role of IDE in the pathogenesis of PD.

Keywords: Parkinson's disease, insulin degrading enzyme, insulin, disease duration.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease is characterized by the accumulation of intraneuronal aggregates defined as Lewy bodies in striatal neurons in the brain.[1] Although the basic component of Lewy bodies is alpha-synuclein, it was found that proteins such as tubulin, neurofilament, and ubiquitin were also involved in this structure.[2-5]

There is increasing evidence that insulin may have central effects in addition to known carbohydrate and energy metabolism. In neuropathological studies, it was shown that individuals with PD had intensive insulin receptors in the substantia nigra pars compacta.[6] It was stated that insulin might have effects on neuron growth, neuronal metabolism, dopaminergic pathways, and cognition. The insulin signaling mechanism was stated to modulate some cellular processes that were revealed to be impaired in PD. It was asserted that this process might contribute to the pathology of PD in the brain, similar to insulin resistance in the periphery.[7]

Studies have shown that the activation of insulin-degrading enzyme (IDE), an enzyme that can degrade insulin, can modulate the degradation of alpha-synuclein and other small peptides forming B-pleated sheets.[7,8]

We hypothesized that the accumulation of alpha-synuclein, which is the basic component of intraneuronal aggregates in PD, as a result of decreased IDE activity might be related to the disease or the clinical symptomatology of the disease. At the same time, since it was shown that interaction at a cellular level in PD cases occurs years before diagnosis[9], determining

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specific biomarkers for the diagnosis of the disease in question may be useful for early diagnosis and development of disease-specific curative treatments. For these reasons, it was aimed to assess the correlation between IDE and clinical characteristics of the disease in PD patients.

METHODS

Data collection

In this case-controlled, cross-sectional study, 57 consecutive patients who applied to Yozgat Bozok University Faculty of Medicine, Neurology Outpatient Clinic between June 2019 and June 2020 and were diagnosed with idiopathic PD following the "UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria"[10] were included. Demographic data of the patients, including age, sex, educational status, and BMI, were obtained. Along with the demographic data of the patients, disease characteristics, e.g. age of onset, symptom (tremor, bradykinesia or tremor + bradykinesia) and side (left, right, or bilateral), duration of disease, and disease subtype, were recorded. The modified Hoehn and Yahr (mHY) scale staging, total UPDRS scores, and UPDRS part I (mentation), part II (activities of daily living), part III (motor examination) and part IV (complications of therapy) scores were recorded. The antiparkinsonian drugs taken by the patients were recorded at a levodopa equivalent daily dose. The information obtained from the patients was verified through interviews with patient caregivers. The control group consisted of healthy subjects of similar age and education level with no medical problems. The mini-mental test was applied to the patient and control groups according to their education level. Baseline brief biochemical laboratory values (glucose, creatinine, AST, ALT, HbA1c, TSH) of the patients were examined. Serum samples were taken from all participants for the insulin-degrading enzyme.

Patients with an MMSE score below 24, those with Alzheimer's disease, PD dementia or any type of dementia, those with neurological disease other than PD, those with thyroid disease, type I or type II diabetes mellitus, patients with REM sleep behavior disorder, synucleinopathies (multiple system atrophy) were not included in the study.

The ethics committee approval of Yozgat Bozok University, Faculty of Medicine was obtained for the present research carried out in line with the Declaration of Helsinki (2017-KAEK-189_2019.06.19_11). Written consent forms were obtained from all participants.

Measurement of serum insulin-degrading enzyme (IDE) levels

Blood samples were taken from the antecubital vein as 2 ml and collected in tubes without anticoagulants. Then, the blood samples were centrifuged at 100 x g for 15 min. Serum was collected and stored at -80 °C. The serum IDE level was measured by enzyme-linked immunosorbent assay (ELISA) kits [Bioassay Technology Laboratory, China] following the manufacturer's instructions. The levels of IDE were calculated from a standard curve generated by serial dilution of recombinant human IDE. The assay was linear in the range of 15 ng/mL to 3000 pg/mL. The sensitivity of this test was 8.04 ng/mL. The specificity of the test was checked by including the appropriate positive and negative controls. The serum IDE level was measured on the same day to reduce variance.

