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Research Article

PINK1 Type of Early Onset Parkinson's Disease (EOPD) in Sudanese Patients, 2018

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Abstract

Background: Parkinson's Disease (PD) is a neurodegenerative disorder affecting the motor system. It is a chronic progressive disorder that leads to long standing disability.
Objective: To study the presentations and *PINK1* gene in young Sudanese patients with PD.
Methods: A prospective study was conducted among 31 PD patients at the National center for Neurological Science (NCNS) at Khartoum state. A structured questionnaire was used for data collection. This consisted of personal data, clinical presentations, and investigations. RT-PCR technique was done using G-spin™ kit. *PINK1* gene was detected in most of the samples and was strongly positive. Data was analyzed using SPSS version 21.
Results: The majority of them, 19 (61%), were located in age group 41–50 years; the mean age of onset was 33.4 ± 12 yr; Our of the total number of subjects, 19 (61%) were male and 12 (39%) were female with a ratio 1.6:1 (M:F); 20 (64.5%) were married and 8 (40%) were endogamous married; 5 (62.5%) were second degree and 3 (37.5%) were third degree, 17 (85%) had children and 2 (10%) had children with PD; 22 (71%) had duration more than 12 months and 12 (39%) were more than 40 years old; 29 (93.5%) had tremor, 27 (87.1%) had rigidity, 23 (74.2%) had bradykinesia, and 14 (45%) had positive family history of PD. *PINK1* gene expression was detected in 28 (90.3%) patients. No significant associations were found between *PINK1* expression and age, gender, age at onset, and family history ($P > 0.05$).
Conclusion: This study concludes that early onset PD was common among males than females. The most affected age group was found to be 41–50 years and the mean age of onset was 33.4 yr. Also, the patterns of the clinical features were generally similar to literature. *PINK1* expression was predominant with no significant associations between *PINK1* expression with age, gender, age at onset, and family history.

Keywords: early onset, Parkinson's disease, *PINK1* gene, Sudan

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative illness. It was first described by James Parkinson in his classic 1817 monograph, "*An Essay on the Shaking*

Palsy" [1], PD, also known as paralysis agitans, is a progressive neurodegenerative disease that affects between 100 and 200 per 100,000 people over 40, and over 1 million people in North America alone [2, 3]. It is uncommon in people younger than 40, and the incidence of the disease increases rapidly over 60 years, with a mean age of 70.5 yr at the time of diagnosis [4].

While PD has traditionally been considered a motor system disorder, it is now recognized to be a complex condition with diverse clinical features that include neuropsychiatric and other non-motor manifestations in addition to its motor symptomatology (5).

Young-onset Parkinson's disease (YOPD) is a subtype of PD, occurring at a younger age, with specific symptoms, genetic correlation, and treatment strategies. YOPD is defined as a diagnosis of PD between the ages of 21 and 40 [6]. A positive PD diagnosis under the age of 21 is referred to as "juvenile Parkinson's" (JP). Between 3 and 6% of all PD cases are reported to be YOPD [7]. Although most clinical features of JP and YOPD are the same, increased occurrence of dystonia and PD are found in patients with JP [8]. The overall age and gender adjusted incidence of PD is 13.4 per 100,000, whereas the incidences for people between the ages of 30 and 39, 40 and 49, and 50 and 59 are 0.5, 2.5, and 9.8 per 100,000, respectively [4]. Approximately 20% of YOPD patients have at least one first- or second-degree relative with PD either in the same or antecedent generation [4].

The cardinal features of PD are tremor, bradykinesia, and rigidity. A fourth feature, postural instability, is commonly mentioned, although it does not generally occur until much later in the course of the disease and is thus not included in any published diagnostic criteria for PD [8].

Women and men are affected equally. Age at onset is highly variable, even in individuals with the same pathogenic variant [9]; onset is usually in the third or fourth decade [10]. In the study by Marongiu et al. (2008), the average age at onset in those with two *PINK1* pathogenic variants was 41 yr [11].

Bradykinesia and tremor are the most common presenting signs. In some individuals, the symptoms at onset are symmetric. Dystonia and hyperreflexia may also be present [10].

In addition to Parkinsonism, individuals with the *PINK1* type of YOPD may be prone to psychiatric involvement. Abnormal behavior and/or psychiatric manifestations (in particular, depression and anxiety) occur in about 30 and 15% of affected individuals,

respectively. Other features include hallucinations and dementia [12]. Non-motor symptoms are also frequent, and overall, the clinical signs at examination are also variable [12].

