Molecular Biology Reports
https://doi.org/10.1007/s11033-022-07372-7

ORIGINAL ARTICLE

Expression analysis of vitamin D receptor and its related long non-coding RNAs in peripheral blood of patients with Parkinson's disease

Mahdi Gholipour[1] · Kasra Honarmand Tamizkar[2] · Amirhossein Niknam[3] · Bashdar Mahmud Hussen[4,5] · Solat Eslami[6,7] · Arezou Sayad[8] · Soudeh Ghafouri-Fard[8]

Received: 23 January 2022 / Accepted: 15 March 2022

Abstract

Background Parkinson's disease (PD) is a neurological condition that is associated with abnormal expression of several transcripts. Vitamin D receptor (VDR) is a possible participant in the pathogenesis of PD.

Methods and results In the present research project, we evaluated expressions of *VDR* and three functionally associated long non-coding RNAs with this signaling, namely *SNHG6*, *SNHG16* and *LINC00346* in PD patients versus normal controls. Level of *SNHG6* transcripts was lower in total patients in comparison with total controls (Expression ratio (95% CI) 0.44 (0.17–1.08)) and in male patients compared with male controls (Expression ratio (95% CI) 0.29 (0.13–0.65)). On the other hand, expression of *VDR* was higher in total patients compared with total controls (Expression ratio (95% CI) 10.86 (4.37–26.72)) and in male patients compared with male controls (Expression ratio (95% CI) 22.16 (6.23–78.8)). There was no significant difference in expression of *SNHG16* and *LINC00346* between PD patients and controls. Amounts of *SNHG6* and *VDR* transcripts could differentiate total PD patients from total controls with AUC values of 0.66 and 0.86, respectively.

Conclusions Cumulatively, the results of the present investigation imply dysregulation of VDR signaling in PD and necessitate conduction of further functional studies.

Keywords Parkinson's disease · lncRNA · VDR · SNHG6 · SNHG16 · LINC00346

Introduction

Parkinson's disease (PD) is a neurological condition manifested by bradykinesia, postural instability, rigidity, and resting tremor [1]. This disorder is caused by the defects in the dopaminergic system [2] and establishment of inclusion bodies containing α-synuclein protein in neurons [3]. The main etiology of this disorder is not clarified yet and it is supposed to be due to involvement of several factors including environmental factors [4], oxidative stress [5], abnormalities in mitochondria [6] and dysregulation of immune responses [7]. Several investigations have highlighted the role of vitamin D receptor (VDR signaling in the pathoetiology of neurological diseases [8, 9]. Vitamin D has been

✉ Arezou Sayad
ar.sayad@yahoo.com

✉ Soudeh Ghafouri-Fard
s.ghafourifard@sbmu.ac.ir

1 Men's Health and Reproductive Health Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2 Skull Base Research Center, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3 Phytochemistry Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4 Department of Pharmacognosy, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq

5 Center of Research and Strategic Studies, Lebanese French University, Erbil, Kurdistan Region, Iraq

6 Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran

7 Department of Medical Biotechnology, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran

8 Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Published online: 15 April 2022

shown to influence proliferation and differentiation of neurons, neurodevelopment and synaptic plasticity [10, 11]. Moreover, genetic polymorphisms within *VDR* gene can predispose individuals to PD [12, 13].

Long non-coding RNAs (lncRNAs) as imperative influencers of gene expression have been found to be involved in VDR signaling [14]. A former assay has indicated that vitamin D signaling regulates expression level of a number of lncRNAs in a way consistent with its protective role against skin cancer. Notably, these lncRNAs can be secreted into exosomes, thus being detected in biofluids [14]. Meanwhile, certain lncRNAs have been shown to influence VDR expression or its transcriptional activity [15]. VDR-related lncRNAs have been identified through different approaches, particularly bioinformatics methods [16]. Expression levels of these lncRNAs have also been measured in different disorders such as schizophrenia indicating a possible role for these lncRNAs in disease pathogenesis and putative application as disease markers [17].

In the current study, we have quantified expression of *VDR* as well as three VDR-associated lncRNAs, i.e. *SNHG6*, *SNHG16* and *LINC00346* in the peripheral blood of patients with PD versus healthy controls to unravel their possible association between these transcript and PD pathogenesis.

