PARAXONASE-1 GENE POLYMORPHISM AND PARKINSON'S DISEASE IN PESTICIDES EXPOSED PATIENTS

BY

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ABSTRACT

Background: Chronic low-level exposure to organophosphates is a significant hazard for the human. It was reported to have an adverse neurological complication, including Parkinson's disease (PD). Studying gene-environment interactions may elucidate the complex origins of idiopathic Parkinson's disease. **Aim of the study:** To investigate the relation between PON1 polymorphisms ($PON1_{192Q/R}$) and its enzymatic activity and PD in patients with chronic exposure to organophosphorus pesticides. **Patients and Methods:** This study was conducted on 190 PD and 190 healthy persons. All participants were subjected to urine analysis for dialkyl phosphate metabolites (DAPs) detection, serum paraoxonase/arylesterase activity, and blood samples subjected to isolation of genomic DNA for PON1 polymorphisms genotyping using polymerase chain reaction–restriction fragment length polymorphism method. **Results**: There was no significant difference between patient and control groups regarding socio-demographic characteristics except for occupational history, as 85.3% of cases reported a history of pesticide exposure for more than ten years (56.8%). GC-MS analysis of urine samples revealed that 91.9% of cases have statistically significant detectable DAPs metabolites compared to 24.2% of controls. The mean value of serum PON1/arylesterase activity was statistically significantly lower in PD than in the control. The study revealed that RR homozygotes and R allele subjects showed a higher risk for PD ($P<0.001$ for each). **Conclusion:** The present study indicated that PON1 gene polymorphisms may contribute to susceptibility to Parkinson's disease in Egyptians. Parkinson's disease may importantly be influenced by PON1 polymorphism in interaction with organophosphorus exposure.

Keywords: *Organophosphates, Parkinson's disease, Paraoxonase, Polymorphism*

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INTRODUCTION

Parkinson's disease (PD) is an idiopathic neurodegenerative disorder marked pathologically by the loss of dopaminergic neurons in the midbrain and the existence of Lewy bodies. Even though the exact etiology of PD is unidentified, it is multifactorial with genomic and environmental influences ***(Goldman, 2014; Islam et al., 2021)***.

Oxidative stress has a significant role in dopaminergic neuron disintegration in PD. substantia nigra, through induction of the oxidation-reduction potential in neurons that hinder numerous biological processes, eventually resulting in cell death. Production of reactive oxygen species (ROS) has numerous sources and mechanisms, including dopamine metabolism, mitochondrial dysfunction, neuro-inflammatory cells, aging, and environmental pollution ***(Dias et al., 2013; Vellingiri et al., 2022)***.

Pesticides are poisonous compounds frequently used in agriculture to eliminate insects and plant infections. Also, they are applied in additional procedures, such as food

preservation and environmental uses, such as removing unwanted aquatic weeds and herbs. A third of the world's crop output has been safeguarded using pesticides ***(Samsidar et al., 2018)***. ***Maluin and Hussein (2020)*** stated that two million tons of insecticides are utilized annually.

Although several pesticides have long been barred in Western countries due to safety concerns, they are widely used in Egypt and subject to low safety controls ***(Mansour, 2004)***. Currently, 40% of Egyptian workers work in agriculture, increasing the probability of being in close contact with pesticides ***(Rohlman et al., 2014)***. Additionally, Egypt has a three-fold residence in rural over urban, with a prevalence of 2,500–2,750 PD patients per 100,000 in various governorates ***(Khedr et al., 2012; Khedr et al., 2015)***. Comparatively speaking, this is a huge gain, especially when compared to nearby Arab Nations ***(Benamer et al., 2008)***.

Nevertheless, occupational pesticide exposure has been a problem, especially chronic low-dose exposure brought on by disregarding safety precautions or improper pesticide handling ***(Damalas and Koutroubas, 2016)***.

Pesticides are recognized to have a neurotoxic effect by inhibiting central cholinesterase. In addition, they have been implicated in oxidative stress, dopamine transporters alterations, dysfunction of the mitochondria, α-synuclein fibrillation, and neuro-inflammation ***(Vellingiri et al., 2022)***.

Six dialkyl phosphates (DAP), the final products of the metabolism of most organophosphorus (OP) chemicals, are eliminated in the urine. According to ***Wessels et al. (2003)***, there are six metabolites, including Dimethylphosphate, dimethylthiophosphate, dimethyl dithio phosphate, diethylphosphate, diethylthio phosphate, and diethyldithiophosphate. DAP metabolites in the urine offer insightful data on cumulative pesticide exposure ***(Barr et al., 2004)***. ***Forsberg et al. (2011)*** presented them as a biomarker of human exposure to organophosphorus.