Statistical analysis

All statistical analyses were conducted by SPSS 20.0 (IBM Corps., Armonk, NY, USA) software. The normality characteristics of the data were analyzed by the Kolmogorov-Smirnov test. The means of the groups were compared by Student's t-test or the Mann-Whitney U-test, depending on the suitability of the data. Categorical data of the groups were compared by performing the chi-square test. The relationships of the parameters with each other were evaluated using Pearson's r test or Spearman's Rho test. The results were considered statistically significant at the $p<0.05$ level.

RESULTS

Forty-four PD patients in total (17 females, 27 males) and 41 healthy volunteers (20 females, 21 males) were enrolled in the research. A total of 13 patients were excluded from the study because 9 patients had newly diagnosed diabetes mellitus and 4 patients had dementia.

The mean age of the patients was 68.50 ± 9.09 years, and the mean age of the control group was 68.78 ± 7.96 years. The patient and control groups were statistically similar in terms of age. No statistically significant difference was found between the groups regarding sex, BMI, waist circumference values, and education level ($p>0.05$).

The mean duration of disease of PD patients was 5.55 ± 3.30 years (1-12), and the age of onset of disease was 63.11 ± 9.83 (37-85) years. The initial symptoms of the patients were tremor at a rate of 63.6%, bradykinesia at a rate of 31.8%, and postural instability at a rate of 4.5%. The disease subtype was tremor-dominant at a rate of 47.7% and bradykinesia-dominant at a rate of 47.7%. The mHY stages of the patients, UPDRS part I, II, III, IV and total scores and LED doses are given in detail in Table 1. The baseline biochemical data of the patient and control groups are also shown.

Table 1: Demographic, clinical and basal laboratory features of patients with Parkinson's disease and healthy volunteers

	Patients with PD (n= 44)	**Control group (n= 41)**	***p***
Age (years)	68.50 ± 9.09	68.78 ± 7.96	0.881
Sex (female/male)	17/27	20/21	0.469
BMI (kg/m^2)	27.54 (25.63-29.51)	29 (28-30.19)	0.053
Education levels (years)	5(0-5)	5(4.82-5)	0.884
Waist circumference (cm)	96.05 (91.25-100.50)	95.53 (94-100)	0.802
Disease duration (years)	5.55 ± 3.30 (1-12)	-	
Age at disease onset (years)	63.11 ± 9.83 (37-85)	-	
Symtoms at disease onset			
• **Tremor**	28/44 (% 63.6)	-	
• **Bradykinesia**	14/44 (% 31.8)		
• **Postural instability**	2/44 (% 4.5)		
Subtype of the disease			
• **Tremor**	21/44 (%47.7)	-	
• **Bradykinesia**	21/44 (%47.7)		
• **Postural instability**	2/44 (% 4.5)		
mHY stage			
• **1-2**	9/44(%20.5)	-	
• **2.5**	11/44(%25)		
• **3**	17/44(%38.6)		
• **4**	7/44 (%15.9)		
UPDRS part I score	3.48 ± 2.22 (0-10)	-	
UPDRS part II score	15.41 ±6.47 (6-30)	-	
UPDRS part III score	22.09 ± 6.86 (12-37)	-	
UPDRS part IV score	3.74 ± 3.24 (0-14)	-	
UPDRS total scores	44.74 ± 15.78	-	
Levodopa dose (LED, mg/day)	509.37 ± 429.44 (0-1982)	-	
MMSE score	27.40 ± 1.48	27.93 ± 1.36	0.133
IDE(birim)	142.75 (92.15-442.46)	130.60 (82.55-325.21)	0.418
Glucose (mg/dL)	95.65 (86.77-102.77)	94.60 (88.75-103.40)	0.721
Hba1C	5.78 (5.41-6.10)	5.77 (5.55-5.93)	0.745
AST (U/L)	17.14 ± 5.03	17.52 ± 4.83	0.786
ALT (U/L)	11.55 (8.25-18.55)	13.90 (11.95-20.25)	0.125
Creatinine (mg/dL)	0.82 (0.74-1.04)	0.86 (0.70-0.97)	0.883
TSH (μIU/mL)	1.26 (0.88-2.20)	1.42 (0.72-2.28)	0.884

