

# The Relationship Between Serum Levels of lncRNA H19, GAS5, HAR1B, LINC01783 and Clinical Signs of Parkinson's Disease

Betul Ozdilek (✉ [ozdilekbetul@gmail.com](mailto:ozdilekbetul@gmail.com))

Istanbul Medeniyet University Faculty of Medicine: Istanbul Medeniyet Universitesi Tip Fakultesi  
<https://orcid.org/0000-0003-1608-9882>

**Ibrahim Alper Kaya**

Istanbul Medeniyet University Faculty of Medicine: Istanbul Medeniyet Universitesi Tip Fakultesi

**Berna Demircan**

Istanbul Medeniyet University Faculty of Medicine: Istanbul Medeniyet Universitesi Tip Fakultesi

**Temel Tombul**

Istanbul Medeniyet University Faculty of Medicine: Istanbul Medeniyet Universitesi Tip Fakultesi

**Handan Ankarali**

Istanbul Medeniyet University Faculty of Medicine: Istanbul Medeniyet Universitesi Tip Fakultesi

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## Research Article

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# Abstract

Long noncoding RNAs (lncRNAs) are highly expressed in brain and alterations in their levels have been shown in many neurodegenerative disorders. Evidence has shown that lncRNAs play role in the onset and progression of Parkinson's disease (PD) and it can be used as a potential therapeutic target for the disease. In this study, we aimed to detect whether the serum expression levels of four candidate lncRNAs; H19, GAS5, HAR1B and LINC01783 are related with the clinical findings and treatment of PD or not. 83 patients and 50 healthy controls, matching in age and gender, are included in this study. We assessed how severe the disease is, by using the Hoehn Yahr (HY) staging and the Unified PD rating scale (UPDRS). Venous blood samples were taken from the participants. Serum samples were centrifuged and stored at  $-80^{\circ}\text{C}$  until analysis. Expression levels of these lncRNAs were analyzed by a real-time PCR instrument in the laboratory. Statistical analysis of the data was performed using SPSS program. There was no significant difference between the PD patients and healthy controls in these lncRNAs serum levels. Age, gender, education level, presence of hypertension, type of onset of the disease and predominance of the right and left side, disease duration and treatment did not differ in lncRNA expression levels. Solely, there was a significant negative correlation between GAS5 and HY and UPDRS scores. Patients with family history had significantly higher levels of LINC01783. This is the first comprehensive study on lncRNAs in Turkish patients with PD. If any altered expression levels of these four lncRNAs were shown in the serum, it would provide an easy way to analyze underlying pathogenesis of the disease and contribute the possible biomarkers of PD.

## Introduction

Parkinson's disease (PD) is the most common chronic and progressive neurodegenerative movement disorder after Alzheimer's disease (AD) and clinically present bradykinesia, rigidity, resting tremor and postural instability. In addition to these motor symptoms, non-motor symptoms such as cognitive, psychiatric, sleep and autonomic nervous system dysfunctions are also frequently observed. PD affects approximately 2-3% of people over the age of 65 [1, 2]. The prevalence of disease is predicted as nearly double in the following 30 years when the aging of the population is considered especially at the point of the extended life expectancy [2]. Therefore, PD represents a heavy burden on patients and their families and also economic burden on society, so that more effective treatments are urgently required. The dominant pathological features in PD are progressive loss of dopamine-producing neurons in the substantia nigra pars compacta (SNpc) and abnormal deposition of the  $\alpha$ -synuclein, Lewy bodies and other proteins [3]. Since the molecular mechanism of neurodegeneration in PD has been mostly unknown, it is clear that further investigations are required. Which molecules can be biomarkers for this neurodegeneration have been studied and long noncoding RNAs (lncRNAs) are thought to be specific biomarkers for PD diagnosis, clinical stages, prognosis and therapeutic targets [4–7]. Besides, there are a few studies conducted on the role of various lncRNAs in the pathogenesis of PD [8–17].

