EREDETI KÖZLEMÉNY

ORIGINAL ARTICLE

Clinical significance of serum lncRNA H19, GAS5, HAR1B and linc01783 levels in Parkinson's disease

Ozdilek BETUL[1, 2], Ibrahim Alper KAYA[3], Berna DEMIRCAN[3], Temel TOMBUL[1, 2], Handan ANKARALI[4]

[1]Department of Neurology, Istanbul Medeniyet University Faculty of Medicine, Istanbul, Turkey
[2]Clinic of Neurology, Ministry of Health Goztepe Training and Research Hospital, Istanbul, Turkey
[3]Department of Medical Biology, Istanbul Medeniyet University Faculty of Medicine, Istanbul, Turkey
[4]Department of Biostatistics, Istanbul Medeniyet University Faculty of Medicine, Istanbul, Turkey

PRINT | English | https://doi.org/10.18071/isz.76.0189 | www.elitmed.hu

Correspondent:
Betul OZDILEK, MD,
Ministry of Health Goztepe Training and Research Hospital Neurology Clinic
Egitim Mah. Doktor Erkin Cad. 34722 Kadikoy, Istanbul, Turkey
Phone: +90 216 606 52 10
E-mail address: ozdilekbetul@gmail.com
https://www.orcid.org/0000-0003-1608-9882

Érkezett:
2023. január 6.
Elfogadva:
2023. március 15.

Background and purpose – Long noncoding RNAs (lncRNAs) are highly expressed in the 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. Our purpose was to detect whether the serum levels of four candidate lncRNAs H19, GAS5, HAR1B and LINC01783 are related with the clinical findings and treatment of PD or not.
Methods – 83 patients and 50 healthy controls were included in this study. We assessed how severe the disease is, by using Hoehn Yahr (HY) staging and Unified PD rating scale (UPDRS). Venous blood samples were taken from the participants. Serum samples were centrifuged and stored at -80°C until analysis. Expression levels of these lncRNAs were analyzed by a real-time PCR instrument after RNA isolation and complementary DNA synthesis in the laboratory.
Results – There was no significant difference between PD patients and healthy controls in these lncRNAs' serum levels. Just as sociodemographic characteristics, also onset type and right or left predominance of the disease, its duration and treatment did not differ in lncRNA levels. Solely, there was a significant negative correlation between GAS5 and HY and UPDRS scores. Patients with family history of PD had significantly higher levels of LINC01783.

A szérum lncRNS H19-, GAS5-, HAR1B- és LINC01783-szintjeinek klinikai jelentősége Parkinson-kórban

Ozdilek B, MD; Kaya IA; Demircan B; Tombul T, MD; Ankarali H

Háttér és cél – A hosszú nem kódoló RNS-ek (lncRNS-ek) nagymértékben kifejeződnek az agyban, és szintjük változását számos neurodegeneratív betegségben kimutatták. Bizonyított, hogy az lncRNS-ek szerepet játszanak a Parkinson-kór (PD) kialakulásában és progressziójában, és potenciális terápiás célpontként használhatók. Célunk annak kimutatása volt, hogy a négy lncRNS, a H19, a GAS5, a HAR1B és a LINC01783 szérumszintje összefügg-e a Parkinson-kór klinikai tüneteivel és kezelésével, vagy sem.
Módszerek – A vizsgálatba 83 beteget és 50 egészséges kontrollt vontunk be. A betegség súlyosságát a Hoehn–Yahr- (HY-) stádiumbeosztás és az egységes PD minősítési skála (UPDRS) segítségével vizsgáltuk. A résztvevőktől vénás vérmintát vettünk. A szérummintákat centrifugáltuk és –80 °C-on tároltuk az elemzésig. Az lncRNS-ek expressziós szintjét valós idejű PCR-rel elemeztük az RNS izolálása és a laboratóriumban végzett komplementer DNS-szintézis után.
Eredmények – Nem volt szignifikáns különbség a Parkinson-kóros betegek és az egészséges kontrollok között ezeknek az lncRNS-eknek a szérumszintjében. A szociodemográfiai jellemzőkhöz hasonlóan a betegségkezdet típusa, jobb vagy bal oldali túlsúlya, fennállásának időtartama és kezelése sem különbözött az lncRNS-szintek alapján. Egyedül a GAS5 és a HY, valamint az UPDRS-pontszámok között volt szignifikáns negatív korreláció. A családi anamnézisben