Materials and methods

Cases and control

Each study group contained 50 individuals (female/male ratio: 14/36 and 24/26 in cases and controls, respectively). PD cases were recruited from University-affiliated hospitals and were diagnosed according to criteria proposed by the International Parkinson and Movement Disorder Society [18]. None of recruited individuals had current or chronic infections, malignancies or any systemic disorders. Control subjects had no individual or family history of PD, other neurological disorders, malignancies or autoimmune disorders. The study protocol was approved by ethical committee of Shahid Beheshti University of Medical Sciences. All enlisted individuals signed the informed consent forms.

Assessment of expression of genes

A total of 4 mL of venous blood was gathered from PD cases and healthy subjects in EDTA-containing tubes. Cellular RNA was retrieved from blood specimens using GeneAll extraction kit (Seoul, South Korea). Subsequently, total RNA was converted to cDNA using BioFact™ kit (Seoul, South Korea). Expression of *VDR*, *SNHG6*, *SNHG16* and *LINC00346* were measured in all samples using PCR master mix provided by Ampliqon company (Denmark) according to our previous study [17]. StepOnePlus™ RealTime PCR System (Applied Biosystems, Foster city, CA, USA) was used as the thermal cycle system.

Statistical analysis

SPSS v.18.0 (SPSS Inc., Chicago, IL) was used for statistical assessments. GraphPad Prism (version 9.0, La Jolla California, USA) was used for depiction of graphs. Expressions of *VDR* and 3 lncRNAs in each sample were quantified using the Efficiency adjusted Ct of the normalizer gene—Efficiency adjusted Ct of the target gene (comparative – ΔCt method). The normal/gaussian distribution of the values was accessed by the Shapiro–wilk test. A non-parametric test (Mann–Whitney U test) or parametric unpaired t test was used to identify differentially expressed genes between the patients and healthy controls. Also, the two-way ANOVA and Tukey post hoc tests were used to analyze the effects of main factors (disease and gender) and their interaction on gene expression levels in patients and control subgroups. – ΔCt values in were plotted the figures in the format of box and whisker plots. Median [line], mean [cross], interquartile range [box], and minimum and maximum values were depicted.

The fold change of the test sample relative to the control sample was calculated using the $2^{-\Delta\Delta Ct}$ formula and was shown as mean and 95% CI of mean in the figures and table.

The correlation between transcript levels of studied genes was judged using regression model. Bonferroni correction was applied for multiple comparisons. The partial correlation between expression levels of studied genes and clinical/demographic data was described by R and P values.

The receiver operating characteristic (ROC) curves were depicted to appraise the diagnostic power of expression levels of *VDR* and *SNHG6* lncRNA. The significance of difference in mean values of expressions of *VDR* and 3 lncRNAs (mean of –ΔCt values) between two subgroups of patients was evaluated using the t-test.

Results

General data

General data of enlisted cases are shown in Table S1. Control group included 24 females and 26 males with age range of 37 to 80 (mean ± SD: 36.54 ± 11.85).

Expression assay

Figure 1 shows relative expression levels of *SNHG6*, *SNHG16*, *LINC00346* and *VDR* in total PD patients versus controls groups.