lncRNAs are a class of noncoding RNAs that consist of more than 200 nucleotides in length and represent one of the largest fractions in the human genome [18, 19]. Studies have shown that lncRNAs

interact with DNA, RNA and protein molecules to regulate gene expression at the epigenetic, transcriptional and posttranscriptional level in cellular homeostasis. Even though studies are still in the preliminary stages, lncRNAs are considered to play a role in development, differentiation, aging and apoptosis activities, immune system and cancer. lncRNAs are highly expressed in various parts of the central nervous system (CNS) and alterations in lncRNA levels have been shown in many neurodegenerative disorders such as AD, PD, Huntington's disease, amyotrophic lateral sclerosis and stroke [20–26]. Accumulating evidence has shown that lncRNAs play role in the onset and progression of PD and it can be used as a potential therapeutic target for the disease. Somewhat abnormal expression levels of these lncRNAs were detected in the samples of brain tissue, cerebrospinal fluid (CSF), blood and even saliva. Serum samples are accessible by minor invasive procedures and offer the possibility of a cheap, fast and quick way of identifying disease-related biomarkers. Whereas the number of the studies on lncRNAs has significantly increased in recent years, identified lncRNAs' numbers in PD patients have also increased [8–12, 14, 15, 24]

We downloaded the experimentally validated disease-to-lncRNA associations according to the lncRNADisease 2.0 database and about 27 of lncRNAs were specifically linked to PD [26]. When reviewed literature data, we decided to select and analyze four of these lncRNAs; H19, GAS5, HAR1B and LINC01783 (ENST00000415386) which have been implicated in neurodegenerative disorders. In this study, we aimed to analyze serum expression levels of these four lncRNAs in patients with PD and to find whether those are associated with motor, non-motor symptoms and treatment.

## Materials And Methods

This study was conducted in accordance with the ethical standards of the Declaration of Helsinki, and national and international guidelines for researches with human subjects. Protocol was approved Istanbul Medeniyet University (T-GAP-2019-1548) and Goztepe Training and Research Hospital (2019/0223) Institutional Review Boards. All of the subjects recruited to study provided a written informed consent agreeing to participate the project.

## Participants

Eighty-three PD patients who were followed up in a movement disorders outpatient clinic and fifty healthy controls, matched for age and gender, included in the study. The controls were recruited from the patients' spouses or individuals who wanted to participate in the study. The participants ranged from 40 to 80 years in age and have at least five years of education.

Patients were diagnosed as having PD based on the Movement Disorder Society Clinical Diagnostic Criteria and regularly followed up by the same experienced neurologist [27, 28]. The patients have had this diagnosis for at least 2 years and have been taking antiparkinsonian treatment regularly for the last six months. Demographic information, clinical findings and scales were recorded in their databank in each follow-up. Data collection included demographic and clinical information such as age, gender, years

of education, duration of disease and treatment, positive family history of PD and levodopa equivalent daily dose (LEDD, mg/day) [29]. Exclusion criteria were as follows: (1) presence of other neurological disorders; (2) to be diagnosed with diabetes mellitus, coronary heart disease, ischemic or hemorrhagic stroke, infectious disease, malignant tumor, glaucoma, severe visual and hearing impairment; (3) to receive any anti-inflammatory or immunosuppressive drugs; (4) presence of psychiatric disorder such as moderate to severe depression with a score of 17 or higher on the Geriatric Depression Inventory, psychosis and recent delirium diagnosed with a structured clinical interview; (5) the subjects whose scores less than 24 on the Mini-Mental State test; (6) any history of alcohol and/or substance abuse; (7) to have a brain surgery for PD or another reason. The same exclusion criteria were applied to the controls, and they had negative family history of movement disorders. We only included hypertension comorbidity for the whole participants.

## Assessment Of Motor And Non-motor Symptoms In Pd Patients

A complete neurological examination was performed and Turkish versions of the scales were administered to PD patients during "on" periods before collecting blood samples. Onset sign of disease (bradykinesia or tremor) and their lateralization (left or right) and the presence of postural instability and gait dysfunction were noted. Disease severity was measured using the Hoehn and Yahr (HY) staging scale [30]. Scores varied between 1 and 5, higher scores mean higher disease severity. Current clinical symptoms were assessed with the Unified PD Rating Scale (UPDRS). It has four parts. Parts I is about mental function disorder and mood, II about motor activities of daily living, III about motor examination, IV about motor complications. PD patients rated items on a Likert scale ranging from 0 *normal: symptom not present* to 4 *severe: symptom present and precludes patient's ability to carry out normal activities or social interactions or to maintain previous standards in personal and family life*. A total of these four parts were calculated by total score [31]. All patients were using dopamine agonist therapy. The presence or absence of motor fluctuations, dyskinesia, hallucinations, delusions and dopamine dysregulation syndrome was recorded.

## Blood Sample Collection

From each participant, ~5 mL venous blood samples were collected into serum vacutainer tubes with gel and clot activator in the morning following 12 hours of fasting. Serum samples were kept at room temperature for one hour, and then centrifuged for 10 min at 4000× g for serum separation. Supernatant serum was stored at -80°C until analysis.

