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RESEARCH ARTICLE

INSERTION C V317RFS*75 WITH FRAME SHIFT MUTATION, WAS SHOWN TO BE MORE FREQUENT IN PINK1 GENE IN SUDANESE PATIENTS.

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Abstract

Introduction: Descriptions of Parkinson's disease has been known as far as 5000 BC and is the second most common neurodegenerative disorder after Alzheimer disease, and the most common movement disorder. In 1997, a genetic linkage analysis showed that chromosome 6q25.2-27 harboured an unidentified gene responsible for autosomal recessive juvenile Parkinsonism (ARJP) in 13 Japanese families. One year later, the Shimizu group cloned the AR-JP gene, PARK2, and identified more Japanese AR-JP patients with either an exon 4 or a large-scale deletion between exons 3 and 7.

The objective of this study is to screen Pink1 gene exon 4 in early onset Parkinson's disease among Sudanese patients.

Material and methods: This is a pilot study that included 51 samples from early onset Parkinson's disease patients diagnosed clinically at the National Centre of Neurological Sciences, during the period of December 2016 to December 2017.

Results: ins. C in exon 4 of PINK1 gene was detected in 51.9% of the samples, C>T mutation (rs142183624) was found in 41% of the samples. In addition to that, polymorphisms; G>C was detected in one sample, and T>C (L314P) in two samples.

Conclusion: Insertion C V317Rfs*75 with frame shift mutation, was shown to be more frequent in Pink1 gene in our data.

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Introduction:-

Descriptions of Parkinson's disease has been known as far as 5000 BC and is the second most common neurodegenerative disorder after Alzheimer disease, and the most common movement disorder. In the United States, at least half a million people are diagnosed as having PD. [1]. Both prevalence and incidence of PD vary greatly across age groups. PD is less common before 50 years of age and increases steadily with age thereafter up to the ninth decade. Hence the rate of Parkinson's disease rises sharply after the fifth decade [2]

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Tremor is the most common and easily recognised symptom of PD. Tremors are unilateral, occur at a frequency between 4 and 6 Hz, and almost always are prominent in the distal part of an extremity. [3] Rigidity is characterised by increased resistance, usually accompanied by the “cogwheel” phenomenon, particularly when associated with an underlying tremor, present throughout the range of passive movement of a limb. [4] Bradykinesia refers to slowness of movement and is a hallmark of basal ganglia disorders. [5] Although it is well known that lack of dopamine causes the motor symptoms of Parkinson's disease, it is not clear why the dopamine-producing brain cells deteriorate. Genetic and pathological studies have revealed that various dysfunctional cellular processes, inflammation, and [stress](#) can all contribute to cell damage. [5] In addition, abnormal clumps called Lewy bodies, which contain the protein alpha-synuclein, are found in many brain cells of individuals with Parkinson's disease. [5] The function of these clumps in regards to Parkinson's disease is not understood. In general, scientists suspect that dopamine loss is due to a combination of genetic and environmental factors.

Pink1 gene:-

In 1997, a genetic linkage analysis showed that chromosome 6q25.2-27 harbored an unidentified gene responsible for autosomal recessive juvenile parkinsonism (ARJP) in 13 Japanese families [6]. One year later, the Shimizu group cloned the AR-JP gene, *PARK2*, and identified more Japanese AR-JP patients with either an exon 4 or a large-scale deletion between exons 3 and 7. [7] Other patients of various ethnicities with early-onset PD were soon reported to also harbour *PARK2* mutations with varying deletions or point mutations that cause *PARK2* protein loss of function [8] *PARK2* contains 12 exons that encode the 465 amino acid protein, Parkin [7]. Parkin is an E3 ubiquitin ligase with an amino-terminal ubiquitin-like (Ubl) domain and a carboxyl-terminal ubiquitin ligase domain [9]. Study done by Rasol et al, mentioned that, The PINK1/Parkin pathway is one of the best known pathways involved in mitochondrial repair and removal of damaged mitochondria [10, 11]. The first phenotypic studies relating PINK and Parkin to Parkinson's disease came from drosophila; PINK1 mutants produced motor dysfunction phenotypes in drosophila including defects in climbing and flying ability, wing and flight muscle defects and some decrease in the number of dopaminergic neurons [12], and electron microscopy studies indicated that PINK1 and Parkin null flies exhibited defects in mitochondrial morphology [13].