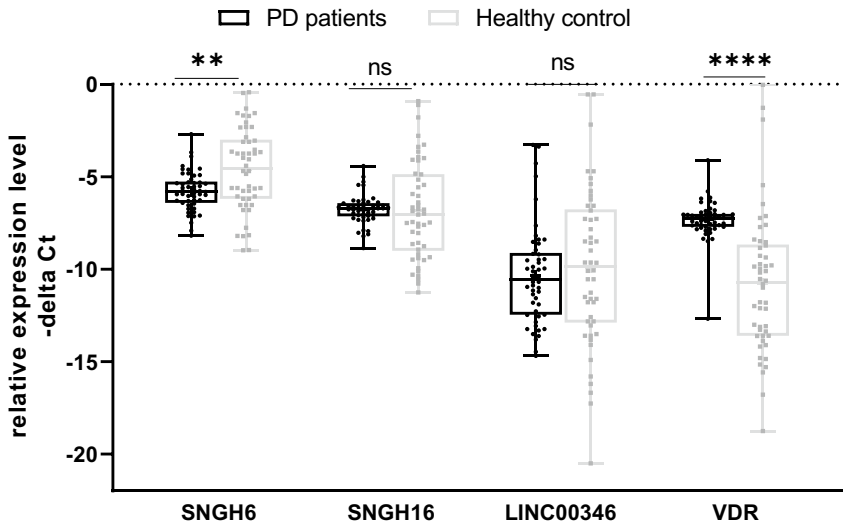

Fig. 1 Relative expression of *SNHG6*, *SNHG16*, *LINC00346* and *VDR* in patients with Parkinson (total) and controls (total) as described by –ΔCt values (Ct Housekeeping gene-Ct Target gene)

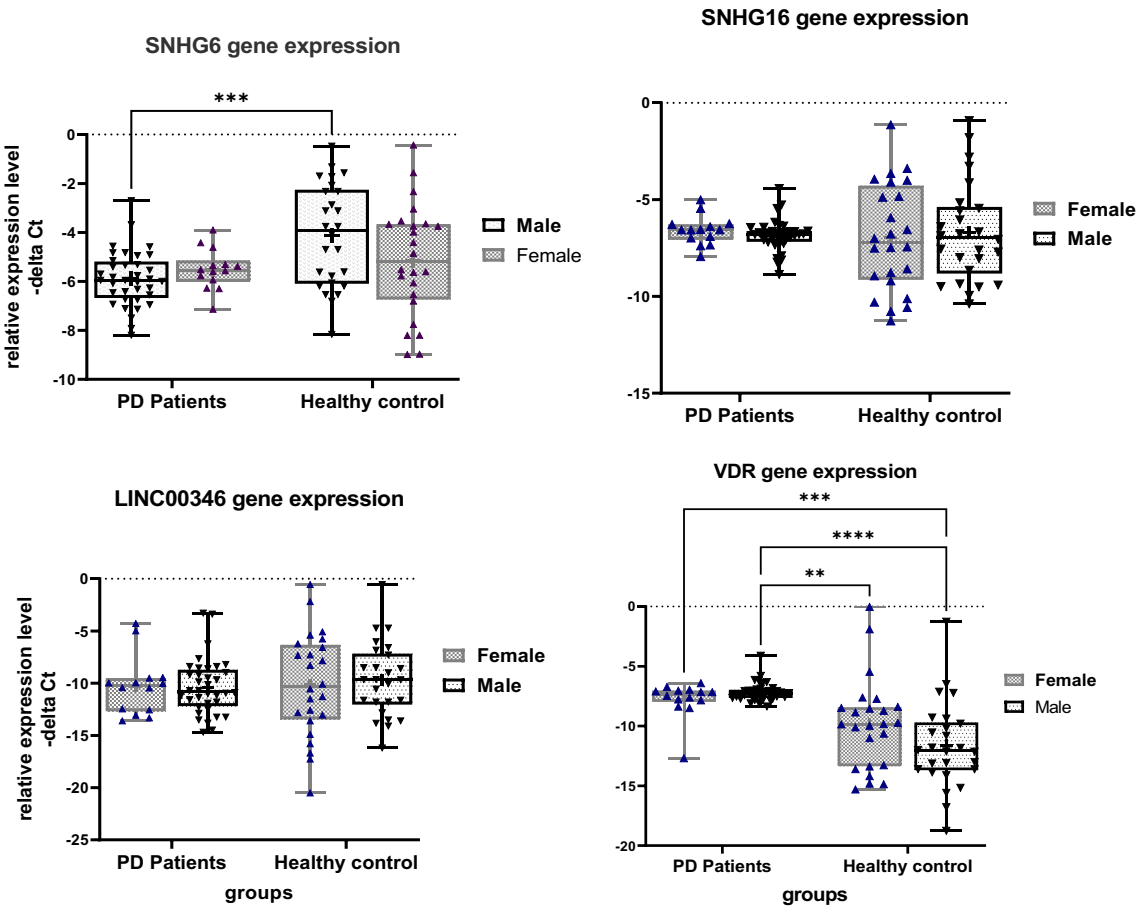

Fig. 2 Relative expression levels of *SNHG6*, *SNHG16*, *LINC00346* and *VDR* in PD patient's subgroups (male and female) versus controls subgroups (male and female) (*P value < 0.05, **P value < 0.001 and ****P value < 0.0001)