On average, the response to levodopa is better than in other forms of Parkinson disease [13]; however, the incidence of levodopa-induced dyskinesia may be greater in individuals with *PINK1*-associated YOPD than in those with Parkinsonism of different etiologies [14].

1.1. Problem statement

There are several genes that have been implicated in PD. Some rare mutations are implicated in the onset of PD in a young age, usually before the age of 30; these genes are PARK2, PARK6, and PARK7. Their abnormal gene products appear to affect the function of the energy factory of the cell-the mitochondrion. Well understanding of clinical presentation and causes of PD will lead to early diagnosis and give new ideas about new modality of treatment.

1.2. Objective

To study *PINK1* gene in early onset PD and its clinical presentations in Sudanese patients at the National Center for Neurological Science (NCNS).

2. Methods

This was a prospective study conducted at the NCNS between December 2016 and December 2018. Sudanese patients aged < 50 yr and diagnosed with PD were included. Patients developing PD at age > 50 yr, patients with idiopathic PD, non-Sudanese patients, and those who were unable or refused to answer the questionnaires were excluded from the study. The sample size was 31 patients and data were collected through a questionnaire.

2.1. Study variables

Patient's age, gender, cause of disease, severity of symptoms, duration of the disease, and motor abnormalities.

2.2. Techniques

2.2.1. Blood collection

5 ml of venous blood was collected in sterile containers and put in vaccination containers and transported to laboratory for performance of genetic analysis.

2.2.2. Genetic analysis (PINK1)

DNA extraction: The extraction of DNA was carried out by using G-spin^{TM} total DNA extraction kit. Briefly, 20 µl of Proteinase K and 5 µl of RNase Solution were added to 200 µl of the whole blood sample. Then, 200 µl of Buffer BL was added, mixed thoroughly, incubated at 56°C for 10 min and centrifuged; 200 µl of absolute ethanol was added to the mixture and mixed gently by pipette (5–6 times) and then centrifuged; 700 µl of Buffer WA was added and centrifuged (repeated twice). And then 100 µl of Buffer CE was added for elution.

Real-time PCR process: Extracted DNA samples were then amplified by adding 2 µl of DNA, 1 ml of forward primer, 1 ml of reverse primer, and 20 ml of water. Then, the mixture was put in tubes placed in thermal cycler and amplification was performed. The collected data were organized into a master sheet and then entered in the computer using the Statistical Package for Social Sciences (SPSS) version 21. The results obtained were presented in tables and figures. Chi-square test was used as significance test and the level of significance was considered as P-value < 0.05.

2.3. Ethical consideration

Consent was taken from all participants and from the National Centre for Neurological Science. Participation to the study was completely voluntary and confidentiality was considered.

3. Results

Of the total of 31 patients, the majority of them were located in age group 41–50 yr, 19 (61%) of the subjects were male and 12 (39%) were female with a ratio 1.6:1 (M: F),

22 (71%) had duration more than 12 months and 12 (39%) had age more than 40 yr, followed by 11 (35%) in age group 20–40 yr and 8 (26%) less than 20 yr. Mean 33.6 ± 12.9 yr (Figures 1–3).