Objective:-

To screen Pink1 gene mutations in early onset Parkinson's disease (EOPD) among Sudanese patients.

Material and Method:-

This is a pilot study that had been performed at the National Centre for Neurological Sciences during December 2016 to December 2017.

The study included samples from 51 early onset Parkinson's disease patients diagnosed clinically during the above mentioned period. The study was conducted in accordance with the guidelines of the local ethical committee. Blood samples were taken in sterile containers that contained (EDTA) anti coagulant and processed for DNA extraction. Clinical and demographic data were collected using predesigned structured interview questionnaire. Furthermore 31 samples were sent to (Macrogen Korea) for sequencing. The personal data of all patients were obtained from the registry data base in the National Centre of Neurological Sciences, and the laboratory data were collected from Parkinson's patient's blood PCR results.

DNA extraction from blood samples:-

The DNA extraction was done by using guanidine chloride method, two millilitre of peripheral blood from patient with Parkinson's disease was placed into Falcon tube (15 ml), 10 ml from red cell lyses buffer was added, then the tubes were gently mixed by using vortex mixer, then centrifuged at 6000 RPM for 10 minutes; this step was repeated until clear pallet was obtained, then 2 ml from White cell lysis buffer, 1 ml from guanidine chloride, 350 µl of ammonium acetate and 20 µl of proteinase K were added, the tubes were vortexed and then incubated at 37 °C over night. After incubation the tubes were vortexed and 2 ml from pre chilled chloroform was added, the tubes were mixed by using vortex mixer, after that the tubes were centrifuged at 6000 RPM for 10 minutes, then the supernatant was transferred into a new Falcon tube (15 ml), 8 ml of pre chilled ethanol was added to each tube with gentle mixing to precipitate the DNA, for completion of DNA precipitation the tubes were incubated at -20 °C for 2 hours, after incubation the tubes were centrifuged at 6000 RPM for 10 minutes, then the ethanol was poured into disposal bottle, after that 4 ml of 70% alcohol was added and the tubes were centrifuged at 6000 RPM for 10 minutes, the 70% alcohol was poured into disposal bottle, and the tubes were blotted on filter paper, and then left

to air dry. After completion of drying 100 µl of Elution buffer was added, then after that the tubes were incubated at 4 °C for completion of DNA elution.

PCR amplification of PINK 1 gene:-

Primers for PINK 1 gene exon 4 were designed using primer3 software, forward primer and reverse primer were used for amplification of PINK 1 gene. Using 2% Agarose gel electrophoresis amplified PCR products were visualized

Results:-

Demographic and clinical results:-

In the present study males were 33 and females were 18 (Table 1). The most affected age group ranging from 41-50 years in 56.8% of the studied material (Table 2). In this study all patients were presented with the cardinal features of Parkinsonism (tremor, bradykinesia and rigidity), in addition to that, the tremor symptoms in this study was shown to be the first symptom, that was found in 84% of the patients (Table 3).

Molecular results:-

The sequencing results showed that, ins. C in exon 4 of PINK1 gene was detected in 51.9% of the samples at position (V317Rfs*75) Fig. (1)

Rs142183624 was detected in 41% of the samples. In addition to that, polymorphisms; G>C was detected in one sample, and T>C (L314P) in two samples. Figures (2, 3). The effect of mutations upon the proteins was displayed in figures (4, and 5). Figure 6, shows results obtained by Mutation **taster program**

Table 1:-shows the frequency distribution of gender among the patients

		gender			
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	male	33	64.7	64.7	64.7
	female	18	35.3	35.3	100.0
	Total	51	100.0	100.0	

Table 2:- shows the frequency distribution of age group among the patients

Age group	Frequency	Percent
20-30 years	10	19.6%
31-40 years	12	23.6%
41-50 years	29	56.8%
Total	51	100%

Table 3:-shows the frequency distribution of cardinal symptoms of Parkinson's disease among the patients

Symptoms	Frequency	Percent
Tremor	43	84.3%
Bradykinesia	3	5.9%
Rigidity	5	9.8%
Total	51	100%