To analyze the effects of main factors (disease and gender) and their interaction on gene expression levels in patients and controls subgroups, a two-way ANOVA with Tukey post hoc tests were used. Figure 2 shows relative expression levels of *SNHG6*, *SNHG16*, *LINC00346* and *VDR* in PD patients' subgroups versus controls subgroups.

Two-way ANOVA analysis indicated significant main effects of disease on the expressions of *SNGH6* (F = 8.76, P = 0.004) and *VDR* (F = 32.34, P < 0.0001). However, there was no significant effect of gender on expression levels of *SNGH6* and *VDR*. Also, significant two-way interaction of gender × disease was found for *VDR* gene expression (F = 4.35, P = 0.04). There were no significant effects of gender, diseases and their interaction on expression levels of *SNHG16* and *LINC00346* genes in subgroups.

SNHG6 was under-expressed in total patients compared with total controls (Expression ratio (95% CI) 0.44 (0.17–1.08)) and in male patients in comparison with male controls (Expression ratio (95% CI) 0.29 (0.13–0.65)). On the other hand, expression of *VDR* was higher in total patients in comparison with total controls (Expression ratio (95% CI) 10.86 (4.37–26.72)) and in male patients compared with male controls (Expression ratio (95% CI) 22.16 (6.23–78.8)). There were no significant differences in expression of *SNHG16* and *LINC00346* between PD patients and controls (Table S2).

No significant correlation was detected between expression levels of genes and age, Hoehn & Yahr stage, disease duration, MMSEor UPDRS score (Table 1).

There was significant correlation between expression level of *SNHG16* and *SNHG6* among patients (P value < 0.0001). Expressions of other genes were not correlated with each other after correction for multiple comparisons (Table 2).

Table S3 summarizes the AUC, sensitivity and specificity values of *SNHG6* and *VDR* transcript levels in PD and among sex-based subgroups.

Discussion

In the present research project, we estimated expression of *VDR*, *SNHG6*, *SNHG16* and *LINC00346* in PD patients versus normal controls. *SNHG6* was under-expressed in total patients compared with total controls and in male patients compared with male controls. *SNHG6* has been shown to affect neuronal apoptosis. A combinatorial in vitro and in vivo study has shown that suppression of *SNHG6* expression leads to higher neuron viability, inhibits neuron apoptosis and reduces caspase-3 activity in oxygen glucose deprivation-induced neurons [19]. This lncRNA can function as a molecular sponge for miR-181c [19], a miRNA that has a protective role in a cellular model of PD through regulation of BCL2L11 expression [20]. Thus, if the functional role of *SNHG6* in the development of PD was supposed to be exerted only through modulation of miR-181c levels, it would be expected to detect higher levels of this lncRNA in PD patients compared with controls. Thus, the observed under-expression of *SNHG6*

Table 1 Assessment of partial correlation between expression of *VDR* and 3 lncRNAs and age, disease duration, disease stage, MMSE and UPDRS (controlled for sex)

Parameters	Age		SNHG6 expression		SNHG16 expression		LINC00346 expression		VDR expression		Hoehn & Yahr stage		Disease duration		MMSE		UPDRS	
	R	P value	R	P value	R	P value	R	P value	R	P value	R	P value	R	P value	R	P value	R	P value
Age	1	0	0.035	0.81	0.006	0.96	0.16	0.25	0.16	0.24	0.19	0.18	− 0.09	0.51	− 0.6	< 0.0001	0.11	0.43
Disease duration	− 0.09	0.51	0.32	0.24	0.24	0.08	− 0.07	0.62	0.15	0.29	0.55	< 0.0001	1	0	− 0.38	0.006	0.5	0.0002
Hoehn & Yahr stage	0.19	0.18	0.15	0.28	0.22	0.12	− 0.11	0.42	0.21	0.13	1	0	0.55	< 0.0001	− 0.58	< 0.0001	0.66	< 0.0001
MMSE	− 0.6	< 0.0001	− 0.22	0.12	− 0.25	0.08	− 0.07	0.62	− 0.23	0.1	− 0.58	< 0.0001	− 0.38	0.006	1	0	− 0.33	0.02
UPDRS	0.11	0.43	0.1	0.48	0.01	0.9	− 0.21	0.14	0.05	0.69	0.66	< 0.0001	0.5	0.0002	− 0.33	0.02	1	0