Human paraoxonase 1 (PON1) is an enzyme implicated in lipoprotein metabolism with inhibition formation of oxidized low-density lipoprotein (LDL). It has athero-protective and anti-inflammatory properties. Also, it has a role in the hydrolysis of the bioactive oxons of organophosphate pesticide compounds to aid in chemical detoxification. Differences in PON1 enzyme expression and catalytic activity are caused by polymorphism of the PON1 gene ***(Mota et al., 2019)***.

Polymorphisms of paraoxonase 1 (PON1) in humans are exhibited in the coding area, the promoter region, and the 3'-UTR region. Two polymorphisms have been recognized and investigated in the PON1 gene's coding region: (Q192R) and (L55M). Polymorphisms of the human PON1 gene affect either the coding region responsible for the catalytic efficiency of hydrolysis or the promotor, which affects the degree of PON1 expression ***(Brophy et al., 2001)***.

The status of PON1 for each individual is determined in part by their genotype and enzymatic activity. A polymorphism in the PON1 isoforms causes low or high enzyme activity. According to ***Richard et al. (2013)***, people with increased PON1 activity are more expected to be able to detoxify organophosphate, even though those with decreased PON1 activity are more expected to be poisoned by pesticides and to modulate DNA damage via interactions between genes and environmental pesticide exposure, which may have detrimental effects on their health ***(Godoy et al., 2019; Sharma et al., 2019)***. Consequently, the interaction of PON1 polymorphisms and pesticide exposure significantly alters an individual's vulnerability to diseases ***(Benedetti et al., 2014; Teodoro et al., 2019)***.

THE AIM OF THE WORK

The current study aimed to identify whether chronic exposure to organophosphate pesticide, as evaluated by biologic DAP markers in urine, is linked with the risk of PD due to PON1 (Q192R) gene polymorphism and its enzymatic activity in pesticide-exposed patients in an Egyptian cohort.

PATIENTS AND METHODS

- **Design and study participants:**

This case-control study was conducted retrospectively from January 2020 to December 2022. The study sample was comprehensive due to the rare presentation of idiopathic Parkinson's disease (about 8 cases

per month). All presented cases during the study period fulfilled the inclusion criteria and were asked to join the study. All the participants were Egyptian citizens attending the Neurology Clinic, Faculty of Medicine, Zagazig University. The Institutional Review Board of the Faculty of Medicine, Zagazig University (IRB#:9976-9-1-2022) agreed on the study's protocol and methods. Each participant gave their valid agreement before a medical history was recorded, a clinical examination was performed, and urine and blood samples were taken for biochemical studies. Clinical information was gathered, documented namelessly, and assessed. For every portion of data, records confidentiality was maintained.

- **Inclusion and exclusion criteria:**

One hundred and ninety patients with Parkinson's disease (PD), according to the criteria for the diagnosis of idiopathic PD based on the Movement Disorder Society Clinical Diagnostic Criteria for PD ***(Postuma et al., 2015)***, were included. All patients underwent a detailed history-taking that covered their personal information (age, gender, place of residence), occupation, medical history (year of diagnosis, duration of illness, medications, family history), level of education (literacy, education years), and smoking habits (number of years and quantity of cigarettes or shisha smoked). The Hoehn and Yahr scale was applied to determine the severity of the disease ***(Goetz et al., 2004)***. History of exposure to pesticide and duration of exposure. Patients exhibiting atypical Parkinson's syndromes, known as secondary Parkinson's disease, cerebrovascular stroke, other movement disorders, or other neurodegenerative disorders, such as motor neuron disease or Alzheimer's, were also excluded. Seventeen cases were missed during the study due to several causes, either uncooperative, due to personal causes or cognitive affection, or unclear history. Only one hundred and seventy-three cases of PD. complete the study.

As a control group, one hundred and ninety neurologically healthy subjects with similar age, gender, residence, and occupation were chosen. The control group was chosen from healthy individuals considering not involve direct relatives of the patients or having any family history of neurological diseases. Age matching was done in five years intervals.

All patients and the control group were subjected to urine samples to detect organophosphorus metabolites, which were used to identify exposure, and a blood sample test to assess and investigate the PON1 Q192R gene polymorphism and its enzymatic activity.