Conclusion – Serum lncRNA GAS5 level may be a possible biomarker for disease severity in PD patients.

PD-vel rendelkező betegeknél szignifikánsan magasabb volt a LINC01783 szintje.
Következtetés – A szérum-lncRNS GAS5-szintje a betegségsúlyosság lehetséges biomarkere lehet Parkinson-kóros betegeknél.

Keywords: Parkinson's disease, neurodegeneration, serum lncRNA, biomarker

Kulcsszavak: Parkinson-kór, neurodegeneráció, szérum-lncRNS, biomarker

Parkinson's disease (PD) is the second most common progressive neurodegenerative movement disorder after Alzheimer's disease (AD) in the world. It is clinically characterized by 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. The prevalence is predicted as to be nearly double in the following 30 years when the aging population is considered especially to be at the point of the extended life expectancy[1]. 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 α-synuclein, Lewy bodies and other proteins[2]. Studies have identified a variety of molecular mechanisms involved in PD pathogenesis, including abnormal ubiquitin-proteasome system function, mitochondrial dysfunction, oxidative stress, dysfunction of calcium homeostasis and synaptic transmission, and neuroinflammation. The combined action of these mechanisms causes toxic effects, autophagy and apoptosis of dopaminergic neurons in the SNpc[2, 3]. In recent years, long noncoding RNAs (lncRNAs) have gained more importance in studies of PD pathogenesis[3–13]. lncRNAs are a class of noncoding RNAs that consists more than 200 nucleotides in length and represents one of the largest fractions in the human genome. Studies have shown that lncRNAs interact with DNA, RNA and protein molecules to regulate gene expression at the epigenetic, transcriptional and posttranscriptional levels in cellular homeostasis[3]. lncRNAs are highly expressed in various parts of the central nervous system (CNS) and alterations in their levels have been shown in many neurodegenerative disorders such as AD, PD, Huntington's disease, amyotrophic lateral sclerosis and stroke[14–16]. Accumulating evidence has shown that lncRNAs play role in the onset and progression of PD and they 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[15]. Serum samples, easily accessible by minor invasive procedures, offer the possibility of a cheap, fast and quick way in the experimental studies of lncRNAs.

Soreq et al. in 2014, for the first time, utilized a whole-transcriptome RNA sequencing to determine all the transcripts that code proteins in leukocytes and lncRNAs in PD patients and controls. 13 of lncRNAs showed differentiated levels of expression in PD[4]. It is so clear that the number of lncRNAs reported in PD has significantly increased in recent years[4–8, 10, 12]. 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[14]. When reviewed literature, we decided to select and analyze four of these lncRNAs; H19, GAS5, HAR1B and LINC01783 (Gene ID: 100132147, ENST00000415386) which have been implicated in PD. lncRNA H19 is seen to be associated with PD pathogenesis and progression. It is reported to be protective against apoptosis[9, 11]. lncRNA GAS5 has also been shown to be associated with inflammatory responses[13]. So GAS5, HAR1B and LINC01783 are abundant and expressed in brain samples[6]. In this study, we aimed to analyze serum expression levels of these four lncRNAs in patients with PD and to search whether those are associated with motor, non-motor symptoms, treatment and prognosis of the disease.