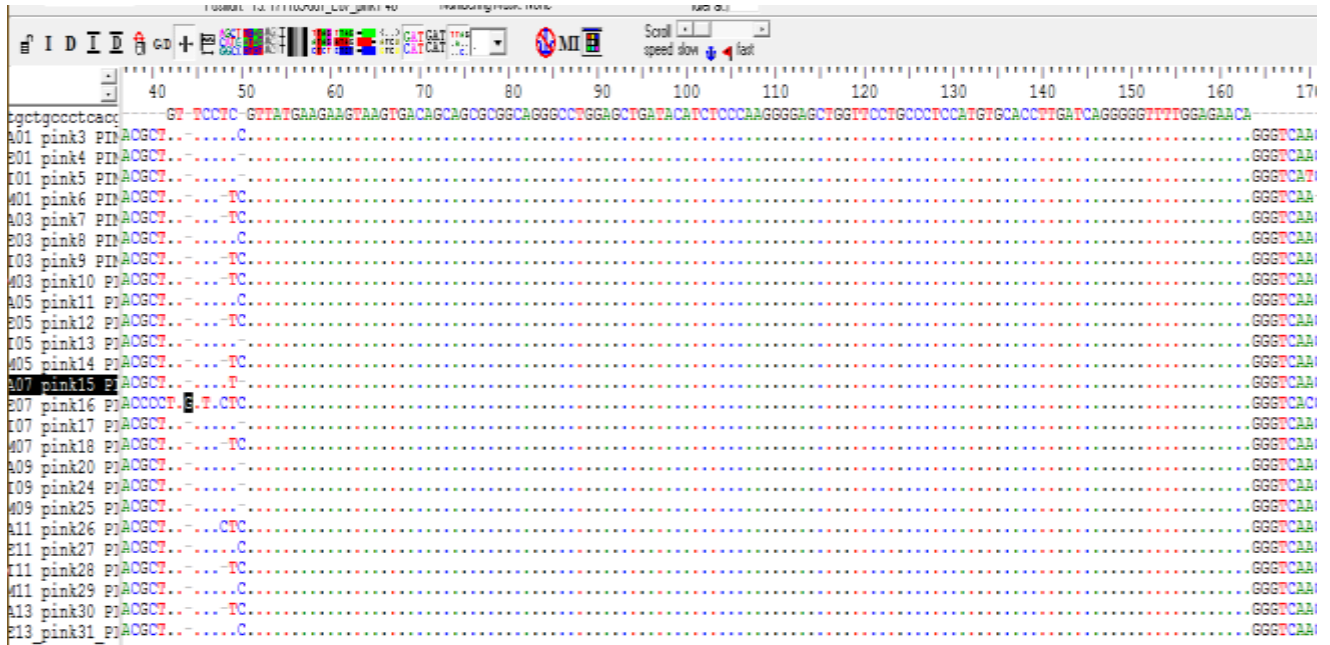


Fig. 1:-shows the multiple alignments of the PINK1 gene reference sequence with the sequenced DNA of the samples, ins. C and substitution C>T were indicated with arrow, in Sudanese patients with Parkinson’s disease