A- Urinary organophosphorus metabolites detection:

For each subject in the study, 10 ml of void urine (first morning) was collected in a polypropylene container ***(Kissel et al., 2005)***. The containers were prewashed for over three hours in 10% nitric acid, followed by two rinses in filtered water. The samples were stored at -20^{o}C for organophosphorus metabolite detection. ***Tarbah et al. (2004)***, stated that DAP metabolites are stable under storage at -20^{o}C. Preparation was made according to the method designated by ***Hardt and Angerer (2000)***. Briefly, urine was thawed before analysis, and the internal standard was added. To make the internal standard, 50 mg of dibutylphosphate was thawed in 50 mL of methanol, and the mixture was then diluted with water to a concentration of 10 mg/L. Then, HCL was used for acidification, followed by two liquid/liquid extraction cycles with a 1:1 mixture of diethyl ether and acetonitrile. The organic layer was vaporized until desiccation, and the deposit was thawed in dehydrated acetonitrile. The DAP metabolites were derivatized using pentafluorobenzyl bromide (PFBBr). Water and hexane were added, and the DAPs were shifted to the hexane portion by shaking the tubes. GC-MS examined the hexane portion.

The National Research Centre's Central Lab in El-Dokki, Cairo, conducted the GC-MS analysis. Helium was used as a carrier gas with a splitless injection of 1µL. The temperature programming was as follows: injection port temperature was 260^{o}C; column 90^{o}C for 1 min, formerly raised at a rate 25^{o}C/min to 120^{o}C and kept for 2 minutes, raised at 6^{o}C/min to 180^{o}C and maintained for 25 minutes, then raised at a rate of 10^{o}C/min to 250^{o}C and isothermal for 34 min; transfer

line temperature 300°C. The mass spectrometer's electron impact (EI) ionization mode was used, with electron energy of 70 eV, multiplier 2300 V, and selected ion monitoring (SIM) mode.

B- Assay of PON1/arylesterase activity:

Two ml Blood samples from patients and healthy subjects were drawn into a tube. Calcium EDTA-containing tubes hinder PON1 activity, so they were not used. The samples were centrifuged (3500 rpm for 10 min) then serum samples were preserved at -20°C till examined. Arylesterase enzyme activity was measured at 270 nm spectrophotometry by determining phenylacetate hydrolysis. The results were stated as U/mL ***(Persico et al., 2008)***.

C-Genotyping analysis of PON1 Q192R polymorphism:

Blood sampling:

Blood samples (2 mL of venous blood) from patients and healthy subjects were drawn into EDTA-containing tubes under aseptic circumstances, and they were subsequently kept at -80°C till DNA extraction and genotyping analysis.

DNA extraction:

Each blood sample underwent genomic DNA extraction using the QIAamp DNA Blood Mini kit (Cat-No: #51104; Qiagen, Valencia, California, USA) per the manufacturer's recommendations. The DNA purity and concentration were assessed separately via electrophoresis (0.7% agarose gel) and spectrophotometer (ND1000; NanoDrop Technologies, Wilmington, Delaware, USA).

PON1 Q192R polymorphism Genotyping:

Genotyping of PON1 Q192R polymorphism was examined via the polymerase chain reaction-restriction fragment length polymorphism (PCR - RFLP) method ***(Hasselwander et al., 1999)***. The following primers were used: ACC: 5'-TAT TGT TGC TGT TGT TGA R: 5'-CAC and G-3' TC-3': GCT AAA CCC AAA TAC ATC. Thermo Fisher Scientific, Massachusetts, USA, provided the PCR master mix, 1.5 liters of each primer (10 pmol/L), and 4.5 liters of sterile deionized water for the 20-liter PCR reaction. In a thermal cycler, the following cycling conditions were carried out. (Biometra, G¨ottingen, Germany): Denaturation takes place first for 5 minutes at 95°C, then by 35 cycles of one minute each at 95°C, 58°C, and 72°C, with a final extension lasting seven minutes at 72°C. The amplified products were digested by FastDigest AlwI restriction enzymes from New England Biolabs in Massachusetts, USA, for 15 minutes at 37°C. The pertinent fragments were parted on a 2.5% agarose gel and observed under U.V. light in a gel credentials procedure (BioDocAnalyze, Biometra, G¨ottingen, Germany).