Materials and methods

Participants

83 PD patients who were followed up in outpatient clinic and 50 healthy controls, matched for age and gender, were included in the study. All participants agreed and signed a written informed consent before enrollment. The controls were recruited among 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[17]. The patients have had this diagnosis for at least two years and have been taking antiparkinsonian treatment regularly for the last six months. Demographic information, clinical findings and scales were recorded in the databank at each follow-up. Data collection included demographic and clinical information such as age, gender, years of education, duration of disease and treatment, family history of PD and levodopa equivalent daily dose (LEDD, mg/day)[18]. Exclusion criteria were as follows: (1) presence of other neurological disorder; (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 and psychosis diagnosed with a structured clinical interview; (5) 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 to have negative family history of movement disorders. We only included hypertension as comorbidity for the whole of participants.

Assessment of the clinical findings of 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 signs of disease (bradykinesia or tremor) and their lateralization (left or right) and presence of postural instability and gait dysfunction were noted. Disease severity was measured using the Hoehn and Yahr (HY) staging scale and Unified PD Rating Scale (UPDRS). HY scale provides assessment of disease progression through a staging which ranges from 0 (no sign of disease) to 5 (severe). PD usually starts from unilateral (Stage 1), to bilateral form without balance difficulties (Stage 2), followed by the presence of postural instability (Stage 3), the loss of physical independence (Stage 4), and being wheel-chair or bedbound unless aided (Stage 5). HY scale had also been used to categorize PD as early stage (Stage 1 and 2), moderate stage (Stage 3) and late stage (Stages 4 and 5)[19]. UPDRS provides a comprehensive assessment of disability and impairment by evaluating the most pertinent clinical findings of PD. The scale consists of 42 items in four parts. Part I assesses the mental function and mood, and contains 4 items. Part II concerns motor activities of daily living, and contains 13 items. Part I and II were evaluated completely as a patient questionnaire. Part III is motor examination, containing 14 items and was evaluated completely by a specialist. Part IV assesses motor complications, containing 11 items and was evaluated by a specialist once a PD subject had started PD medication. Part IV assesses two motor complications: dyskinesias and motor fluctuations which are strongly related to the duration of the disease, levodopa dose and the duration of levodopa treatment. PD patients rated items on a Likert scale ranging from 0 as normal: symptom not present to 4 as severe: symptom is 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[20]. All patients were using dopamine agonist therapy.

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 1 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 µL of QIAzole lysis reagent was added to 100 µL of the serum sample and the whole reaction mixture was incubated for 5 min at room temperature. Then, 100 µL chloroform was added to the lysate tube, vortexed for 15 s and was incubated for 2 min at room temperature. Thereafter, we performed centrifugation at 12000×g for 15 min at 4°C. ~ 300 µL of the upper aqueous phase was removed and the mixture was transferred to a new collection tube, then 450 µL of 100% ethanol was added. Then, 700 µL of the mixture was added to the RNeasy MinElute spin column in a 2 mL collection tube and was centrifuged at 8000×g for 15 sec at room temperature. After the mixture had been completely transferred to the column, we added 700 µl of buffer RWT to each column and centrifuged at 8000×g for 15 sec at room temperature. Next, 500 µl buffer RPE was added and centrifuged at 8000×g for 15 sec. Finally, 500 µL of 80% ethanol prepared with RNase-free water was added to the column and centrifuged at 8000×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 µL 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 µL buffer GE2 completed with RNase-free water to 14 µl 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 µL with the addition of 6 µL BC5 to each 14 µL of the mixture at 42°C for 15 min and 95°C for 5 min. Thereafter, the cDNA was diluted with 80 µL 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 QuantiTech 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 µL 2× QuantiTect SYBR Green Master Mix, 1 µL 10× miScript Universal Primer, 1 µL 10× primer assay, 1 µL RNase-free water and 2 µL of cDNA. Reaction mixture was prepared in 0,1 mL strip tubes and caps (Qiagen, Germany) in a total volume of 10 µL 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 sec, 55 °C for 30 sec and 72 °C for 15 sec. 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[21]. lncRNA CT levels were exported appropriately into Qiagen GeneGlobe Data Analysis Center. CT cut off value was set to 40. For normalization, β-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 ± standard deviation, 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 findings 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 the 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 the control group than

