



Pesticide Exposure and Its Association with Parkinson's Disease: A Case–Control Analysis

Ali Samareh^{1,2,3} · Hossein Pourghadamyari^{1,3} · Mohammad Hadi Nemtollahi^{1,3} · Hossein Ali Ebrahimi Meimand⁴ · Mohammad Erfan Norouzmahani^{3,5} · Gholamreza Asadikaram^{1,3}

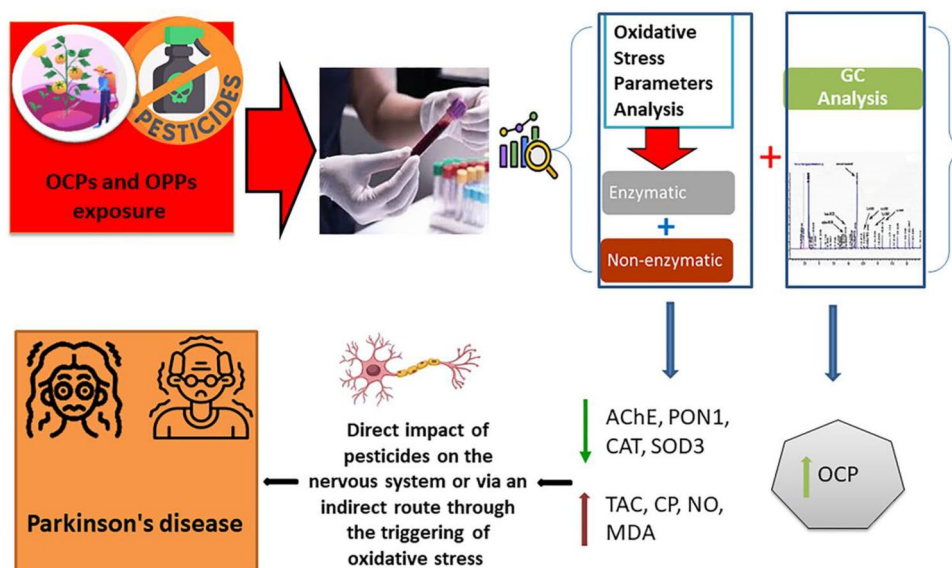
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Abstract

Parkinson's disease (PD) is a complex disorder that arises from genetic and environmental factors. The current investigation endeavors to investigate the role of exposure to organochlorine (OCPs) and organophosphate pesticides (OPPs), recognized as the main environmental elements, in the genesis of PD. In this case–control study, 29 PD patients and 51 healthy subjects were involved. Gas chromatography was performed to measure the serum levels of organochlorine chemicals (2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, α -HCH, β -HCH, and γ -HCH). Furthermore, acetylcholinesterase (AChE) activity, arylesterase activity of paraoxonase-1 (PON-1), and several oxidative stress (OS) markers were assessed. The levels of OCPs in the PD patients were significantly higher than in the control subjects. In addition, AChE activity, arylesterase activity of PON-1, catalase activity, and superoxide dismutase 3 activity in PD patients were significantly less than controls. However, the levels of carbonyl protein, total antioxidant capacity, malondialdehyde, and nitric oxide in PD patients were higher than the controls. The findings of this investigation have indicated that OCPs and OPPs exposure could contribute to the development of Parkinson's disease. This potential linkage could either be established through the direct impact of these pesticides on the nervous system, leading to neurotoxicity, or via an indirect route through the triggering of OS.

Graphical Abstract

Pesticide exposure and its association with Parkinson's disease: a case–control analysis. The study reveals that pesticide exposure may significantly increase the possibility of developing Parkinson's disease.