STATISTICAL ANALYSIS:

The data was digitized and examined statistically by the Statistical Package for Social Science (SPSS) program, version 16 (SPSS Inc., Chicago, IL). Frequencies and percentages represented the qualitative data. Quantitative data were represented using the mean and standard deviation. The chi-square test (X2) was applied for the comparison of qualitative data, the independent sample-t test was applied for comparing between two means of normally distributed data or Mann Whitney U test for not normally distributed data, and the Analysis of variance ANOVA (F) test were applied to compare more than two means of normally distributed data or Kruskal Wallis for not normally distributed data. The odds ratios (ORs) and Z test of significance were used at 95% confidence intervals (CIs) for quantifying the risk related to polymorphisms in patients and controls. Multivariate regression analysis was used to analyze significant predictors among studied cases. The test results were significant when the p-value was less than 0.05 and highly significant when the p-value was less than 0.001.

RESULTS

Table (1) illustrates the socio-demographic features and occupational history among PD patients and the healthy group. There was no significant statistical difference between both groups concerning age, gender, residence, Smoking, and history of head trauma. Regarding occupational history, (85.3%) of PD patients and (72.1%) of the healthy group described a history of exposure to pesticides (p=0.002). Nearly half were exposed to pesticides for over ten years (P<0.001).

Table (1): Socio-demographic characteristics and occupational history among PD patients and control group:

Variables	PATIENT (n=190)	CONTROL (n=190)	P
	No. (%)	No. (%)	
Age group (years) 30 - 40 >40 - 50 >50 – 60	 7 (3.7%) 55 (28.9%) 128 (67.4%)	 15 (7.9%) 65 (34.2%) 110 (57.9%)	0.1
Gender Males Females	 145 (76.3%) 45 (23.7%)	 153 (80.5%) 37 (19.5%)	0.33
Age at the diagnosis (M±SD)	53±7.8		
Disease duration (M±SD)	5.23±2.03		
Rural living: -Most of life. -childhood	 139 (73.2%) 51 (26.8%)	 135 (71.1%) 55 (28.9%)	0.62
Smoking	30 (15.8%)	39 (20.5%)	0.22
Head trauma	8 (4.2%)	11 (5.8%)	0.44
Severity of disease by (Hoehn and Yahr scale): 1-2.5 3-5	 2.6 ± 0.7 117 (61.6%) 73 (38.4%)		
Occupational exposure to pesticide Yes No	 162 (85.3%) 28 (14.7%)	 137 (72.1%) 53 (27.9%)	0.002
Duration of exposure to pesticide <5 year 5-10 Years ≥10 years	 21 (11.1%) 61 (32.1%) 108 (56.8%)	 55 (28.9%) 58 (30.6%) 77 (40.5%)	<0.001

**: P-value<0.05 is significant **: P-value<0.001 is highly significant.*

Organophosphorus Metabolites Detection in Urine:

As shown in **table (2)**, GC-MS analysis of urine samples revealed that 91.9% of patients had statistically significant detectable DAPs metabolites compared to 24.2% of controls (p<0.001).

Serum PON1/arylesterase:

The serum PON1/arylesterase activity mean value was statistically significantly lesser in PD cases (67.7±25.3 U/ml) than in the healthy group (93.8±55.9 U/ml) (p<0.001) **(Table 3)**.

PON1 Q192R polymorphism and Parkinson's disease risk:

In this Egyptian cohort, all three PON1 Q192R gene polymorphism genotypes were observed; QQ Wild-type homozygous (undigested fragment of 99 bp), QR heterozygous (99 bp, 69 bp, and 30 bp), and RR mutant homozygous (digested bands consisting of 69 bp and 30 bp) after restriction digestion. Note that 30 bp was faded and not shown in the gel **(Figure 1)**.

The PON1 Q192R genotype distribution and allele frequencies are shown in **table (4)**, where QQ (homozygous), QR (heterozygous), and RR (homozygous mutated) were observed in 18.5%, 16.8%, and 64.7% of PD patients, respectively, versus 74.2%, 11.1% and 14.7% in control subjects, respectively. The R mutated allele was also detected in 73.1% of PD patients and 20.3% of controls. Regarding the PD risk development, the wild genotype QQ and wild allele Q were used as references. The study revealed that subjects who are QR heterozygotes showed a lower risk for PD (OR 6.08, 95% CI 3.08-12.01, P<0.001) while RR homozygotes and R allele showed higher risk for PD (OR 17.62, 95% CI 10.02–30.99, P<0.001) (OR 10.51, 95% CI 7.58-15.12, P<0.001) respectively.