Table 1. *Demographic and clinical profile of patients with PD and controls*

Variable	PD group	Control group	P-value
Age (years)[a]	63 ± 8.75	61.34 ± 8.32	*0.283*
Gender, male (%)	51 (61)	27 (54)	*0.398*
Education (years)[a]	7.23 ± 3.48	8.82 ± 4.10	*0.024**
History of hypertension (%)	28 (66)	12 (24)	*0.236*
Disease duration (years)[a]	8.04 ± 4.14		
HY stage[a]	2.13 ± 0.65		
LEDD (mg/day)[a]	933.84 ± 466.59		
UPDRS-I[a]	1.35 ± 1.23		
UPDRS-II[a]	9.16 ± 5.16		
UPDRS-III[a]	13.14 ± 6.56		
UPDRS-IV[a]	2.60 ± 3.49		
UPDRS-Total[a]	26.28 ± 13.57		

[a] data are presented as the mean ± SD
* Significance at $p < 0.05$
Abbreviations: PD: Parkinson's disease; HY: Hoehn & Yahr stage score; LEDD: levodopa equivalent daily dose; UPDRS: Unified Parkinson's Disease Rating Scale

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

lncRNA	N	PD group	N	Control group	P-value*
H19	83	0.03 (0.01–0.17)	50	0.03 (0.01–0.10)	0.352
GAS5	83	0.07 (0.03–0.23)	50	0.13 (0.05–0.30)	0.592
HAR1B	25	0.06 (0.02–0.14)	12	0.01 (0.00–0.07)	0.117
LINC01783	33	0.03 (0.01–0.20)	26	0.03 (0.00–0.09)	0.253

Data are expressed as median (25–75% percentiles).
*: Independent samples t-test
Abbreviation: N: number

among PD patients (p = 0.024). All participants were right-handed.

51 (61%) of the 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 patients had used dopaminergic treatment. Only 3 patients were not treated with dopamine agonists and 61 (74%) had received pramipexole treatment, while the others 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

Relative serum expression levels ($2^{-\Delta\Delta CT}$) of lncRNAs H19, GAS5, HAR1B and LINC01783 in PD patient and control groups are given in **Table 2**. HAR1B and LINC01783 had many unmeasurable values in the serum samples. 25 PD patients and 12 controls for HAR1B, 33 patients and 26 controls for LINC01783 had measurable values in the laboratory analysis. No significant difference was observed in the levels of these lncRNAs between the two groups.

For 3 PD patients and 3 controls the normalization value using β-actin could not be obtained from the laboratory analysis so they were estimated by the missing value method. At comparison of normalization values of PD group (27.44 ± 2.893) to control group (27.60 ± 2.027), no significant difference was found (p = 0.697). This result desired further analysis to be performed. Distribution of normalization values in PD patient and control groups is given in **Figure 1**. When figure is examined, it is seen that the normalization values in the control and PD groups show a slightly right-skewed distribution. Normalization values were mostly below 30. The mean levels of lncRNAs that normalization values below 30 in PD patient and control groups are given in **Table 3** and no significant difference was found.

Correlation analysis between 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