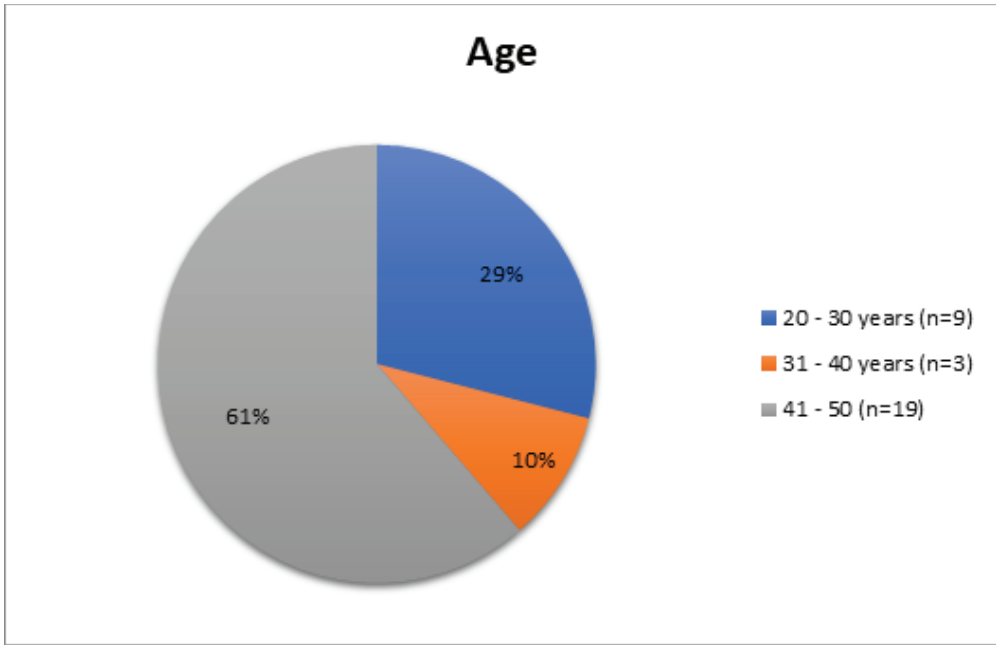

Figure 1: Age distribution among *PINK1* early onset PD patients (*n* = 31).

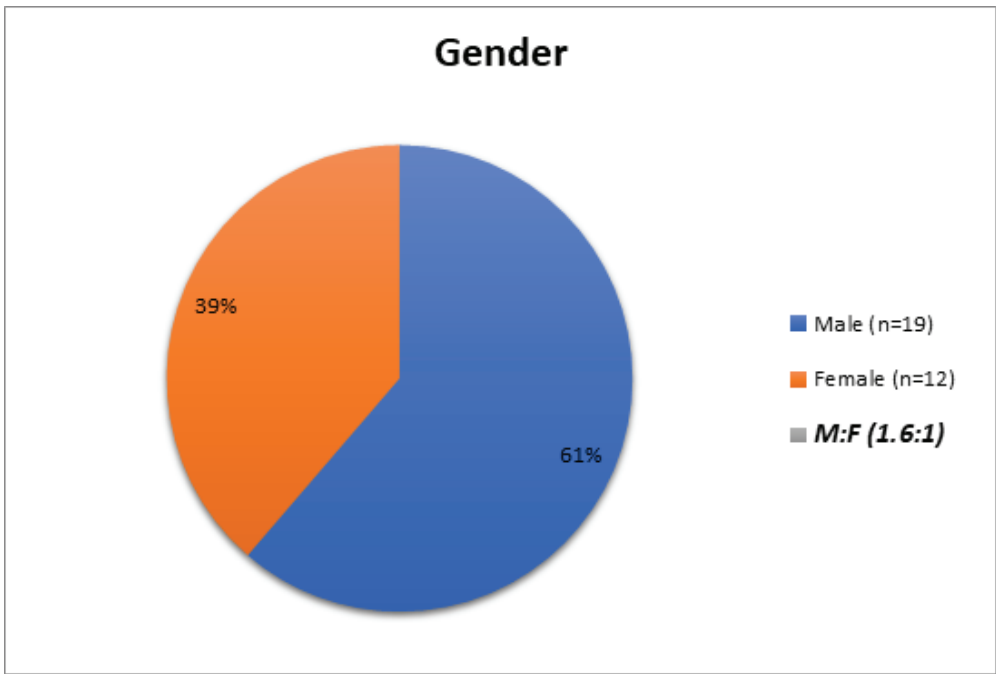

Figure 2: Gender distribution among study group (*n* = 31).

PINK1 gene expression: 28 (90.3%) patients showed positive result and 3 (9.7%) showed negative results (Figure 4).