As regards the association between the PON1 Q192R allele and genotype frequencies and the baseline features of the disease **(Table 5)**, there was a significantly high association between PON1 Q192R and Hoehn and Yahr scale, age of diagnosis and Arylesterase activity (U/ml) (p<0.001). RR genotype and R allele associated with high Hoehn and Yahr

scale, early age of diagnosis, and low amylesterase activity.

T**able (6)** shows a significant positive correlation between the Hoehn and Yahr scale of disease severity and patient's age, duration of disease or pesticide exposure, and R allele of PON1-Q 192R. At the same time, there was a significant negative correlation between the age of diagnosis and arylesterase activity. But after applying Multivariate Analysis for significant predictors of the studied group, the age of diagnosis became a non-significant predictor. At the same time, the other factors were still statistically significant predictors for disease severity.

Table (2): Detectable urinary dialkylphosphates (DAPs) metabolites among both studied groups

Dialkylphosphates (DAPs) metabolites	PD patients (173)		Controls (190)		P value
	N	%	N	%	
Detectable DAPs	159	91.9	46	24.2	<0.001**
Non -detectable DAPs	14	8.1	144	75.8	

***: P-value<0.001 is high significant*

Table (3): The serum PON1/arylesterase activity among both studied groups

Arylesterase activity (U/ml)	PD patients (N=173)	Controls (N=190)	P value
Min –Max	22.63 – 174.72	53.57 – 214.61	<0.001**
Mean ± SD	67.7 ± 25.3	93.8 ± 55.9	
Median	58.4	92.5	

***: P-value<0.001 is high significant*

Reference value: high arylesterase activity >145 U/ml, moderate activity (124-145 U/ml) and low activity <124 U/ml

Table (4): Distribution of PON1 Q192R allele and genotype frequencies in PD patients (N = 173) and the control subjects (N = 190)

Groups PON1 Q192R	PD cases		Control		P	OR (95% CI)
	No.	%	No.	%		
Genotypes						
QQ	32	18.5	141	74.2	-----	1
QR	29	16.8	21	11.1	<0.001**	6.08 (3.08-12.01)
RR	112	64.7	28	14.7	<0.001**	17.62 (10.02-30.99)
Total	173	100	190	100		
Alleles						
Q	93	26.9	303	79.7	<0.001**	10.51 (7.58-15.12)
R	253	73.1	77	20.3		
Total	346	100	380	100		

*OR = Odds Ratio, 95% CI = 95% confidence interval **: P-value<0.001 is high significant*

Table (5): The association between PON1 Q192R allele and genotype frequencies and the baseline features of the disease.

Demographical Data	PON1 Q192R			P			P
	QQ (N=32)	QR (N=29)	RR (N=112)		Q (N=93)	R (N=253)	
Age	52.2±3.23	50.4±5.66	52.1±6.88	0.39** NS	52.3±5.12	53.5± 6.11	0.092$$
Male Gender (n=132)	25 (78.1%)	20 (69.0%)	87 (77.7%)	0.59# NS	-------	-------	------
Hoehn & Yahr Scale	2.4 ± 0.23	3.1 ± 0.55	4.3 ±0.88	<0.001** HS	3.21± 0.62	4.65 ±1.01	<0.001$$ S
Duration of pesticide exposure	9.52 (4-22)	10.12 (5-19)	10.55 (6-22)	0.341* NS	10.22 4-22	10.85 5-22	0.942$ NS
Disease duration	6.12±0.78	6.23±1.11	6.55±1.23	0.11** NS	6.25± 1.65	6.65±1.98	0.083$$ NS
Age of diagnosis	53.8±4.23	52.3±5.87	48.5±6.77	0.002 ** S	51.1±6.18	50.4±7.18	0.405$$ NS
Arylesterase activity (U/ml)	63.3 (35.4-174.7)	59.4 (26.1-146.2	53.8 (22.6-112.3)	<0.001* HS	67.4 35.4-174.7	55.8 22.6-146.2	<0.001$ S

***ANOVA (F) test of significance #Chi-square test *Krusskal-Walis test NS: P-value>0.05 is not significant*
$$Independent t-test S: P-value<0.05 is significant $Mann-Whitney test HS: P-value<0.001 is high significant

Table (6): Univariate and multivariate regression analysis for predictors of disease severity assessed by Hoehn and Yahr scale:

Variables	Univariate		Multivariate		
	r (P-value)	P Value	B	SE	P
Age	0.523	0.004	0.417	0.163	0.01
Age of diagnosis	-0.623	0.001	0.170	0.103	0.32
Disease duration	0.334	0.03	-0.168	0.079	0.034
Pesticide exposure duration	0.423	0.004	-0.349	0.099	0.001
PON1- 192 Q/R	0.441	0.007	0.781	0.280	0.007
Arylesterase activity (U/ml)	-0.512	0.002	2.87	0.984	0.004
	r=0.896 , r^2 =0.803 ANOVA P<0.000* Durbin-Waston ratio=1.768				

NS: P-value>0.05 is not significant S: P-value<0.05 is significant

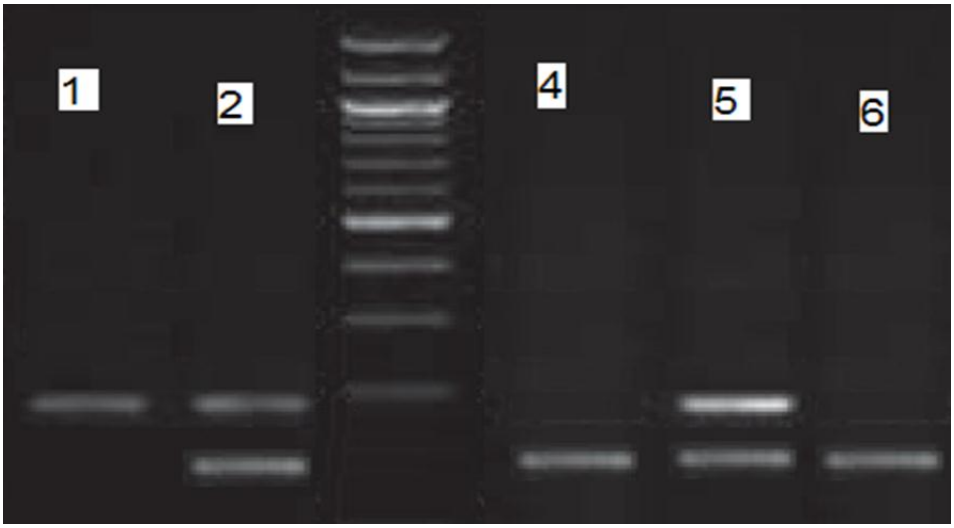

Figure (1): Agarose gel electrophoresis of the genotyping of PON1 Q192R polymorphism using PCR-RFLP method. M: DNA markers (100 bp); Lane 1: QQ wild- type genotype yielded 1 band of 99 bp. Lane 2 and Lane 5: QR heterozygous genotype of 99 bp and 63 bp. Lane 4 and Lane 6: RR homozygous mutant of 69 bp.

DISCUSSION

Environmental facets have been hypothesized to be conceivably related to the PD development, and pesticides mainly organophosphorous (OP) substances are one of the utmost predominant environmental exposures associated with PD ***(Vellingiri et al., 2022)***. The current study aimed to identify whether the PON1 Q192R gene polymorphism and its enzymatic activity, which is included in the detoxification of the pesticides, are related to PD in occupational pesticide-exposed persons.

In the current work, analysis of the socio-demographic data of our patients demonstrated that (67.4%) of patients were older than 50 years old, which correlates with the mean age of PD patients in other studies done in Central California by ***Narayan et al. (2017)***, in Egypt by ***George et al. (2017)*** and ***Tanner et al. (2011)*** and ***Beck et al. (2012)*** conducted studies in Germany found that between the ages of 30 and 70 years, there was a noticeable rise in the risk of PD and history of occupational pesticide usage. These findings contradict the PD prevalence reported by ***de Rijk et al. (2000)***, which was 1.8% in people under 65. Although there is little doubt that getting older raises the risk of PD, the fundamental process of that disease is different from normal aging. Because pesticides are used earlier in Egypt than in European nations, there is a variation in the age at which people are exposed.

Despite having a smaller stratum size and lesser levels of exposure for women, neurodegenerative disorders like PD were present in both sexes. According to ***Parrón et al. (2011)***, ***George et al. (2017)***, and ***Narayan et al. (2017)***, the prevalence was generally higher in male subjects and those living in rural areas. Men comprised 76.3% of the population, while women comprised 23.7%. A sex-specific genomic signature was discovered in dopaminergic neurons of PD patients through gene expression studies: the upregulated genes in women are mostly found in signal transduction and maturation of neurons, even though, in men, the upregulated genes encode proteins implicated in disease development, as PINK1 and alpha-synuclein ***(Cantuti-Castelvetri et al., 2007)***.