Keywords Organochlorine pesticides · Organophosphate pesticides · Parkinson's disease · Oxidative stress · Pesticide exposure

Abbreviations

PD	Parkinson's disease
AChE	Acetylcholine esterase
BMI	Body mass index
GC	Gas chromatography
OS	Oxidative stress
PON-1	Paraoxonase-1
RBC	Red blood cell
TAC	Total antioxidant capacity
CP	Carbonyl protein
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
CAT	Catalase
OCP	Organochlorine pesticide
α -HCH	Alpha-hexachlorocyclohexane
β -HCH	Beta-hexachlorocyclohexane
γ -HCH	Gamma-hexachlorocyclohexane
2,4-DDE	2,4-Dichlorodiphenyldichloroethylene
4,4-DDE	4,4-Dichlorodiphenyldichloroethylene
2,4-DDT	2,4-Dichlorodiphenyltrichloroethane
4,4-DDT	4,4-Dichlorodiphenyltrichloroethane
OPPs	Organophosphate pesticides

Introduction

Parkinson's disease (PD) is considered the 2nd most prevalent neurodegenerative disorder resulting from loss of function and structure of dopaminergic neurons in the substantia nigra of the midbrain (Wang et al. 2020). Both motor symptoms (e.g., resting tremors, rigidity, bradykinesia, and postural instability) and non-motor manifestations (e.g., psychosis, sensory symptoms, autonomic dysfunction, and sleep disturbance) are described as the main characteristics of the disease (Wang et al. 2020). Although the current estimated prevalence of the disease is 0.1–0.2% worldwide (Goel et al. 2022), the main cause of PD is largely unknown. Nevertheless, it is believed that PD is a multifactorial disease resulting from the combined impact of environmental and genetic factors. Exposure to toxic chemicals and head injuries can increase the risk of PD, while certain lifestyle factors may reduce it (Goldman et al. 2019).

Pesticide exposure is assumed to be one of the main environmental factors leading to PD (Chin-Chan et al. 2015). These chemicals, which are frequently used to control or exterminate pests, are classified based on the presence of active substances, such as carbamates, chlorinated

hydrocarbons, and organophosphates (Samareh et al. 2022; Abdollahdokht et al. 2021). Indeed, synthetic and organic pesticides are classified into three main groups based on their structure, including organophosphates, organochlorines, and organonitrogens (Kamboh et al. 2016). The organophosphate pesticides (OPPs), which are widely used all over the world, are derivatives of phosphoric acid esters able to cause chronic and acute toxic effects in non-target organisms (Samareh et al. 2022; Kamboh et al. 2016). One of the acute effects of these pesticides is neurotoxicity, and the usual target is acetylcholinesterase (AChE) (Kamboh et al. 2016). AChE causes the breakdown of acetylcholine in synapses after nerve impulses. Meanwhile, exposure to OPPs inhibits AChE activity via phosphorylation of the hydroxyl group of serine at the active site (Camacho-Pérez et al. 2022). Recent studies have shown that AChE activity and, therefore, acetylcholine levels change in patients with PD (Baik et al. 2022; Shim et al. 2021). These pesticides can also inhibit paraoxonase-1 (PON1) activity (Hofmann et al. 2009). PON-1 (EC 3.1.8.1.) is a 355 amino acid glycoprotein with three activities, including lactonase activity (against lactones and peroxides), paraoxonase activity (hydrolyzing organophosphates such as paraoxon), and arylesterase activity. The deficiency in PON-1 activity may cause oxidative stress and systemic inflammation, as well as neurodegenerative diseases, such as multiple sclerosis, Alzheimer's disease (AD), PD, and amyotrophic lateral sclerosis (ALS) (Menini and Gugliucci 2014). Moreover, organochlorines (OCPs), the other class of pesticides that are lipophilic, chlorinated, aromatic, or aliphatic hydrocarbons with a long environmental half-life, represent crucial hazardous properties (Samareh et al. 2022). Three main categories of OCPs, including dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes (HCH), and chlorinated cyclodienes, could disrupt the function of central (CNS) and peripheral nervous systems (PNS) via the induction of neuronal depolarization in the PNS and/or interrupt gamma-aminobutyric acid function in CNS (Samareh et al. 2022; Paydar et al. 2019). In addition, OCPs such as dieldrin may cause adverse consequences in the dopamine system (Richardson et al. 2006).