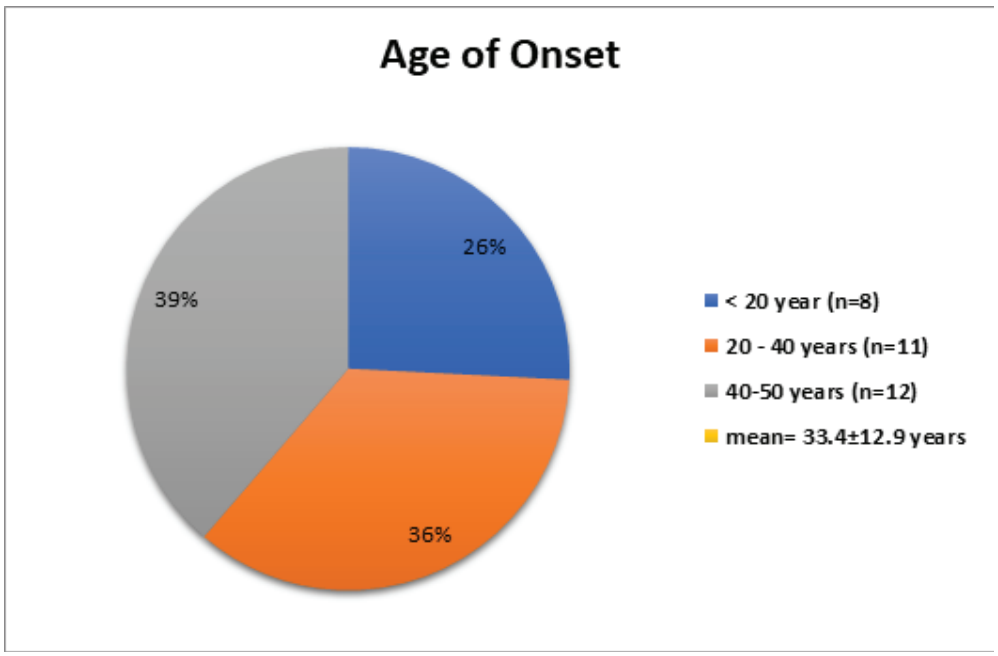

Figure 3: Gender distribution among study group (*n* = 31)The age of onset of *PINK1* type early onset PD (*n* = 31).

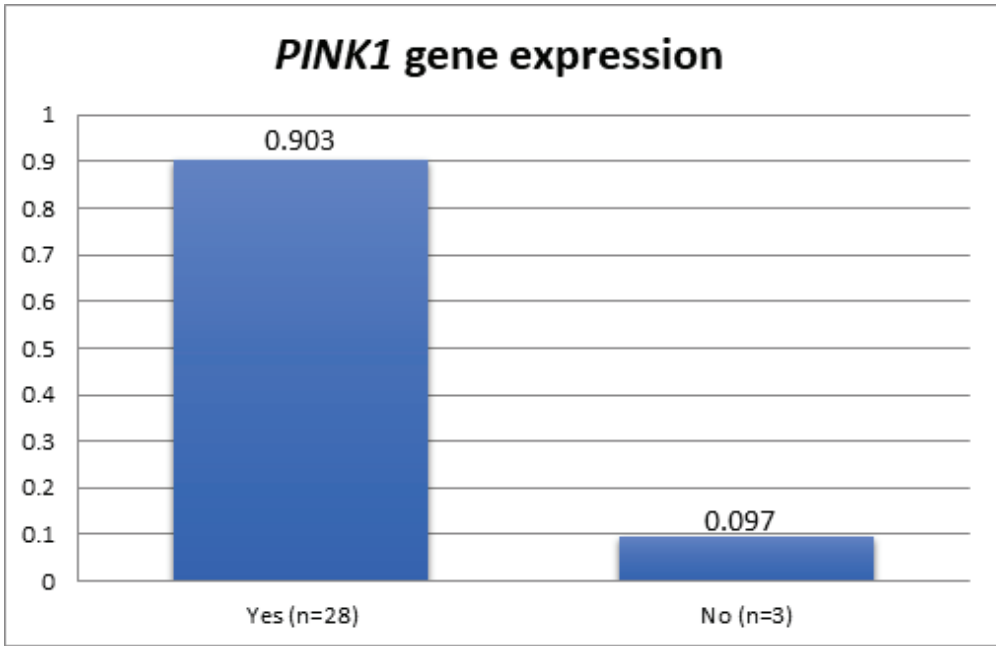

Figure 4: *PINK1* gene expression distribution among the study group.

The association between PINK1 gene and age at onset: 100% of the patients in age group less than 20, 81.8% in age group 20–40 yr, and 91.7% of the patients in age group 40–50 ys expressed *PINK1* gene. But the difference was statistically insignificant (*P* = 0.408; Figure 5).