Additionally, the D1:D2 ratio was higher in females than males in entirely brain striatum regions examined except the insula, signifying that D1 domination may afford one enduring to disorders (such as; dependence) nevertheless susceptible to others ***(Cullity et al., 2019)***.

The results of our work regarding occupational pesticide exposure agree with those of ***Ritz et al. (2016)*** and ***Narayan et al. (2017)***, which found that OP pesticide exposure at work raises the PD risk. Our results also support the predictions ***Rutz and Krieger (1992)*** and ***Narayan et al. (2017)*** made regarding the exposure length. High P.D. risks were reported with job activities like mixing and loading as they were recognized as higher exposure risks.

Bonetta (2002), ***Richardson et al. (2009)***, ***and Freire and Koifman (2012)***, showed a higher PD incidence among persons exposed to pesticides. A meta-analysis that included 4 cohort, 39 case-control, and 3 cross-sectional studies by ***van der Mark et al. (2012)*** concluded that the chance of PD development increased with pesticide and herbicide exposure.

In the current work, out of 173 cases of PD included in the study, 159 showed positive urinary dialkyl phosphate metabolites, which were evaluated as a biomarker of chronic organophosphate exposure. Although red blood cell choline esterase activity can also be used as a marker of chronic organophosphates exposure, it has the advantage of difficult interpretation due to inter-and intra-individual variation and the lack of baseline values for individuals ***(Wessels et al., 2003)***. According to ***Meeker et al. (2005)***, urinary organophosphate metabolites are frequently the favored method for monitoring exposure since sample gathering is easy and non-invasive and can be measured easily. In addition, ***Cocker et al. (2002)*** stated that urinary organophosphate metabolites are more sensitive than choline esterase activity because they can be estimated at lesser exposure levels.

Additionally, the serum PON1/arylesterase activity mean value in PD cases was significantly lower than in the healthy group ($p<0.001$). This was in line with findings

made by ***Sözmen et al. (2002)***, who discovered that in organophosphate-exposed patients and controls, respectively, PON1/arylesterase activity was determined to be 114.2±67.4 nmol/ml/min and 152.9±78.9 nmol/ml/min ($p<0.05$). This investigation revealed a significant inter-individual variation in the serum PON1/arylesterase activity means, with values in PD cases and controls ranging from 22.63 to 174.72 U/ml and 53.57 to 214.61 U/ml, respectively.

According to ***Singh et al. (2011)***, PON1/arylesterase activity towards phenylacetate was significantly lesser in employees than in controls ($p<0.001$). Even though considering exposure at work.

Furthermore, PON1/arylesterase enzyme activity was shown to be significantly lesser in PD cases than in the control group, according to studies by ***Benmoyal-Segal et al. (2005); Ranjbar et al. (2005); Kirbas et al. (2014); Mota et al. (2019)***. Paraoxonase-1 (PON1) is an essential free-radical scavenger. The accumulation of pesticide residues in the blood might enhance reactive oxygen species production, which declines serum arylesterase activity in the exposed group ***(Mecdad et al., 2011)***.

In this study, concerning PON1 Q192R genotyping, the homozygous mutant gene (RR) was the most predominant genotype in the examined PD cases, then (QQ), then heterozygous (QR), their frequencies were 112 (64.7%), 32 (18.5%) and 29 (16.8%), respectively. This was in agreement with ***Kondo and Yamamoto (1998)***, who reported a significant rise in the RR polymorphism in PD cases compared to the control group. Moreover, an Egyptian study done by ***Abdel Hamid et al. (2017)*** reported the frequencies of PON1 Q192R polymorphism genotyping, homozygous mutated (RR), heterozygous (QR), and homozygous (QQ) were 58.4%, 14.5%, and 27.1% in chronic kidney disease patients of unidentified etiology, respectively.

While ***Mota et al. (2019)*** reported no significant variances in the frequency of the Q192R polymorphisms and alleles among the P.D. patient and control, also, ***Clarimon et al. (2004)*** and ***Akhmedova et al. (2001)*** did not observe significant variances in the frequency of 192 genotypes. Ethnic populations are known as a reason for differences among PON1 genotypes, which may be a possible explanation for controversies between these studies.