BMI, body mass index; mHY, Modified Hoehn and Yahr Staging Scale; UPDRS, Unified Parkinson's Disease Rating Scale; LED, levodopa equivalent dose; MMSE, mini mental state examination; IDE, Insuline degrading enzyme; AST, aspartate aminotransferase; ALT, alanine transaminase; TSH: thyroid stimulating hormone.

The MMSE score of PD patients was 27.40 ± 1.48, and that of the control group was 27.93 ± 1.36. The level of IDE was 142.75 (92.15-442.46) in the patients and 130.60 (82.55-325.21) in the control group. There was no statistically significant difference between the two groups concerning the MMSE score and serum IDE level ($p<0.05$).

The correlation between the level of IDE and age, BMI, waist circumference, duration of disease, age of onset, symptom and side of the disease, mHY stages, UPDRS scores, LED dose, and MMSE score in PD patients was evaluated. (Table 2) There was a weak negative correlation between IDE and the duration of disease (p:0.049, r:-0.299), and UPDRS part I (p=0.049, r=-0.299 and p=0.003, r=-0.431, respectively), while no significant difference was revealed in terms of other parameters.

When the tremor-dominant subtype (n=21), bradykinesia-dominant subtype (n=21) and control group were evaluated as three groups, no significant difference was found in terms of serum IDE (p=0.625).

DISCUSSION

This study shows that the level of IDE is not different from the control group in patients with PD. There was no relationship between motor symptoms, the disease subtype (tremor/bradykinesia-dominant), disease stage and IDE. It was suggested that as the duration of disease increased in PD patients and as the affected mentation, behavior and mood became evident in these patients, the level of IDE decreased.

IDE is present in the cytosol, peroxisomes, and plasma membrane of many cells. It was reported that in the brain, IDE is predominantly expressed in neurons. In a study, IDE expression was determined in cultured human cerebrovascular endothelial cells.[11] IDE is a zinc-metalloendopeptidase enzyme that is responsible for insulin catabolism but degrades other polypeptides such as amylin and beta-amyloid.[12,13] It is thought that IDE dysregulation may have an effect on aggregopathies and neurodegenerative diseases. It was shown in various studies that IDE can modulate the degradation of alpha-synuclein and other small peptides forming B-pleated sheets.[7,8] However, in our study, we detected that insulin-degrading enzyme level was not different from the control group in PD patients. It was shown in vitro that IDE becomes more active in the presence of the C-terminal of alpha-synuclein and that there is nonproteolytic interaction between IDE and alpha-synuclein. It was stated that electrostatic attraction affects the enzyme activity because the C-terminal of alpha-synuclein is more acidic.[8] In

Table 2: The correlation of insulin-degrading enzyme with clinical features of the Parkinson's disease and minimental status test score

	p	r/rho
Age (years)	0.481	-0.109
BMI (kg/m^2)	0.127	-0.234
Waist circumference (cm)	0.693	0.061
Disease duration (years)	**0.049**	**-0.299**
Age at disease onset	0.879	-0.024
Symtoms at disease onset	0.196	-0.198
Subtype of the disease	0.250	-0.177
mHY stage	0.152	-0.220
UPDRS part I score	**0.003**	**-0.431**
UPDRS part II score	0.102	-0.250
UPDRS part III score	0.236	-0.182
UPDRS part IV score	0.923	-0.015
UPDRS total score	0.107	-0.216
Levodopa dose (LED, mg/day)	0.731	-0.053
MMSE total score	0.830	-0.033

