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Investigation of the expression of long non-coding RNA in Parkinson's disease

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Abstract

Background Parkinson's disease is the second most common age-related neurodegenerative disease after Alzheimer's. Pathogenic factors in Parkinson's include inflammation and oxidative stress, which lead to dopaminergic cell apoptosis. The case–control study aims to determine the expression level of long non-coding RNAs (lncRNAs) of the apoptosis pathway in Parkinson's patients compared to healthy individuals. In the case–control study, 50 patients with Parkinson's disease were examined, along with 50 healthy individuals matched in age and sex. In both groups, the expression of long non-coding RNAs, including taurine upregulated 1 (TUG1), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), nuclear-enriched abundant transcript 1 (NEAT1), and growth arrest-specific 5 (GAS5), was compared using real-time polymerase chain reaction (PCR).

Results The ratio of MALAT1, NEAT1, and TUG1 gene expression in the case group was statistically significantly higher than in healthy individuals. The ratio of GAS5 gene expression in people with Parkinson's disease was lower, with a statistically significant difference. The ratio of HULC gene expression was higher in the case group, but it did not show a statistically significant difference with the control group.

Conclusion The involvement of long lncRNAs that increase apoptosis may play a role in the pathogenesis of the disease, which may be used for identification and therapeutic purposes.

Keywords Apoptosis, Long non-coding RNA, Parkinson's disease, Neurodegeneration

Introduction

Parkinson's disease (PD) involves the central nervous system, mainly affecting the motor nervous system [1]. Although extensive studies have been conducted concerning its etiology, the main cause of the disease is still unknown. Its important causes include genetics, environmental pollution, age, external toxins, internal toxins caused by the metabolism of neurons, and the presence of free radicals. Genetics plays an important role in Parkinson's [2, 3]. Previous findings show that approximately 15% of patients have one of their first-degree relatives who also have Parkinson's [4], so genetic studies in these patients play an important role in understanding its pathogenesis. Currently, the available treatment option for these patients is to reduce the symptoms of the disease instead of preventing the disease from progressing5. Therefore, it is necessary to carry out continuous research efforts on strategies aimed at explaining the pathogenic mechanism at the molecular level and providing effective treatment for Parkinson's.

Long non-coding RNAs are non-coding RNAs that have extensive activities in the cell, most of which include regulating the expression of other genes [6]. Among these, RNAs include taurine upregulated 1(TUG1), metastasis-associated lung adenocarcinoma transcript 1(MALAT1), growth arrest-specific 5(GAS5), nuclear-enriched abundant transcript 1 (NEAT1) and highly upregulated in liver cancer (HULC), most of which are

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expressed in brain cells and neurons and are related to neuronal disorders [7]. Although genetic factors have been directly and indirectly identified in some of these patients, the molecular mechanism of the disease has not been fully determined yet. There is much evidence that lncRNAs play a role in synaptic plasticity gene regulation, cognitive function, and memory [8]. In addition, lncRNAs may act as valuable biomarkers for Parkinson's disease and other neurological diseases [9]. Gene expression changes of lncRNAs in Parkinson's patients can help in the etiology of the disease and in finding potential therapeutic targets and potential biomarkers for diagnosis [10]. The study aims to identify the role of mentioned genes in connection with Parkinson's disease and to know the new pathways involved in the pathogenesis and etiology of the disease.

Material and methods

The study was conducted with a case–control design in 2021 in Hamadan (western Iran). A written consent form was obtained from the people participating in the study. Ethics Committee of Hamadan University of Medical Sciences with ID IR.UMSHA.REC.1398.990 approved. The individuals in the case group were patients with Parkinson's disease, who were diagnosed by a specialist doctor (with great experience in this field) and referred to the neurology department of Beast Hospital (Hamadan, Iran). The individuals in the control group were the companions of the patients who were matched with the case group in terms of age and gender.

RNA extraction and cDNA synthesis

A trained nurse collected 5 cc of blood from each person in two groups, control and case, from the brachial vein under aseptic conditions. The blood was divided into two tubes: 3 cc of blood was transferred to a tube without anticoagulant, while 2 cc of blood was transferred to a tube containing an anticoagulant called ethylene diaminetetra acetic acid (EDTA) [11].

First, complete cellular RNA was extracted from whole blood samples. Then, the purity of the extracted RNA was checked by the Nanodrop device (NanoDrop® ND-1000 UV–Vis Spectrophotometer, US) [12]. After confirmation, the reverse transcription reaction is performed according to the protocol included in the cDNA synthesis kit. In the next step, the real-time polymerase chain reaction(PCR) technique was used to check the expression level of the desired LNCRNAs compared to the reference gene (HPRT-1).