In this study, the Q allele frequency in the P.D. cases and healthy control group was 93 (26.9%) and 303 (79.7%), whereas the mutant R allele frequency was 253 (73.1%) and 77 (20.3%), respectively. This was in agreement with ***Abdel Hamid et al. (2017)***, and in agreement with different studies carried out on Egyptian and Turkish individuals displayed reliable data and showed that persons with genotype PON1 RR and R alleles were in increasing danger of organophosphorus poisoning (either acute or chronic) ***(Zayed et al., 2015; Tawfik Khattab et al., 2016; Sunay et al., 2015)***. In contrast, PON1 QQ genotype carriers in Indian research displayed greater susceptibility to organophosphorus poisoning. Exposure to various organophosphorus chemicals with distinct harmful effects and ethnic diversity are two additional factors that may contribute to inconsistent results among studies ***(Mackness et al., 2003; Usman et al., 2021)***.

This work showed that subjects chronically exposed to organophosphorus and PON1 Q192R heterozygous showed a low risk for developing PD while RR homozygous showed a high risk for PD. This was consistent with ***Kondo and Yamamoto (1998)***, who reported a significant upsurge of the PON1 R allele in Parkinson's cases from Japan compared to the control group.

Regarding PON1Q192R genotyping in the studied group, the serum PON1/arylesterase showed the lowest activity in the homozygous mutated gene (RR) followed by heterozygous (QR), which was followed by (QQ) in PD patients. ***Nakanishi et al. (2003)*** demonstrated that the average individual's serum activities of the PON1(Q192) genotype displayed increased arylesterase and declined paraoxonase activity when compared to the PON1(R192) genotype. People with allozyme Q have better defensive efficacy against tissue injury triggered by oxidized LDL than those with R allozyme ***(Shokri et al., 2020)***. Conversely, individuals with Q allozyme have a lesser binding capacity, approximately three

times between PON1 and HDL, than R allozyme ***(Kotur-Stevuljevi'c et al., 2020)***.

On the contrary, ***Mota et al. (2019)*** elaborated that serum PON1/arylesterase activities in PD patients were higher in the RR genotype without significant difference. In contrast, ***Singh et al. (2012)*** found that the PON1 activity was increased in the R/R genotypes with significant differences. Moreover, ***Sirivarasai et al. (2007); López-Flores et al. (2009); Gupta et al. (2011)***, showed that PON1 polymorphisms do not affect arylesterase activity.

The fact that PON1/arylesterase activity may differ up to forty-fold within specified inhabitants and the protein levels may differ up to thirteen-fold for a solitary PON1 192 genotypes can be used to explain these contradictory findings regarding the impact of PON1 polymorphism on PON1/arylesterase activity ***(Bednarska-Makaruket al., 2013)***. In addition, various variables, including genetic variations in other genes, exposure to environmental pollution, or epigenetic modifications, may impact the PON1 expression ***(Huen et al., 2011)***. The large inter-ethnic heterogeneity in PON1 polymorphism reported by ***Rahmani et al. (2002)*** may be the reason for these conflicting results.

Our findings showed that PD patients who carry the RR genotype and R allele have higher disease severity, younger age of onset, and lower arylesterase enzyme activity than those who carry the QR and QQ Genotypes and Q allele, allowing us to investigate whether the clinical profile of the PD patient might be related to the PON1gene polymorphism. This demonstrated that a worse prognosis relates to the RR genotype and R Allele. Additionally, multivariate regression analysis supported our findings and demonstrated that the PON1-192 Q/R gene polymorphism was the most effective predictor of disease severity as assessed by the Hoehn and Yahr scale.

Despite these results, there are some limitations facing this work; relatively small sample size, it was conducted at a single institute, some self-reported covariates such as smoking habit and organophosphorus exposure, and consequently, recall bias is unavoidable.

CONCLUSION

The present study indicated that PON1 gene polymorphisms may contribute to susceptibility to Parkinson's disease in Egyptians. Parkinson's disease may importantly be influenced by PON1 polymorphism in interaction with organophosphorus exposure.

RECOMMENDATIONS

We recommend increasing the awareness of farmers and workers towards the proper dealing and handling of such pesticide products to reduce pesticide exposure to prevent and reduce the risk of PD. Also, we recommend upcoming researches that better understand the roles of the PON1 gene in PD and possible pathogenic mechanisms of PD.

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