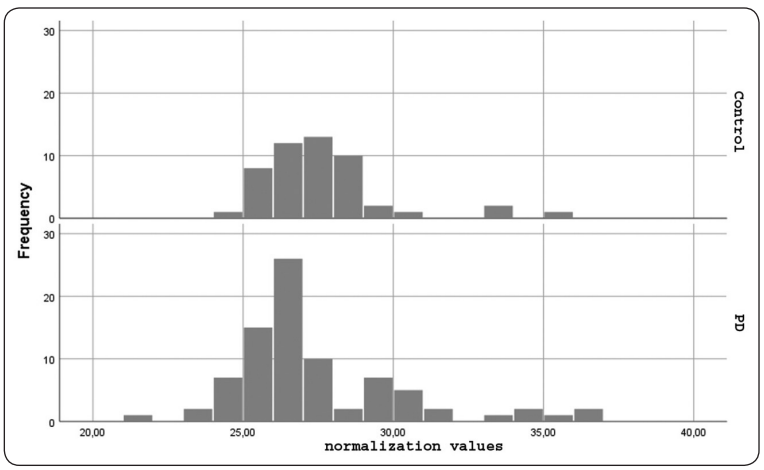

Figure 1. *Distribution of normalization values in PD patient and control groups*

relationship was found between other lncRNAs in PD patients **(Table 4)**.

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 shows that the relative expression of lncRNA GAS5 levels were significantly 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 positive family history ($p = 0.047$). However, the other three lncRNA levels were found in similar amounts in patients with and without positive family history.

Table 3. *The mean levels of lncRNAs that normalization values below 30 in PD and control groups*

lncRNA	Group	N	Mean ± SD	P-value*
H19	PD	75	0.115 ± 0.262	*0.764*
	Control	47	0.100 ± 0.256	
GAS5	PD	75	0.232 ± 0.648	*0.832*
	Control	47	0.257 ± 0.571	
HAR1B	PD	22	0.388 ± 1.546	*0.444*
	Control	12	0.040 ± 0.068	
LINC01783	PD	29	0.078 ± 0.124	*0.499*
	Control	25	0.139 ± 0.464	

*: Independent samples t-test
Abbreviation: N: number

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

Correlation analysis	H19	GAS5	HAR1B	LINC01783
H19		r = 0.441 p < 0.001**	r = 0.072 p = 0.751	r = 0.626 p < 0.001**
GAS5			r = 0.530 p = 0.011*	r = 0.598 p < 0.001**
HAR1B				r = 0.164 p = 0.631
LINC01783				

Data were analyzed by Spearman (for non-parametric data) and Pearson (for parametric data) correlation.
* Significance at p < 0.05; ** Significance at p < 0.001

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 are 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 patients and controls.

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

Discussion

Although there is a significant progress to understand the mechanisms which lead to PD, it is still challenging to determine specific biomarkers enabling accurate diagnosis, classification and risk factors of the disease, and prediction of probable patients. All these findings indicate that detecting lncRNAs' profiles have the potential to become a biomarker for diagnosis, prognosis and therapeutic target for PD[8, 10, 12, 16]. In this study, we determined the levels of lncRNAs H19, GAS5, HAR1B and LINC01783 in the sera of PD patients.

Studies have reported that lncRNAs are expressed in many regions of the brain[4, 5]. Soreq et al. reported that 13 lncRNAs showed differentiated levels of expression in PD patients and four of these lncRNAs' levels were reversed after deep brain stimulation treatment. In this study it was stated that U1 levels (ENST00000415386 which is LINC01738) were differentiated in PD patients' amygdala and leukocytes[4]. *Kraus* and colleagues studied lncRNA expression levels in brain tissue of 20 PD patients postmortem and 10 healthy controls. It was identified that GAS5 and HAR1B were abundant and expressed in brain samples, so they were used as normalizers[6]. In

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

Variable	Coefficient (β)	SE	Wald (x^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	*0.050**	2.362	0.97–5.74
Education (years)	–0.149	0.056	7.123	*0.008**	0.861	0.77–0.96
Hypertension (present / absent)	0.608	0.460	1.746	0.186	1.837	0.74–4.52

* Significance at $p < 0.05$
Abbreviations: SE: standard error

Table 6. *Logistic regression analysis of H19, GAS5 and risk factors for PD group that normalization values below 30*

Variable	Coefficient (β)	SE	Wald (x^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	*0.005**	0.852	0.76–0.95
Hypertension (present / absent)	–2.646	1.752	2.281	0.131	0.071	0.65–4.19