## Laboratory Analysis

# Total RNA isolation

The lncRNAs were isolated from serum samples of PD patients and healthy controls using the miRNeasy serum / plasma kit (Qiagen, Germany) according to the manufacturer's instructions. Briefly, 500  $\mu$ L of QIAzol lysis reagent was added to 100  $\mu$ L of the serum sample and incubated the whole reaction mixture for 5 min at room temperature. Then, 100  $\mu$ L chloroform was added to the lysate tube, vortexed for 15 s and incubated it for 2 min at room temperature. Thereafter, we performed centrifugation at 12,000 $\times$  g for 15 min at 4°C. ~ 300  $\mu$ L of the upper aqueous phase was removed and the mixture transferred to a new collection tube, then 450  $\mu$ L of 100% ethanol was added. Then, 700  $\mu$ L of the mixture was added to the RNeasy MinElute spin column in a 2 mL collection tube and centrifuged at 8000 $\times$  g for 15 s at room temperature. After the mixture had completely transferred to the column, we added 700  $\mu$ L of buffer RWT to each column and centrifuged at 8000 $\times$  g for 15 s at room temperature. Next, 500  $\mu$ L buffer RPE was added and centrifuged at 8000 $\times$  g for 15 s. Finally, 500  $\mu$ L of 80% ethanol prepared with RNase-free water was added to the column and centrifuged at 8000 $\times$  g for 2 min. A full speed centrifugation was performed to dry the membrane with an open cover for 5 min. The filtrate and collection tube were discarded at each step. Total RNA was eluted by centrifugation for 1 min at full speed using 14  $\mu$ L of RNase-free water. Each sample was evaluated by nanodrop for its purity and concentration.

## Complementary DNA (cDNA) synthesis

After RNA extraction, cDNA was generated using Qiagen cDNA RT2 First Strand Kit. All reverse transcription quantitative polymerase reaction (RT-qPCR) was set up on ice. For each sample, 100 ng of total RNA and 6  $\mu$ L buffer GE2 completed with RNase-free water to 14  $\mu$ L of final volume was incubated at 37°C for 5 min and then kept on ice for 1 min. RT procedure was completed in a total volume of 20  $\mu$ L with the addition of 6  $\mu$ L BC5 to each 14  $\mu$ L of the mixture at 42°C for 15 min and 95°C for 5 min. Thereafter, the cDNA was diluted with 80  $\mu$ L nuclease-free water for the later use in qPCR.

## RT-PCR

Expression levels of serum lncRNAs were analyzed using Rotor-Gene® Q instrument with 2.1.0.9 software and QuantiTect SYBR Green PCR Kit (Qiagen, Germany). qPCR was performed in duplicates, including RT controls to evaluate DNA and nontemplate controls to avoid background signal. The qPCR reaction was set up with minimal changes according to the manufacturer's instructions as follows: 5  $\mu$ L 2x QuantiTect SYBR Green Master Mix, 1  $\mu$ L 10x miScript Universal Primer, 1  $\mu$ L 10x primer assay, 1  $\mu$ L RNase-free water and 2  $\mu$ L of cDNA. Reaction mixture was prepared in 0,1 mL strip tubes and caps (Qiagen, Germany) in a total volume of 10  $\mu$ L for each reaction. The following conditions were used for qRT-PCR to amplify the lncRNAs: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 55°C for 30 s and 72°C for 15s. Finally, melt analysis from 55 to 95°C temperature in Rotor-Gene Q instrument with 72 well plate was performed.

## Analysis of lncRNA qPCR Data

The relative expression level of each lncRNA was calculated according to the cycle threshold (CT) value by using  $2^{-\Delta\Delta CT}$  method [32]. The lncRNA CT levels were exported appropriately into Qiagen GeneGlobe Data Analysis Center. CT cut off value was set to 40. For normalization,  $\beta$ -actin was chosen appropriately. Results were obtained using the Qiagen Data Analysis Center web tool.

## Statistical analysis

All measured variables were subjected to normality testing using Shapiro–Wilk normality test. Descriptive values were expressed as mean  $\pm$  standard deviation (SD), median (25–75% quartiles) or count and percent frequencies. Independent samples t-test was used to compare differences between PD patients and control group with regard to numerical variables. The relationships between the categorical variables were evaluated by using Chi-squared test, or Fischer exact test. Multivariate binary logistic regression was performed to identify the significant predictors of PD risk. A  $p$ -value of less than 0.05 was considered statistically significant. Statistical calculations were performed using SPSS (ver. 23).