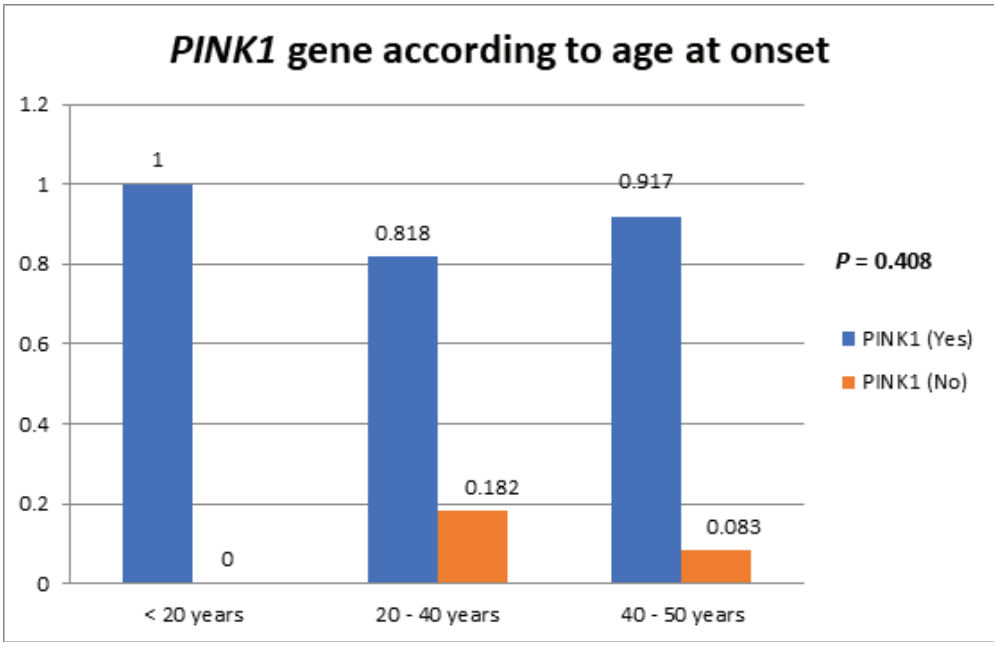

Figure 5: *PINK1* gene expression according to the age at onset.

Symptoms: 29 (93.5%) patients had tremor, 27 (87.1%) had rigidity, 23 (74.2%) had bradykinesia, and 24 (77.4%) had tremor as initial symptoms.

27 (87.1%) patients had hand tremor, it was bilateral in 24 (85.7%) and symmetrical in 6 (25%), 12 (54.5%) had both types of rigidity (cogwheel and lead pipe), 30 (97%) had monotonous speech (Tables 1 and 2); 20 patients (64.5%) were married, 8 (40%) were endogamous married and 5 (62.5%) were second-degree and 3 (37.5%) were third-degree relative; 17 (85%) patients had children, 2 (10%) had children with PD **Table(3).**

TABLE 1: The symptoms of *PINK1* type early onset PD.

Symptoms	N	%
Tremor	29	93.5
Rigidity	27	87.1
Bradykinesia	23	74.2
Other	4	12.9

Family History: All of the patients have positive family history (100%) of PD and had 1–3 members of family with PD: 8 (57.1%) of them were first-degree members; 28.6% were first- and second-degrees members, and 14.4% were third degree (Table 4).

Family members' symptoms: 14 (100%) had rigidity, 13 (92.2%) had tremor, and 4 (28.6%) had bradykinesia.

Table 2: The signs of *PINK1* early onset PD among the patients.

Signs	N	%
Tremor site		
• Hand	27	**87.1**
• Head	1	**3.2**
Bilateral hand tremor	24	**85.7**
• Symmetrical	6	**25**
• Non-symmetrical	18	**75**
Rigidity type (n = 22)		
• Cog-wheel	9	**40.9**
• Lead pipe	1	**4.5**
• Both	12	**54.5**

Table 3: Distribution of marital status, endogamous marriage and degree of relativity in endogamous marriage among the study group.

	N	%
Marital status (n = 31)		
• Married	20	64.5
• Unmarried	11	35.5
Endogamous marriage (n = 20)		
• Yes	8	40
• No	12	60
Degree of relativity in endogamous marriage (n = 8)		
• Second degree	5	62.5
• Third degree	3	37.5

Initial symptoms: 12 (85.7%) of them developed tremor and 2 (14.3%) developed rigidity.

Age and gender of the family members: 9 (64.3%) were in age group 20–35 yr, followed by 21.4% that were in age group 36–50 yr and 14.3% > 50 years; 7 (50%) were males.

Brain imaging: 20 (64%) patients underwent CT brain, which was normal, 11 (36%) underwent MRI brain, which was also normal.

Types of treatment: 23 (74%) patient used combined akisol and levocare, 5 (16%) used Akisol, and 3 (10%) used levocare alone.

Association between *PINK1* gene expression age: all of the patients (100%) in age group –40 yr expressed *PINK1* gene, followed by 89.5% in the age group 41–50 yr, and 88.9% in age group 20–30 yr, the difference was statistically insignificant (P = 0.836). **Gender**:

Table 4: Characteristics of family members of *PINK1* early onset PD.