Along with the direct destruction of neural activity, pesticides cause cellular damage by disrupting the intracellular oxidative balance through the excess production of reactive oxygen species (ROS) (Kiani et al. 2022). ROS is considered one of the pivotal factors in the progression of PD and other aging-related diseases (Sule et al. 2022; Li et al. 2013). Indeed, it is widely suggested that oxidative stress

can be associated with several neurodegenerative diseases, such as PD, AD, ALS, and Huntington's disease (Li et al. 2013). Therefore, several studies have evaluated the level of ROS and cellular antioxidant defenses in neurological pathological states. Superoxide anion radicals, hydroxyl radicals, hydrogen peroxide, nitric oxide, etc. are examples of destructive oxidative molecules that are physiologically eliminated by the cooperation of a variety of antioxidant defenses, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), etc. (Kiani et al. 2022; Samareh et al. 2022). Moreover, total antioxidant capacity (TAC) is one of the most important parameters to measure the capability of cellular non-enzymatic antioxidant defenses (Samareh et al. 2022). Oxidative stress may also irreversibly generate protein carbonyl derivatives (carbonyl proteins, CPs), including aldehydes and ketones, through amino acid oxidation that may be implicated in neurodegenerative diseases (Picklo Sr et al. 2002). In addition, the measurement of the level of malondialdehyde (MDA), the most mutagenic end product of lipid oxidation, is used to assess lipid peroxidation and oxidative stress intensity (Samareh et al. 2022).

Population growth, increasing demand for agricultural products, and limited agricultural resources have led to an increase in the use of pesticides, which may play a role in various disorders such as neurodegenerative diseases (Chin-Chan et al. 2015). Therefore, to investigate the possible role of pesticides in PD, the current study aimed to measure the concentrations of some OCPs, such as 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, α -HCH, β -HCH, and γ -HCH, and also the erythrocyte AChE and PON-1 activity, which indicates exposure to OPPs, in patients with PD compared to the control group.

Materials and Methods

Subjects' Ascertainment

This case-control study was conducted to investigate the association between pesticide exposure and PD. Out of 80 samples, 29 samples from Parkinson's patients were used as case groups, and 51 samples were enrolled from healthy subjects as controls. In this regard, patients and healthy subjects were justified with the aims of the research scheme, and informed consent was obtained. Then the demographic, clinical, age, and sex determination checklist was completed.

The inclusion criteria for patients were the diagnosis of PD by a seasoned neurologist based on the criteria of the International Parkinson and Movement Disorder Society and also the manifestation of bradykinesia, rigidity, and tremor. These criteria for control subjects were the absence of a specific disease and receiving no medication. The patient's exclusion criteria were cerebellar abnormalities, treatment

with a dopamine receptor blocker or dopamine-reducing agent consistent with drug-induced parkinsonism, and diagnosis of alternative causes of parkinsonism that could cause symptoms. The exclusion criteria for healthy participants specifically required them to be disease-free and not on any medication.

Data Collection and Sampling

Subjects were interviewed by a neurologist to provide demographic characteristics including body mass index (BMI), family history, farming, confirmation of the presence (patients) or absence (controls) of PD, and disease duration (if PD exists). All participants were weighed barefoot on a verified electronic scale, and the scale was recalibrated before each weigh-in. A stadiometer was used to measure height, and BMI was calculated from the weight (kg)/height (m)² formula.

10 mL of venous blood was collected from all participants, 500 μ l of which was poured into tubes containing EDTA to assess AChE activity in red blood cells, and the remainder was centrifuged at 750 g for 7 min after keeping it at room temperature and forming a clot. Separated serum samples were stored in appropriate tubes at -70°C for further analysis.