## Results

### Demographic and clinical features of the PD patients and healthy controls

A total of 133 participants, including 83 PD patients and 50 healthy controls, were included in the study. The demographic profile of the patients and controls and clinical findings are described in Table 1. No significant difference was observed in age ( $p = 0.283$ ), gender ( $p = 0.398$ ) and presence of hypertension ( $p = 0.236$ ) between PD and control groups. But duration of education was found significantly longer in control group than PD patients ( $p = 0.024$ ). All participants were right-handedness and there were no alcohol users.

Table 1  
Demographic and clinical profile of the patients with PD and controls

Variable	PD group	Control group	P-value
Age (years) <sup>a</sup>	63 ± 8.75	61.34 ± 8.32	0.283
Gender, male (%)	51 (61)	27 (54)	0.398
Education (years) <sup>a</sup>	7.23 ± 3.48	8.82 ± 4.10	0.024*
History of hypertension (%)	28 (66)	12 (24)	0.236
Disease duration (years) <sup>a</sup>	8.04 ± 4.14		
HY stage <sup>a</sup>	2.13 ± 0.65		
LEDD (mg/day) <sup>a</sup>	933.84 ± 466.59		
UPDRS-I <sup>a</sup>	1.35 ± 1.23		
UPDRS-II <sup>a</sup>	9.16 ± 5.16		
UPDRS-III <sup>a</sup>	13.14 ± 6.56		
UPDRS-IV <sup>a</sup>	2.60 ± 3.49		
UPDRS-Total <sup>a</sup>	26.28 ± 13.57		
<i><sup>a</sup> data are presented as the mean ± SD</i>			
<i>* Significance at p &lt; 0.05</i>			
Abbreviations: PD, Parkinson's disease; HY, Hoehn & Yahr stage score; LEDD, levodopa equivalent daily dose; UPDRS, Unified Parkinson's Disease Rating Scale			

51 (61%) of these 83 PD patients were suffering from a tremors-rigid form of the disease and this tremors-rigid form had a right sided onset in 61% of them. Besides, 66% of patients with a bradykinesia form of PD had a right-sided onset, too. 12 (15%) of the patients had a family history of PD. According to HY stages, patients were distributed as follows: 13 (16%) patients in stage 1; 46 (55%) in stage 2 and 24 (29%) in stage 3. All of the patients had used dopaminergic treatment. Only three patients were not treated with dopamine agonists and 61 (74%) had received pramipexole treatment, while the others with ropinirole. 10 (12%) of the patients had dopamine dysregulation syndrome, 28 of them (34%) dyskinesia and 36 (43%) of them motor fluctuations.

## Serum expression levels of lncRNAs H19, GAS5, HAR1B and LINC01783

The normalized value of 3 PD patients and 3 controls could not be obtained from the laboratory analysis so they were estimated by the missing value method. Comparison of normalized values of PD group ( $27.44 \pm 2.893$ ) to control group ( $27.60 \pm 2.027$ ), no significant difference was found between the two groups ( $p = 0.697$ ). This result desired further analysis to be performed. Distribution of normalized values in PD patients and control groups was given in Figure 1. When figure is examined, it is seen that the normalized values in the controls and PD group show a slightly right-skewed distribution.

Relative serum expression levels ( $2^{-\Delta\Delta CT}$ ) of four lncRNAs; H19, GAS5, HAR1B and LINC01783 in PD patients and control groups are given in Table 2. No significant difference was observed in the levels of these lncRNAs between the two groups.

Table 2  
Relative serum expression levels of H19, GAS5, HAR1B and LINC01783 in the PD patients and control groups.

<b>lncRNA</b>	<b>N</b>	<b>PD group</b>	<b>N</b>	<b>Control group</b>	<b>P-value*</b>
<b>H19</b>	83	0.03 (0.01-0.17)	50	0.03 (0.01-0.10)	0.352
<b>GAS5</b>	83	0.07 (0.03-0.23)	50	0.13 (0.05-0.30)	0.592
<b>HAR1B</b>	25	0.06 (0.02-0.14)	12	0.01 (0.00-0.07)	0.117
<b>LINC01783</b>	33	0.03 (0.01-0.20)	26	0.03 (0.00-0.09)	0.253
<i>Data are expressed as median (25–75% percentiles).</i>					
<i>*: Independent samples t-test</i>					

Since the normalized value of 8 PD patients and 3 healthy controls couldn't be obtained from the laboratory analysis, they were estimated by the missing value method. The two groups were compared in terms of these four lncRNAs and no significant difference was found (Table 3).