* Significance at $p < 0.05$
Abbreviations: SE: standard error

recent studies, it was shown that lncRNA H19 can play protective roles against dopaminergic neuronal loss and apoptosis in mice models with PD[9, 11]. 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 tumors and plays an oncogenic role by inhibiting apoptosis. Moreover, GAS5 also takes place in the development of inflammation-related disorders by activating microglia and increasing the expression levels of inflammatory cytokines. Microglia-induced neuroinflammation plays a significant role in PD pathogenesis[13]. All these findings highly suggest an involvement of GAS5 in PD development, but further studies are still needed to determine its whole role in the disease. It was reported that HAR1 specifically expressed in Cajal-Retzius neurons in the human neocortex during a period of 7 to 19 gestational weeks, a crucial time for cortical neuron specification and migration[15]. It upregulates reelin. A recent study found that PD-related genes associated with lncRNAs were decreased in the SN and cerebellum of patients, just as consistent with the results obtained in peripheral blood cells and CSF of patients with PD[23].

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 expression levels of these four lncRNAs were examined by 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. lncRNA GAS5 serum level may be a possible biomarker for disease severity in PD patients. Other studies in the literature did not report any significant relationship[6, 9, 12, 13]. 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. Searching serum lncRNAs levels may help to understand PD pathogenesis better and more in detail, but there have been few studies in the literature so far. It can be important contribution to the development of potential biomarkers for PD and also to the identification of new therapeutic targets, although the mechanisms by which lncRNAs play role in complex physiological and pathological cases such as PD have not been elucidated fully yet.

FUNDING – This study was supported by a grant from the Research Fund of Istanbul Medeniyet University (Grant number T-GAP-2019-1548).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE – All procedures performed in this study were approved by the Goztepe Training and Research Hospital Institutional Review Boards (2019/0223), and informed consent was obtained from all participants.

The manuscript was posted as a PREPRINT at Research Square (doi: 10.21203/rs.3.rs-1120131/v1).

CONFLICT OF INTEREST – The authors declare no conflicts of interest.