Disease duration was classified into 3 ranges (1–5, 6–10 and more than 10 years)

Table 2 Correlation between expressions of *VDR* and 3 lncRNAs in patients and controls (R values are presented; after correction for multiple comparisons (Bonferroni correction), P value < 0.0083 was regarded as significant)

SNHG16	Controls	0.27	0.057				
	Patients	0.64	<0.0001*				
LINC00346	Controls	0.24	0.08	0.31	0.02		
	Patients	0.16	0.24	0.09	0.51		
VDR	Controls	-0.02	0.87	0.21	0.16	0.27	0.057
	Patients	-0.09	0.51	0.13	0.36	0.04	0.77
		r	P value	r	P value	r	P value
		SNHG6		*SNHG16*		*LINC00346*	

p value less than 0.05 (typically ≤ 0.05) is statistically significant

Expression levels of *SNHG6* and *VDR* could differentiate total PD patients from total controls with AUC values of 0.66 and 0.86, respectively (Fig. 3). Both values were lower among females (Fig. 4), compared with males (Fig. 5)

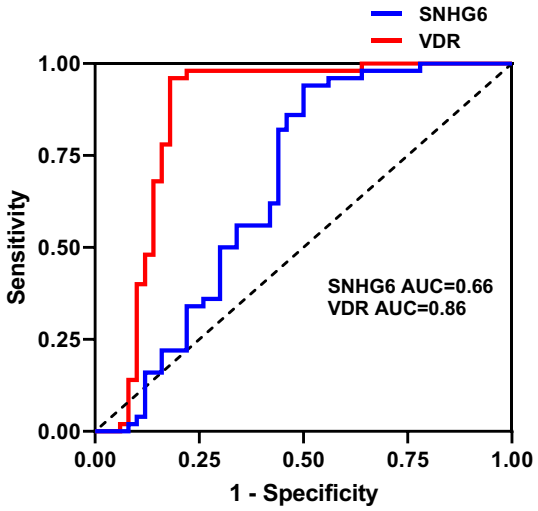

Fig. 3 ROC curves of *SNHG6* and *VDR* transcript levels in PD

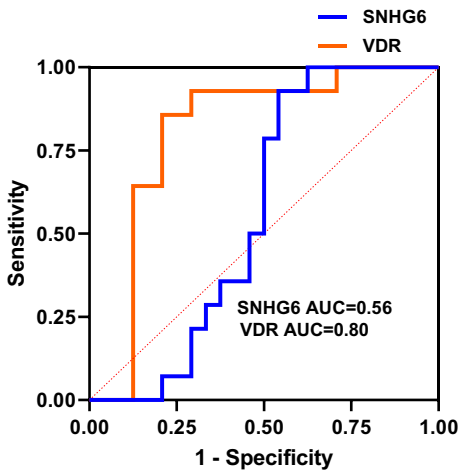

Fig. 4 ROC curves of *SNHG6* and *VDR* transcript levels in females

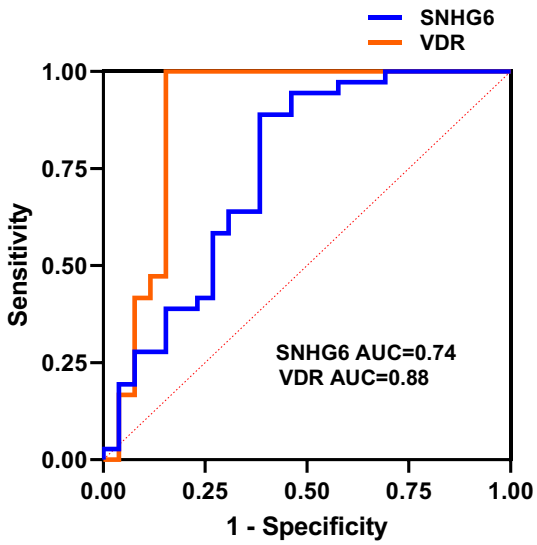

Fig. 5 ROC curves of *SNHG6* and *VDR* transcript levels in males

in the circulation of PD patients implies that this lncRNA affect PD pathogenesis through other mechanisms rather than sponging miR-181c. Other possible explanation for this observation is discrepancy between its peripheral levels and its level in the nervous system. Future studies in neurons and animal models of PD are necessary for discovery the underlying mechanism for participation of *SNHG6* in the pathoetiology of PD.

On the other hand, expression of *VDR* was higher in total PD cases compared with total healthy individuals and in male patients compared with male controls.

Differences in the expression of *SNHG6* and *VDR* exist only between male patients and male controls. This observation might indicate differential roles of these two

genes in the pathetiology of PD among males and females. In other words, these two genes might only participate in the pathogenesis of PD only among males. Previous studies have suggested sex-specific roles for some lncRNAs, particularly in the context of neuropsychiatric disorders. For instance, expression of neuronal-enriched lncRNA *LINC00473* has been shown to be decreased in the prefrontal cortex of depressed females but not males. Studies in animal models have also confirmed this sex-specific function of *LINC00473*. In fact, *LINC00473* has been identified as a female-specific induced of stress resilience which is abnormal in depressed females [21]. Another study has reported a sex-specific elevation of neurodevelopment-related lncRNAs in the circulation of patients with schizophrenia [22]. Gender has been also shown to affect the impact of *VDR* variants on susceptibility to multiple sclerosis [23]. Similar to mentioned studies, the observed sex-specific dysregulation of *SNHG6* and *VDR* in the current study might indicate specific roles of these genes in the development or progression of PD. This observation is also supported by the proposed sex differences in epidemiology, disease susceptibility and clinical profile of PD [24]. In fact, clinical and molecular investigations have evidently supported the concept that females are protected from PD compared with males [24].