Table 3  
The mean levels of lncRNAs that normalized value below 30 in PD and control groups.

lncRNA	Group	N	Mean $\pm$ SD	P-value*
<b>H19</b>	Control	47	0.100 $\pm$ 0.256	0.764
	PD	75	0.115 $\pm$ 0.262	
<b>GAS5</b>	Control	47	0.257 $\pm$ 0.571	0.832
	PD	75	0.232 $\pm$ 0.648	
<b>HAR1B</b>	Control	12	0.040 $\pm$ 0.068	0.444
	PD	22	0.388 $\pm$ 1.546	
<b>LINC01783</b>	Control	25	0.139 $\pm$ 0.464	0.499
	PD	29	0.078 $\pm$ 0.124	
*: Independent samples t-test				

*Correlation analysis between the serum levels of H19, GAS5, HAR1B and LINC01783 in the PD patients*

There was a significant positive correlation between H19 and GAS5 and LINC01783. In addition, a significant positive correlation was found between GAS5 and HAR1B and LINC01783. Apart from that, no meaningful relationship was found between other lncRNAs in PD patients (Table 4).

Table 4  
Correlation analysis between the serum levels of H19, GAS5, HAR1B and LINC01783 in the PD patients.

Correlation analysis	H19	GAS5	HAR1B	LINC01783
<b>H19</b>		r = 0.441 <i>p</i> < 0.001**	r = 0.072 <i>p</i> = 0.751	r = 0.626 <i>p</i> < 0.001**
<b>GAS5</b>			r = 0.530 <i>p</i> = 0.011*	r = 0.598 <i>p</i> < 0.001**
<b>HAR1B</b>				r = 0.164 <i>p</i> = 0.631
<b>LINC01783</b>				
<i>Data were analyzed by Spearman (for non-parametric data) and Pearson (for parametric data) correlation.</i>				
* Significance at <i>p</i> < 0.05; ** Significance at <i>p</i> < 0.001				

The relationship between disease duration, onset sign and lateralization, positive family history, disease severity (HY score) and clinical status (UPDRS score), LEDD in patients and lncRNA H19, GAS5, HAR1B and LINC01783 levels were examined. The results showed that the relative expression of lncRNA GAS5 levels were significant negatively correlated with HY and UPDRS II, III and total scores ( $r = -0.243, p = 0.027$ ;  $r = -0.286, p = 0.009$ ;  $r = -0.232, p = 0.035$ ;  $r = -0.225, p = 0.041$ , respectively). No significant relationship was found between these four lncRNAs and other characteristics of the PD. LINC01783 was found to be significantly higher in those with a positive family history ( $p = 0.047$ ). However, the other 3 lncRNA levels were found in similar amounts in patients with and without family history.

## Logistic regression analysis to predict PD risk

Considering the age, gender, duration of education and presence of hypertension in PD patients and controls together with lncRNA H19 and GAS5, re-evaluation was performed with a multivariate logistic regression model and the results shown in Table 5. lncRNA HAR1B and LINC01783 were not included in the model as there were many unmeasurable values in the laboratory analysis (25 PD patients and 12 controls for HAR1B, 33 patients and 26 controls for LINC01783). We found that male individuals had significantly higher risk for PD. In addition, as the duration of education increases, the risk of PD decreases. lncRNA H19 and GAS5 do not appear to have a discriminating role between patient and control groups.

Table 5  
Logistic regression analysis of H19, GAS5 and risk factors for PD

Variable	Coefficient ( $\beta$ )	SE	Wald ( $\chi^2$ )	P-value	Odds Ratio	95% CI
H19	0.151	0.289	0.272	0.602	1.163	0.66-2.04
GAS5	0.007	0.021	0.108	0.742	1.007	0.96-1.04
Age	0.015	0.025	0.350	0.554	1.015	0.96-1.06
Gender (Male / Female)	0.860	0.453	3.599	<b>0.050*</b>	2.362	0.97-5.74
Education (years)	-0.149	0.056	7.123	<b>0.008*</b>	0.861	0.77-0.96
Hypertension (present / absent)	0.608	0.460	1.746	0.186	1.837	0.74-4.52
<i>* Significance at <math>p &lt; 0.05</math></i>						
Abbreviations: SE, standard error						

After the individuals with normalized values above 30 were excluded from the data, correlation between the age, gender, duration of education, presence of hypertension and lncRNA H19 and GAS5 were evaluated with the multivariate logistic regression model and the results were given in Table 6. Same results were found. Since there are many unmeasurable values of the HAR1B and LINC01783, they were not included in the model.