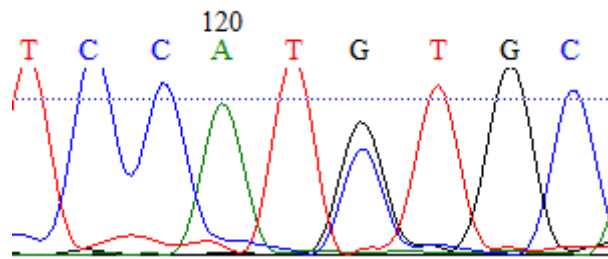


Fig. 2:- shows polymorphism G>C in Sudanese patients with Parkinson’s disease

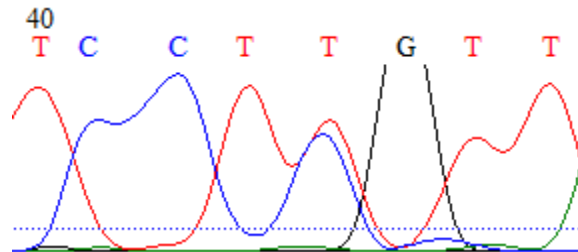


Fig. 3:- shows polymorphism T>C in Sudanese patients with Parkinson’s disease

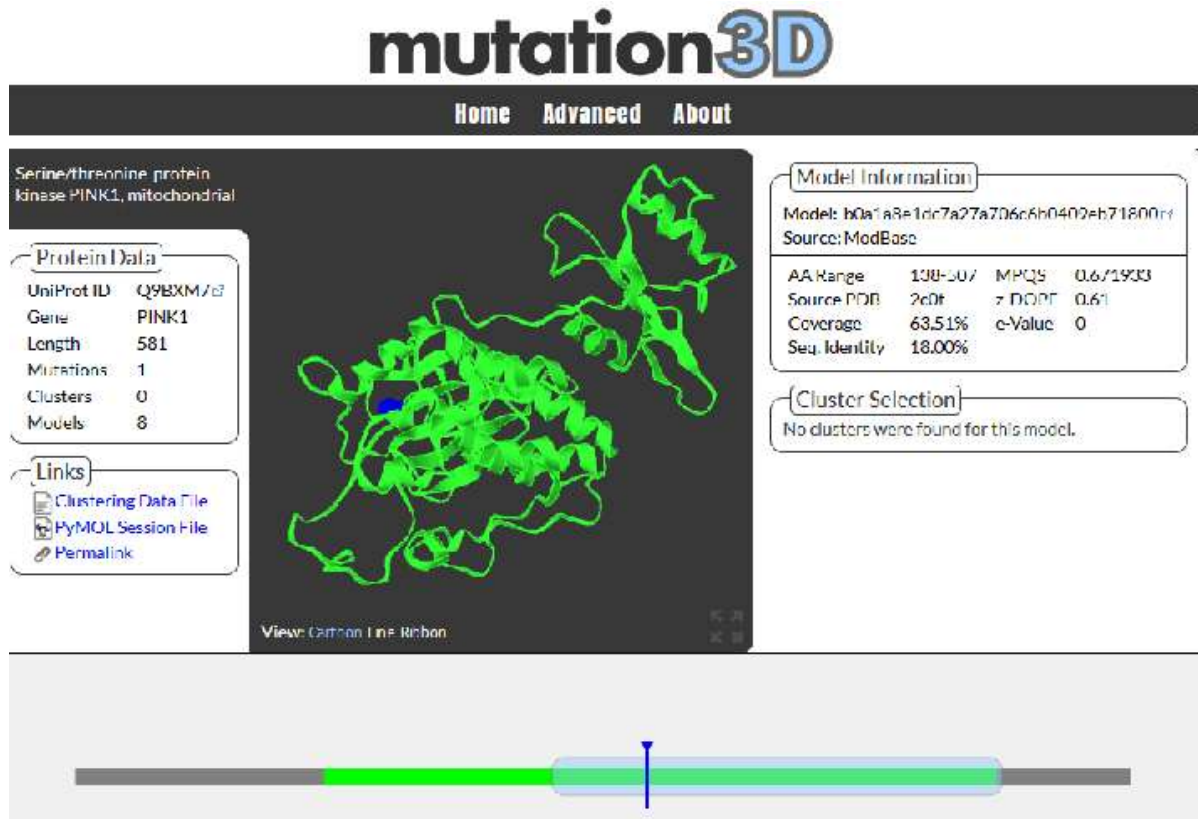


Fig. 4:-shows the location of polymorphism T>C upon the protein (L314P), using **Mutation 3D program**

alteration type	insertion
alteration region	CDS
DNA changes	c.948_949insC cDNA.1042_1043insC g.11207_11208insC
AA changes	V317Rfs*75
position(s) of altered AA if AA alteration in CDS	317 (frameshift or PTC - further changes downstream)
frameshift	yes
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC. known disease mutation at this position, please check HGMD for details (HGMD ID CM065381)
regulatory features	H3K36me3, Histone, Histone 3 Lysine 36 Tri-Methylation
phyloP / phastCons	PhyloP PhastCons (flanking) -0.445 0.891 (flanking) 5.02 0.997 explain score(s) and/or inspect your position(s) in in UCSC Genome Browser

Fig. 5:-shows the alteration type, AA changes, position and type of insertion C mutation, using **Mutation taster program**

original gDNA sequence snippet	ATGGCCGGACGCTGTTCCCTCGTTATGAAGAAGTAAGTGAC
altered gDNA sequence snippet	ATGGCCGGACGCTGTTCCCTC C GTTATGAAGAAGTAAGTGAC
original cDNA sequence snippet	ATGGCCGGACGCTGTTCCCTCGTTATGAAGAAGTATCCCTG
altered cDNA sequence snippet	ATGGCCGGACGCTGTTCCCTC C GTTATGAAGAAGTATCCCTG