References

1. *Poewe W, Seppi K, Tanner CM, et al*. Parkinson disease. Nat Rev Dis Primers 2017;3:17013. https://doi.org/10.1038/nrdp.2017.13
2. *Michel PP, Hirsch EC, Hunot S*. Understanding dopaminergic cell death pathways in Parkinson disease. Neuron 2016;90:675-91. https://doi.org/10.1016/j.neuron.2016.03.038
3. *Wang DQ, Fu P, Yao C, et al.* Long non-coding RNAs, novel culprits, or bodyguards in neurodegenerative diseases. Mol Ther Nucleic Acids 2018;10:269-79. https://doi.org/10.1016/j.omtn.2017.12.011
4. *Soreq L, Guffanti A, Salomonis N, et al*. Long non-coding RNA and alternative splicing modulations in Parkinson's leukocytes identified by RNA sequencing. PLoS Comput Biol 2014;10:e1003517. https://doi.org/10.1371/journal.pcbi.1003517
5. *Majidinia M, Mihanfar A, Rahbarghazi R, et al*. The roles of non-coding RNAs in Parkinson's disease. Mol Biol Rep 2016;43:1193-204. https://doi.org/10.1007/s11033-016-4054-3
6. *Kraus TFJ, Haider M, Spanner J, Steinmaurer M, Dietinger V, Kretzschmar HA*. Altered long noncoding RNA expression precedes the course of Parkinson's disease-a Preliminary report. Mol Neurobiol 2017;54:2869-77. https://doi.org/10.1007/s12035-016-9854-x
7. *Chi LM, Wang LP, Jiao D*. Identification of differentially expressed genes and long noncoding RNAs associated with Parkinson's disease. Parkinsons Dis 2019;6078251. https://doi.org/10.1155/2019/6078251
8. *Lyu Y, Bai L, Qin C*. Long noncoding RNAs in neurodevelopment and Parkinson's disease. Animal Model Exp Med 2019;2:239-51. https://doi.org/10.1002/ame2.12093
9. *Zhong L, Liu P, Fan J, Luo Y*. Long non-coding RNA H19: physiological functions and involvements in central nervous system disorders. Neurochem Int 2021;148:105072. https://doi.org/10.1016/j.neuint.2021.105072
10. *Acharya S, Salgado-Somoza A, Stefanizzi FM, et al.* Non-coding RNAs in the brain-heart axis: the case of Parkinson's disease. Int J Mol Sci 2020;21:6513. https://doi.org/10.3390/ijms21186513
11. *Jiang J, Piao X, Hu S, Gao J, Bao M*. LncRNA H19 diminishes dopaminergic neuron loss by mediating microRNA-301b-3p in Parkinson's disease via the HPRT1-mediated Wnt/β-catenin signaling pathway. Aging (Albany NY) 2020;12:8820-36. https://doi.org/10.18632/aging.102877
12. *Lv Q, Wang Z, Zhong Z, Huang W.* Role of long noncoding RNAs in Parkinson's disease: putative biomarkers and therapeutic targets. Parkinsons Dis 2020;5374307. https://doi.org/10.1155/ 2020/ 5374307
13. *Xu W, Zhang L, Geng Y, Liu Y, Zhang N.* Long noncoding RNA GAS5 promotes microglial inflammatory response in Parkinson's disease by regulating NLRP3 pathway through sponging miR-223-3p. Int Immunopharmacol 2020;85:106614. https://doi.org/10.1016/j.intimp.2020.106614
14. *Bao Z, Yang Z, Huang Z, Zhou Y, Cui Q, Dong D.* LncRNADisease 2.0: an updated database of long non-coding RNA-associated diseases. Nucleic Acids Res 2019;47:1034-7. https://doi.org/10.1093/nar/gky905
15. *Maniati MS, Maniati M, Yousefi T, Ahmadi-Ahangar A, Tehrani SS*. New insights into the role of microRNAs and long noncoding RNAs in most common neurodegenerative diseases. J Cell Biochem 2019;120:8908-18. https://doi.org/10.1002/jcb.28361
16. *Chen Y, Li Z, Chen X, Zhang S*. Long non-coding RNAs: from disease code to drug role. Acta Pharm Sin B 2021;11:340-54. https://doi.org/10.1016/j.apsb.2020.10.001
17. *Postuma RB, Berg D, Stern M, et al*. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord 2015;30:1591-601. https://doi.org/10.1002/mds.26424
18. *Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE*. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010;25:2649-53. https://doi.org/10.1002/mds.23429
19. *Goetz CG, Poewe W, Rascol O, et al*. Movement disorder society task force report on the hoehn and yahr staging scale: status and recommendations. Mov Disord 2004;19:1020-8. https://doi.org/10.1002/mds.20213
20. *Fahn BS, Elton R*. Committee. Unified Parkinson's disease rating scale. In: Recent developments in Parkinson's disease. Florham Park, NY: Macmillan Health Care Information; 1987. p. 153-64.
21. *Livak KJ, Schmittgen TD*. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ Method. Methods 2001;25:402-8. https://doi.org/10.1006/meth.2001.1262
22. *Li XZ, Zhang SN, Lu F, Liu SM*. Microarray expression analysis for the paradoxical roles of Acanthopanax senticosus harms in treating alpha-synucleinopathies. Phytother Res 2016;30:243-52. https://doi.org/10.1002/ptr.5522
23. *Elkouris M, Kouroupi G, Vourvoukelis A, et al*. Long noncoding RNAs associated with neurodegeneration-linked genes are reduced in Parkinson's disease patient. Front Cell Neurosci 2019;13:58. https://doi.org/10.3389/fncel.2019.00058