Table 6

Logistic regression analysis of H19, GAS5 and risk factors for PD group that normalized value below 30

Variable	Coefficient ( $\beta$ )	SE	Wald ( $\chi^2$ )	P-value	Odds Ratio	95% CI
H19	0.942	0.966	0.951	0.329	2.566	0.38-17.04
GAS5	-0.024	0.373	0.004	0.950	0.977	0.47-2.02
Age	0.022	0.026	0.721	0.396	1.022	0.97-1.07
Gender (Male / Female)	0.641	0.460	1.947	0.163	1.899	0.77-4.67
Education (years)	-0.160	0.057	7.732	<b>0.005*</b>	0.852	0.76-0.95
Hypertension (present / absent)	-2.646	1.752	2.281	0.131	0.071	0.65-4.19
<i>* Significance at <math>p &lt; 0.05</math></i>						
Abbreviations: SE, standard error						

## Discussion

Although there has been a significant progress to understand the mechanisms which lead to PD, it is still challenging to determine specific biomarkers enabling accurate diagnose of the disease, classification and risk factors and predict probable patients [33]. Accumulation of a large number of pathological features  $\alpha$ -synuclein, Lewy bodies and other proteins resulted from abnormal proteasome function, mitochondrial dysfunction, oxidative stress, calcium homeostasis, synaptic transmission and neuroinflammation has been shown in multiple studies [4, 5, 15, 34, 35]. The combined action of these mechanisms causes autophagy and apoptosis of dopaminergic neurons in the SNpc, promoting the development of PD [12, 36]. There has been accumulating significant evidence of the important role of lncRNAs in these activities of PD pathogenesis [6, 12, 15]. It is generally perceived that diagnosis and treatment of the disease will be facilitated by detecting lncRNAs' profiles that extracted from serum, plasma, CSF, saliva and tissue samples [24]. In this study, we studied to determine the levels of four of these lncRNAs H19, GAS5, HAR1B and LINC01783 in the sera of PD patients.

The ubiquitin-proteasome system (UPS), a nonlysosomal pathway of protein degradation, removes damaged mutant and aberrant proteins in cells, regulates cell cycle DNA damage and repairs apoptosis.

UPS dysfunction causes abnormal accumulation of protein within the cell and gets an important role in the pathogenesis of PD [12, 37]. Reduced mitochondrial activity promotes free radical formation and enhances the susceptibility of tissues to oxidative stress, resulting in damage to cellular DNA, lipids and proteins. It has been shown that PD patients have high oxidative stress in the brain, meanwhile dopaminergic neurons don't have the ability to control oxidative stress. The process of apoptosis is highly related to mitochondrial dysfunction and oxidative stress, so plays an important role in death of the dopaminergic neurons in the disease [38]. Autophagy-mediated-protein degradation is a process in which the proteins are engulfed within vesicles that fuse with the lysosomes to degrade proteins. Enhanced autophagy can effectively counter PD [12]. Neuroinflammation is so important in PD. The inflammatory cytokines, affecting the integrity of the blood-brain barrier and synaptic plasticity, accelerate the aging and degeneration of dopaminergic neurons [12]. To clarify the pathogenesis of PD and discover new therapeutic targets, it is clearly important to elucidate lncRNA-mediated regulation of the  $\alpha$ -synuclein that linked to some mechanisms such as abnormal modification of  $\alpha$ -synuclein after translation, aggregation of  $\alpha$ -synuclein, toxic effects and degradation [39]. During the inhibition of  $\alpha$ -synuclein toxicity by the traditional Chinese medicine *Acanthopanax senticosus* (AS), a commonly used agent, 341 lncRNAs were differentiated under the stimulation of  $\alpha$ -synuclein in microarray expression analysis. 29 of these lncRNAs were involved in the inhibition of  $\alpha$ -synuclein neurotoxicity mechanism mediated by AS and 19 were potentially related to  $\alpha$ -synuclein neurotoxicity [40]. The potential mechanisms of lncRNAs include the inhibition of PD-linked genes' expressions, the reduction in production of  $\alpha$ -synuclein, the maintenance of autophagy system balance, the delay in the apoptosis of dopaminergic neurons, the alleviation of nerve inflammation and so forth. All these findings indicate that lncRNAs have the potential to become a biomarker and therapeutic target for PD.