Fig. 6:-shows the original DNA sequence and altered DNA sequence snippet of insertion C mutation, using **Mutation taster program**



Fig. 1:- shows the multiple alignments of the PINK1 gene reference sequence with the sequenced DNA of the samples, ins. C and substitution C>T were indicated with arrow, in Sudanese patients with Parkinson’s disease

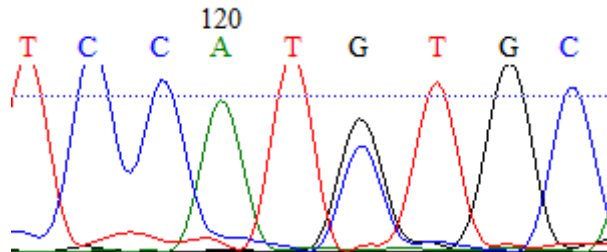


Fig. 2:-shows polymorphism G>C in Sudanese patients with Parkinson’s disease

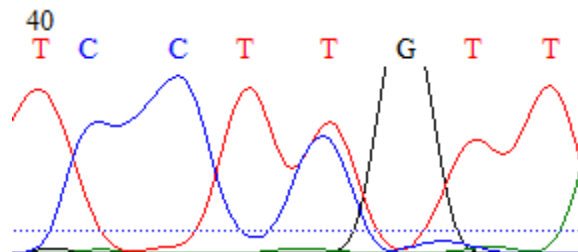


Fig. 3:-shows polymorphism T>C in Sudanese patients with Parkinson’s disease

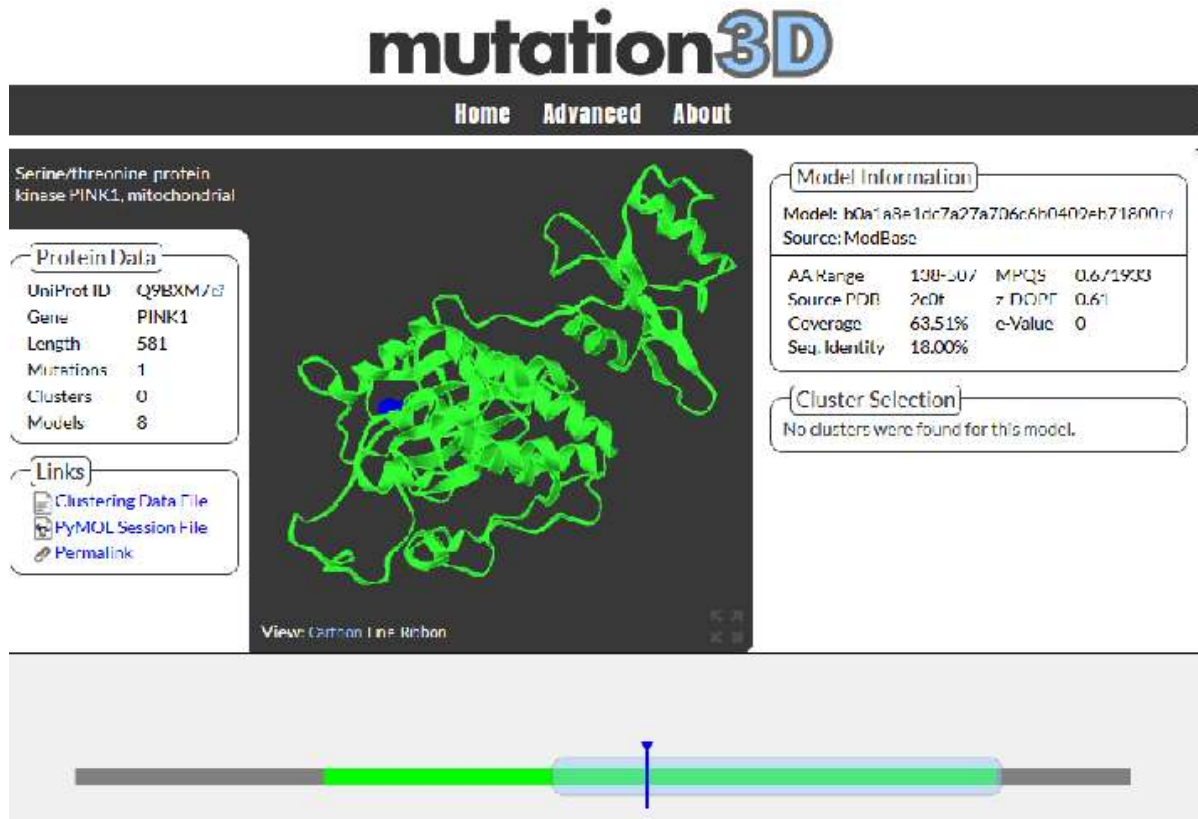


Fig. 4:-shows the location of polymorphism T>C upon the protein (L314P), using Mutation 3D program