*Data are presented as r value. Bold indicates statistically significant values ($p<0.05$).

another study examining the role of ubiquitin in the capacity of IDE to bind and degrade insulin molecules, the results show that ubiquitin plays an allosteric role for IDE and that high ubiquitin levels impair IDE activity.[14] Therefore, we think that there is multidimensional interaction at the cellular level, and this complex pathophysiology cannot be explained by the serum IDE level alone in a cross-sectional evaluation and remains at an insufficient level.

Insulin is thought to have neuroprotective effects on the central nervous system. As a result of the study performed by Kao, it was stated that insulin reduced the aggregation and toxicity by affecting the turnover of alpha-synuclein.[15] It was shown that irregularities in glucose and energy metabolism were an early event in sporadic PD.[16] A meta-analysis of seven population-based cohort studies involving more than 1.7 million people reported a 38% increased risk of PD in patients with diabetes.[17] In a series consisting of 800 patients with PD, concurrent diabetes along with PD was demonstrated to accelerate the progression of both motor and cognitive symptoms.[18] The onset of diabetes prior to PD was considered a risk factor for more severe disease symptoms.[19] The collected data suggest that DM and PD share similar dysregulated pathways.[20,21]

In their recent study evaluating 76 PD patients and 39 controls, Sanchez-Gomez *et al*. found that fasting blood glucose and hemoglobulin A1c levels did not differ from the control group, but the fasting plasma insulin levels in PD patients were lower than in controls. They suggested that insulin resistance was present rather than insulin sensitivity.[22]

Another important finding of our study is that the level of IDE decreases as the duration of PD increases. Mechanisms such as genetic factors, mitochondrial and proteasomal dysfunction, inflammation, oxidative stress and dysfunction of autophagy systems that are involved in the pathophysiology of the disease that has not been fully clarified may result in a decrease in this enzyme activity as the disease progresses.[7,23-25]

No statistically significant difference was revealed between the level of IDE and the age of onset of disease, the onset symptom (tremor or bradykinesia), the disease subtype, the stage of the disease, the treatment dose, the MMSE score, the activities of daily living of the patients (UPDRS part II), UPDRS part III showing the motor examination, the treatment dose received by the patients, and complications (UPDRS part IV). While there was no relationship between the level of IDE and the motor symptoms of the patients, it is an interesting finding that it was correlated with the affected mentation, behavior and mood. We found a relationship with the decrease in IDE levels as the mentation, behavior and mood became more evident in PD patients. A more detailed evaluation of non-motor symptoms in future studies will provide different information.

The study has some limitations. The sample group consisted of a small number of patients. Patients mostly consisted of early-stage patients, and patients with mHY stage 5 were not included in the study. Another limitation is that the enzyme activity was studied from serum samples, and its analysis with cerebrospinal fluid may open up new horizons. A simultaneous and comprehensive evaluation of patients' non-motor symptoms and psychiatric comorbidities will be able to reveal more clearly the relationship of the disease with serum IDE level by developing methods in which enzyme activity can be determined, not the enzyme level.

To our knowledge, this clinical research is the first study investigating the level of IDE in PD patients. Our results showed that the level of IDE in PD patients was not different from the control group, and there was no relationship between IDE and the clinical symptomatology and the stage of the disease. The IDE level was suggested to be associated with a longer duration of disease and affected mentation, behavior and mood. In PD patients, symptoms and functionality interact with many genetic, demographic and ethnocultural and psychosocial factors.[26] The evaluation of these findings with larger patient series or different population will contribute to clarifying the role of IDE in the pathogenesis of PD and to the treatment strategies that can be developed.

DISCLOSURE

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