Soreq et al in 2014, for the first time, utilized a whole-transcriptome RNA sequencing to determine all the transcripts that code proteins in leukocyte and lncRNAs in PD patients and controls. However, they couldn't fully elucidate the detailed functions of the identified lncRNAs. 13 lncRNAs showed differentiated expression levels and selective PD-induced alteration. These researchers also found 4 of these lncRNAs were reversed after deep brain stimulation treatment. Comparisons of the peripheral blood and brain tissue samples of the PD patients and healthy individuals reveal common differences in lncRNAs, with the same expression trends. In this study it was stated that U1 levels (ENST00000415386 which is LINC01738) were differentiated in PD patients' amygdala and leukocytes [8].

A recent study found that PD-related genes associated with lncRNAs decreased in the SN and cerebellum of patients, just as consistent with the results obtained in peripheral blood cells [41]. lncRNAs in the CSF of patients with PD have a higher frequency compared with controls, corroborating previous reports that various lncRNAs performed essential functions in the regulation of PD progression [42]. Both animal and cell models are commonly used in PD researches. Abnormal expression of approximately 756 lncRNAs was detected in the SNpc of the presymptomatic mice. Although these studies cannot exactly replicate the pathological changes in human PD patients, 87 of lncRNAs had significant differentiations in the expression in the SN of PD patients, when compared to the normal tissues [43].

Kraus and colleagues studied lncRNA expression levels in brain tissue of postmortem 20 PD patients and 10 healthy controls [10]. It was identified that GAS5 and HAR1B were so abundant and expressed in brain samples and so, they were used as normalizers. They found that only 5 patients had significant alterations when compared to controls, out of 90 non-coding transcripts investigated in the study. lncRNA H19 upstream conserved regions 1 and 2 were downregulated significantly [10]. Besides, it was shown in recent studies lncRNA H19 can play protective roles against dopaminergic neuronal loss and apoptosis in mice models with PD [13, 17]. lncRNA H19 regulates the p53/Notch1 pathway to inhibit neurogenesis in ischemic stroke [44]. H19 is one of the first lncRNAs, linked to different types of cancer, such as bladder cancer, colorectal cancer, and hepatocellular carcinoma [45]. Moreover, lncRNA H19 regulates neuronal apoptosis in AD, suggesting that the regulation of lncRNA networks have unneglectable influence on AD pathology, that is they may lighten the unclear etiology of AD and lead current drug therapy which is unsatisfactory [46].

GAS5, as a member of the lncRNA family, is located on chromosome 1 of the human genome. Studies have shown that GAS5 is abnormally expressed in many tumor disorders and plays an oncogene role by inhibiting apoptosis. Moreover, GAS5 also takes place in the development of inflammation-related disorders [16]. There have been some studies investigating its role in neurological disorders such as ischemic stroke and AD. Acetylcholine release has a related role in the cholinergic nervous system of AD patients [47]. Microglia-induced neuroinflammation plays a significant role in PD pathogenesis. In another study, it has been shown that GAS5 can activate microglia and increase the expression level of inflammatory cytokines [48]. All these findings highly suggest GAS5 involvement in PD development, but further studies are still needed for GAS5 role in PD.

As being confirmed, lncRNAs are expressed in many regions of the brain [9]. The study demonstrated that HAR1 specifically expressed in Cajal-Retzius neurons in the development human neocortex during a period of 7 to 19 gestational weeks, a crucial time for cortical neuron specification and migration [21]. It upregulates reelin. lncRNA LINC01783 (Gene ID: 100132147) (ENST00000415386) locates in the 1p36.13 region of human genome. Underlying its importance in cancer, in a recent study it was reported that LINC01783 had an association with the proliferation, migration, and invasion of cervical cancer cells [25].

No significant difference was shown when the expression levels of four candidate lncRNAs, H19, GAS5, HAR1B and LINC01783 in the sera of PD patients were studied in comparison to those of healthy controls. However, it was found that there was solely a significant negative correlation between GAS5 and HY stage and UPDRS II, III and total scores when the relationship between the expressed levels of these four lncRNAs were examined in age, gender, duration of education, disease duration, disease onset finding, HY stage, UPDRS scores and treatment doses. Even though this parameter was suggested to have a role in inflammation in other previous studies, in our study we revealed that it was associated with the clinical severity of the disease and there was no correlation with other lncRNAs' levels. Other studies did not report any significant relationship in the literature [10, 13, 15, 16]. It was found that LINC01783 lncRNA was significantly higher only in PD patients with positive family history.

In conclusion, as we know this is the first clinical study in order to explore the expression levels of serum lncRNAs in Turkish PD patients. It may help to understand PD pathogenesis better and more in detail to search serum lncRNAs levels, but there have been few studies in the literature so far. It can suggest important contribution to the development of potential biomarkers for PD and also the identification of new therapeutic targets, although the mechanisms by which lncRNAs role in complex physiological and pathological cases such as PD have not been elucidated fully yet.