Statistical analysis

The data analysis was performed using SPSS version 16 software. A statistically significant level of less than 5% was considered. Quantitative variables were expressed using mean and standard deviation, while qualitative variables were expressed using number and percentage. *T*-test was used to examine quantitative variables between the two groups. To determine the sample size by using the software G Power, considering the first type error of 5% and the test power of 80%, the sample size was estimated to be 50 cases in each group.

Results

The control group consisted of 32 male subjects (64%), while the case group had 37 male patients (74%). However, the difference in the proportion of male patients between the two groups was not statistically significant ($P=0.280$). The average age of individuals in the case group was 55.4 ± 4.5 years, while that of the control group was 55.6 ± 6.6 years. A *t*-test was conducted to compare the two groups, and the results showed that there was no statistically significant difference between them ($P=0.855$). The average duration of PD in the case group was 10.2 ± 5.5 years.

MALAT1 gene expression in patients with Parkinson's disease was higher than in healthy individuals with statistically significant differences. In terms of gender, the average MALAT1 expression in male and female patients was higher than in healthy individuals, but the difference was not significant for males (Table 1).

The level of GAS5 gene expression in patients with Parkinson's disease was lower than in the control group with statistically significant differences. The average GAS5 expression in patients of both genders was found to be lower, with a statistically significant difference (Table 2).

The level of NEAT1 gene expression in patients with Parkinson's disease was higher than in the control group with a statistically significant difference. In terms of

Table 1 Comparison of MALAT1 expression in both groups

MALAT1 expression	Control no	PD patient no	Expression ratio	Standard deviation	*P*-value	95% CI
Total	50	50	0.4600	0.44	< 0.0001	− 2.58 to − 0.801
Male	32	37	0.9341	0.002	0.73	− 2.6 to 2.15
Female	18	13	0.3799	0.467	< 0.0001	− 3.063 to − 1.22

MALAT metastasis-associated lung adenocarcinoma transcript1

Table 2 comparison of GAS5 expression in both groups

GAS5 expression	Control no	PD patient no	Expression ratio	Standard deviation	*P*-value	95% CI
Total	50	50	12.9657	0.67	< 0.0001	3.57–6.21
Male	32	37	24.2782	1.39	< 0.0001	3.36–8.87
Female	18	13	10.1265	0.84	< 0.0001	2.74–6.08

GAS5, growth arrest-specific 5

Table 3 comparison of NEAT1 expression in both groups

NEAT1 expression	Control no	PD patient no	Expression ratio	Standard deviation	*P*-value	95% CI
Total	50	50	0.0340	0.709	< 0.0001	− 8.03 to − 5.25
Male	32	37	0.0626	1.058	< 0.0001	− 7.57 to − 0.415
Female	18	13	0.0237	0.89	< 0.0001	− 9.08 to − 5.58

NEAT1, nuclear-enriched abundant transcript 1

Table 4 comparison of HULC expression in both groups

HULC expression	Control no	PD patient no	Expression ratio	Standard deviation	*P*-value	95% CI
Total	50	50	0.533293	0.48	0.062	(− 1.861 to 0.046)
Male	32	37	0.488354	0.561	0.071	(− 2.161 to 0.092)
Female	18	13	0.240982	0.546	< 0.0001	(− 3.137 to − 0.969)

HULC, highly upregulated in liver cancer

Table 5 Comparison of TUG1 expression in both groups

TUG1 expression	Control no	PD patient no	Expression ratio	Standard deviation	*P*-value	95% CI
Total	50	50	0.580352	.36	0.034	(− 1.51 to − 0.058)
Male	32	37	0.311866	.401	< 0.0001	(− 2.47 to − 0.88)
Female	18	13	1.860899	.446	0.047	(0.014–1.77)

TUG1, taurine upregulated 1

gender, the average NEAT1 expression in patients of both genders was also higher with a statistically significant difference (Table 3). The level of HULC gene expression in patients with Parkinson's disease was higher than in the control group. But there was no statistically significant difference. In terms of gender, the average HULC expression was higher in patients of both genders, but in male patients, the difference was not statistically significant with the control group (Table 4).

TUG1 gene expression in patients with Parkinson's disease was statistically significantly higher than in the control group. In terms of gender, the average TUG1 expression was higher in male and female patients, but in female patients, it was lower than the control group with a statistically significant difference (Table 5).