	N	%
Affected members (n = 14)		
• 1–3 members	14	100
Degree of relativity (n = 14)		
• First degree	8	57.1
• Third degree	2	14.4
• First and second degrees	4	28.6
Age (n = 14)		
• 20–35	9	64.3
• 36–50	3	21.4
• > 50	2	14.3
Gender (n = 14)		
• Male	7	50.0
• Female	4	28.6
• Both	3	21.4
Family blood genetic screening (n = 14)		
No	14	100

91.7% of the female patients and 89.5% male expressed *PINK1* gene, the difference was statistically insignificant (P = 0.672).

Expression of *PINK1* gene according to the family history: 92.9% of those with positive family history and 88.2% with negative family history group expressed *PINK1* gene. The difference was statistically insignificant (P = 0.575). Also, all the patients had third-degree member- and first- and second-degree member-expressed *PINK1* gene, 85.7% of the patients had first-degree relative-expressed *PINK1* gene. The difference was statistically insignificant (P = 0.629) (Tables 5 and 6).

4. Discussion

This study included 31 PD patients, among them males (61%) were affected more than females (39%) with male to female ratio of 1.6:1; this is similar to other study conducted by Khalda et al. in Sudan who found that male:female in PD was 1.5:1 [14].

The peak incidence of PD was found to be in the age group 41–50 yr (61%), mean age was 33.4 yr, which was similar to the literature and Witjas et al. [4, 15].

Also, our study showed that most patients had tremor (93.5%), rigidity (87.1%), and bradykinesia (74.2%); 77.4% had tremor as initial symptoms, this similar to the findings of Khalda et al. [14] and goes well with what was mentioned in the literature [15].

Table 5: Shows the association between *PINK1* gene expression and demographic data of the study group.

	PINK1 gene expression		P-value
	Yes (%)	No (%)	
Age			0.836
• 20–30	8 (88.9)	1 (11.1)	
• 31–40	3 (100)	0 (0)	
• 41–50	17 (89.5)	2 (10.5)	
Gender			0.672
• Male	17 (89.5)	2 (10.5)	
• Female	11 (91.7)	1 (8.3)	
Marital status			0.719
• Married	18 (90)	2 (10)	
• Unmarried	10 (90.9)	1 (9.1)	
Chi-square test was used.			

Table 6: Shows the association between *PINK1* gene with affected family members.

	PINK1 gene expression		P-value
	Yes (%)	No (%)	
Positive family history			0.575
• Yes	13 (92.9)	1 (7.1)	
• No	15 (88.2)	2 (11.8)	
Degree of relativity (n = 14)			
• First degree	6 (85.7)	1 (14.3)	0.629
• Third degree	2 (100)	0 (0)	
• First and second degrees	4 (100)	0 (0)	

Regarding the PD signs, the majority of the subjects (87.1%) had hand tremor, it was bilateral in 85.7% and symmetrical in 25%; 54.5% had both types of rigidity (cogwheel and lead pipe) and this was in favor with what was mentioned in the literature [15].

The current study revealed that 45% of the patients had positive family history to PD, this result was similar to Bentio et al.'s in Denmark [16], and differ from the study of Khalda et al. [14] who found that small percentage of the PD patients (11.7%) had positive family history.

Regarding *PINK1* gene expression, this study presented that 90.3% of the patients showed positive result. This is similar to those reported by Koziorowski et al who found that mutations were identified only in the *PARK2* and *PINK1* genes with the frequency of 84.7% and 82.7% of subjects, respectively, and they conclude that the frequency of the PARK2 and *PINK1* mutations among Polish EO–PD patients seems to be high [17]. Also, mutations in *PINK1* were initially identified in early onset, autosomal recessive kindreds. Point mutations, frame shift mutations, and truncating mutations have been reported

throughout the gene. In addition, no significant associations were found between *PINK1* expression and age, gender, age at onset, and family history ($P > 0.05$). These findings go in line with what was mentioned in literature [18].

5. Conclusion

This study concludes that *PINK1* gene seems predominant in Sudanese patients. It was common among males than females. The most affected age group was found to be in the third and fourth decades. The patterns of the clinical features were generally similar to the literature. *PINK1* expression was predominant with no significant associations between *PINK1* expression and age, gender, age at onset, and family history. *PINK1* gene testing is recommended in all EOPD Sudanese patients.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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