## Declarations

**Disclosure:** The authors report no conflict of interest.

**Author contributions** B Ozdilek and B Demircan contributed in study concept and design; T Tombul provided the funding; B Ozdilek contributed in acquisition of data; B Demircan and IA Kaya contributed laboratory analysis, H Ankarali performed statistical analysis; B Ozdilek wrote the first draft; all authors contributed to the analysis and interpretation of data, critical revision of the manuscript for important intellectual content and approval of the final draft.

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**Availability of Data and Materials** All data generated or analyzed during this study are available from the corresponding author on reasonable request.

**Ethics Approval and Consent to Participate** All procedures performed in this study were approved by Goztepe Training and Research Hospital Institutional Review Boards (2019/0223), and the informed consent was obtained from all the participants.

**Conflict of interest** The authors declare no conflicts of interest.

**Consent for Publication** Not applicable.

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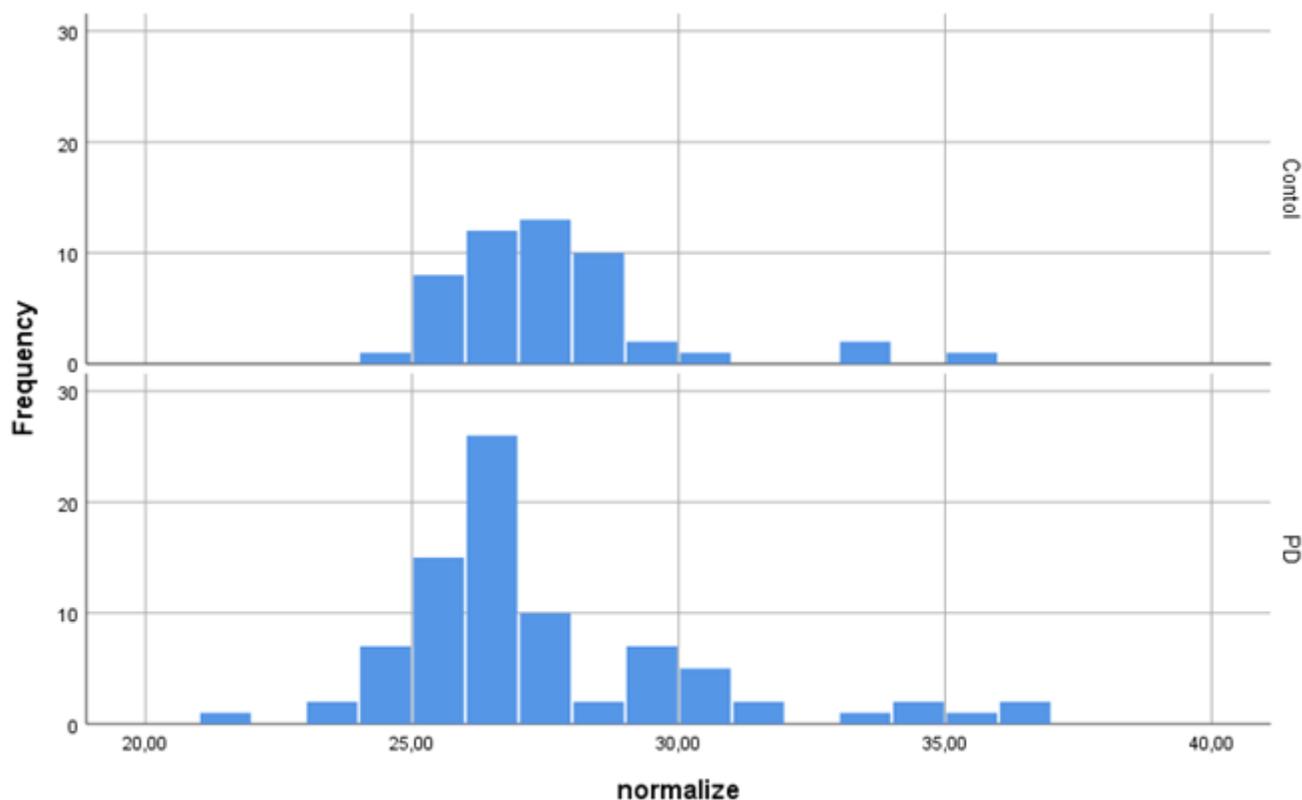
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## Figures



## Figure 1

Distribution of normalized values in PD patients and control groups