alteration type	insertion
alteration region	CDS
DNA changes	c.948_949insC cDNA.1042_1043insC g.11207_11208insC
AA changes	V317Rfs*75
position(s) of altered AA if AA alteration in CDS	317 (frameshift or PTC - further changes downstream)
frameshift	yes
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC. known disease mutation at this position, please check HGMD for details (HGMD ID CM065381)
regulatory features	H3K36me3, Histone, Histone 3 Lysine 36 Tri-Methylation
phyloP / phastCons	PhyloP PhastCons (flanking) -0.445 0.891 (flanking) 5.02 0.997 explain score(s) and/or inspect your position(s) in in UCSC Genome Browser

Fig. 5:-shows the alteration type, AA changes, position and type of insertion C mutation, using Mutation taster program

original gDNA sequence snippet	ATGGCCGGACGCTGTTCCCTCGTTATGAAGAAGTAAGTGAC
altered gDNA sequence snippet	ATGGCCGGACGCTGTTCCCTC C GTTATGAAGAAGTAAGTGAC
original cDNA sequence snippet	ATGGCCGGACGCTGTTCCCTCGTTATGAAGAACTATCCCTG
altered cDNA sequence snippet	ATGGCCGGACGCTGTTCCCTC C GTTATGAAGAACTATCCCTG

Fig. 6:-shows the original DNA sequence and altered DNA sequence snippet of insertion C mutation, using **Mutation taster program**

Discussion:-

Pink1 gene mutations have been set as a causative genetic alteration for Parkinsonism. Pink1 gene is coding for a mitochondrial protein kinase Nickolas wood with collaboration with other investigators from the UK, Italy, Germany, the USA and Spain, found that, Pink1 gene and PARK6 mutations were associated with early onset Parkinson's disease. [14] Enza Maria Valente and colleagues reported that, G>A mutation was found in members of the Spanish family in exon 4 while both Italian families were affected by a similar G-to-A transition in exon 7. [15]

In this study Pink1 gene exon 4 was sequenced for the first time in Sudanese patients with Parkinson's disease. The sequencing results showed that, insertion C at position (V317Rfs*75) was detected in 51% of the patients. rs142183624 (L316L) was detected in 41% of our patients, T>C (L314P) was found in 2 samples and G>C in one sample. Study from Central Norway revealed that, G >A (Gly411Ser) and a novel C>T (Pro498Leu) mutations were been detected in PINK1 gene. [16] Another study from Brazil detected two mutations, G > A (p.A340T) and T > C (p.D391D), the last one (T>C) was been observed in our data. [17] In this study insertion C, was predicted as pathogenic mutation, the prediction was done by using Mutation tester program. Study done by Rafiqua Ben El Haj et al, identified pathogenic mutation in PINK1 gene. [18] Another study identified insertion CAA (c.1602_1603 ins CAA) [19]. International data revealed that, several mutations in Pink1 gene were identified, T313M in Chinese [20], V317I in Caucasian [21], and M318L in North American [22]. Our study showed V317R with frame shift mutation, L316L and L314P mutations. This is preliminary work, and we are aiming to investigate the PINK1 gene in a big sample size in the future.

Conclusion:-

Insertion C V317Rfs*75 with frame shift mutation, was detected in 51% of studied materials, C>T mutation (rs142183624) was found in 41% of the samples at position L316L of the protein.

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Availability of data and materials:-

The original data are stored at the NCNS and corresponding author and can be obtained from them at any time.

Authors' contributions:-

The all authors are participated in this work, the participation included study design, data collection and analysis, drafting and revising the paper. The all authors are approved the final submitted manuscript.

Ethics approval and consent to participate:-

This work was approved by the local ethical committee at the NCNS, Khartoum, Sudan

Competing interests:-

The authors declare that they have no competing interests.

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