Discussion

Most of the studies that have studied the gene expression of long non-coding RNAs in Parkinson's patients have had animal models [13, 14] and the results of human studies are limited and the results of studies conducted in this field are controversial [7, 9, 10]. The case–control study was conducted with aim of determining the expression level of long-pathway non-coding apoptosis RNAs in Parkinson's patients compared to healthy individuals. Based on the knowledge of the authors and a review of existing sources, no study has been published that investigates the expression of long-pathway non-coding apoptosis RNAs in Iranian patients with Parkinson's disease.

Our findings showed that MALAT1 gene expression is higher in patients than in healthy individuals with

a statistically significant difference. It was shown that MALAT1 is expressed in neurons and modulates a set of genes related to dendrite and synapse growth [15]. In 2019, Shaker et al. [16] showed that there is a direct correlation between the expression level of MALAT1 and lnc-DC in patients with multiple sclerosis. In the study of Liu et al. [17], they also showed that MALAT-1 induces apoptosis through miRNA-124 in mouse and human models of Parkinson's disease. MALAT1 is highly expressed in neurons and MALAT1 acts as a regulator of gene expression involved in synapse formation or maintenance. The decrease in MALAT1 gene expression due to the decrease in dopamine in mice has been shown to play a role in neurodegenerative pathogenesis [16].

Our findings showed that the ratio of NEAT1 gene expression in patients is higher compared to healthy people. The gene is expressed in several non-neuronal cells and cell lines. Mello et al. [18] in 2017 showed that NEAT1 has a role in various cellular pathways. One of these paths is the path of neuronal apoptosis. The gene is positively regulated by P53. The findings of another study by Sun et al. [19] in 2016 showed that NEAT1 stimulates lung cancer progression through the regulation of the miR-377-3p-E2F3 pathway. NEAT1 regulates cellular and mitochondrial homeostasis. Changes in the level of NEAT1 have been reported in the brain of Parkinson's patients and animal models of the disease. The increase in NEAT1 gene expression is more observed among patients with long disease duration.

According to the findings of the present study, TUG1 gene expression was higher in patients with a statistically significant difference. The gene plays a role in stimulating proliferation, differentiation, and inhibition of apoptosis and cell invasion [20]. Previous findings show that gene expression is necessary for the survival of neurons, and for this reason, this gene is upregulated in diseases involving the nervous system, especially Huntington's disease, which is activated through p53 [21].

We observed that the ratio of GAS5 gene expression in patients is lower than in healthy individuals with a statistically significant difference. The findings of the conducted studies have also linked GAS5 with the process of apoptosis and it plays a role in the development of some types of cancer [22, 23]. Mourtada et al. [24] in 2008 also showed that GAS5 and its mRNA levels are reduced in breast cancer samples compared to the adjacent healthy tissue, and accordingly, it is known as a tumor suppressor gene. In line with our findings, in 2018, Gharesouran et al. [25]. Showed that the level of GAS5 in patients with multiple sclerosis is higher compared to the expression level of NR3C1 in the control group, which can play an important role in the treatment of people with the disease.

According to the findings of the present study, HULC expression in Parkinson patients was higher than in healthy people. The findings of previous studies show that the gene is not only related to various cancers but also related to cardiovascular diseases, diabetes, autism, and Alzheimer's [8]. Takahashi et al. [26] showed in 2014 that HULC is upregulated in liver cancer. It is also involved in the negative regulation of several miRNAs, including miR-372.

The studies have limitations, including the relatively small volume of examined patients and the limitations of case–control studies to investigate the causal relationship. Also, due to the need for an invasive procedure (blood drawing), some people, especially healthy people, were less willing to participate in the study, which may cause selection bias in the present study. Studies with a larger sample size and with a prospective design and a clinical trial can be helpful in this field. Conducting a study among patients with different clinical symptoms is also valuable for future studies.

Conclusions

With the increase in life expectancy and longevity of humans, the incidence of neurodegenerative diseases has also increased. Therefore, diagnostic methods for the disease using cheap blood tests are very valuable. Although genetic factors have been directly and indirectly identified in some of these patients, the molecular mechanism of the disease has not been fully determined yet. The involvement of long non-coding RNAs (lncRNAs) that increase apoptosis may play a role in the pathogenesis of the disease.

Author contributions

MM reviewed and edited the manuscript, AK conceptualized and designed the study, MME collaborated on the analysis, MKF critically reviewed the manuscript, and all authors reviewed the manuscript and they agree with the final version.

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Availability of data and materials

Not applicable.

Code availability

Not applicable.

Declarations

Ethics approval and consent to participate

Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.990). Consent to participate from all patients was obtained.

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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