There was no significant difference in expression of *SNHG16* and *LINC00346* between PD cases and controls. VDR signaling can contribute in the pathophysiology of PD. Functional assays in an animal model of PD have shown that calcitriol relieves MPP+-and MPTP-associated parthanatos via modulation of the VDR/PARP1 pathway [25]. Other studies have shown abundant expression of VDR and vitamin D hydroxylase in the substantia nigra and their relationship with development of midbrain dopaminergic neurons [26, 27]. The reported over-expression of *VDR* in PD patients in the current study might be a compensatory mechanism to alleviate the pathologic events in the course of PD.

In spite of identification of dysregulation of *VDR* and *SNHG6* in PD patients, no significant correlation was detected between expression levels of genes and age, Hoehn & Yahr stage, disease duration, MMSE or UPDRS score.

However, we identified significant correlation between expression level of *SNHG16* and *SNHG6* among patients but not healthy subjects, implying alterations in the interaction network between these lncRNAs in the context of PD. Expression of other genes was not correlated with each other after correction for multiple comparisons.

Expression levels of *SNHG6* and *VDR* could differentiate total PD patients from total controls with AUC values of 0.66 and 0.86, respectively. Thus, these genes, particularly *VDR* might be used as a diagnostic marker for PD.

Cumulatively, the results of the present investigation imply abnormal activity of VDR signaling in PD and necessitate conduction of further functional studies.

Our study is among the first studies assessing VDR signaling and related lncRNAs in the circulation of PD patients. However, this study has a limitation regarding the small sample size and lack of functional studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07372-7.

Acknowledgements This study was financially supported by Grant from Medical School of Shahid Beheshti University of Medical Sciences.

Author contributions SGF wrote the manuscript and revised it. AS, KHT supervised and designed the study. MG, BMH and AN performed the experiment. SE analyzed the data. All authors read and approved the submitted version.

Funding Not applicable.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences. All methods were performed in accordance with the relevant guidelines and regulations.

Consent of publication Not applicable.

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