Biochemical Factors

For the determination of serum levels of total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and high-density lipoprotein cholesterol (HDL-c), an autoanalyzer (Selectra-XL, Vital Science: Netherlands) and standard kits (MAN Co., Tehran, Iran) were used in a standard laboratory setting, and the low-density lipoprotein cholesterol (LDL-c) concentration was calculated using Friedewald's formula:

$$\text{LDL-c} = \text{Total cholesterol} - \left(\text{HDL cholesterol} + \frac{\text{Triglyceride}}{5} \right)$$

Assessment of OCPs Exposure

For measuring the OCP levels in the serum of subjects, the standards for the analysis of 2,4- and 4,4-Dichlorodiphenyldichloroethylene (DDE), 2,4- and 4,4-Dichlorodiphenyltrichloroethane (DDT), and α -, β -, and γ -Hexachlorocyclohexane (HCH) were supplied by Pestana (Dr. Ehrenstorfer GmbH, Augsburg, Germany). 4,4-Dichlorobenzophenone as the internal standard was purchased from Supelco (Sigma-Aldrich, PA, USA) and anhydrous sodium sulfate from Scharlau Chemie (Barcelona, Spain). OCP amounts were evaluated using the method described by

Zumbado et al. (2005) with certain modifications. Briefly, 20 μL of the 1 mg/mL internal standard, 4,4-dichlorobenzophenone was mixed with 500 μL of serum. Then 2 ml of high-purity n-hexane solvent (99.99 %) was added to the mixture, forming two distinct phases. As OCPs are lipid-soluble compounds, the organochlorines and the internal standard pass through the n-hexane phase. The upper phase was transferred to another tube, and the process was repeated to ensure all the desired amounts were extracted. Thereafter, 200 μL of high-purity (99%) sulfuric acid (H_2SO_4) was mixed with the second tube, which in turn led to the formation of two phases. The upper layer was extracted, and 100 mg of anhydrous sodium sulfate was added for the dehydrating extraction phase. The mixture was then centrifuged at 750 g for 10 min, and the supernatant was transferred to a glass tube where the solvent was evaporated under a biochemical hood. After that, the sample was injected into a gas chromatograph (GC) by adding 100 μL of ethyl acetate and mixing thoroughly. A sample injection of 1 μL was then performed into the GC (Agilent 7890A, USA) using capillary columns (HP-5) and a flame ionization detector. The quantification standard was inspected at the beginning and end of each run, and the limit of detection (LOD) was determined as the composition concentration in the quantification standard divided by 3 times the signal-to-noise ratio.

Erythrocyte AChE Activity Assay

To measure erythrocyte AChE activity, the modified Ellman's method (Kiani et al. 2022) was used with reagents obtained from Sigma (Saint Louis, MO, USA), including 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), acetylcholine iodide, and hyamine 1622. Briefly, normal saline was used to rinse 100 μL of blood sample three times and centrifuged to remove RBCs from plasma that might interfere with the plasma isoform of AChE. Then the washed RBCs were diluted with 6 mL of distilled water, and 100 μL of the dilution was incubated with the reaction buffer containing 20 μM quinidine sulfate, DNTB (0.28 mmol), and 3.2 mmol of acetylcholine iodide at 37 °C for 10 min. To terminate the reaction, hyamine 1622 (1 mL) was added to the solution. DTNB, chromophore, and the thiocholine generated from the reaction produced 5-thio-2-nitrobenzoic acid, which has a maximum absorbance of 440 nm.

Measurement of PON-1 Arylesterase Activity

Sigma (Saint Louis, MO, USA) supplied phenylacetate, which serves as a substrate for the PON-1 enzyme. To measure the arylesterase activity of this enzyme in serum, the procedure proposed by Bobin-Dubigeon et al. (2012) has been recruited. The method comprises measuring phenylacetate hydrolysis as an indicator of enzyme activity. Specifically,

100 μL of serum was mixed with 2 mM calcium chloride, 2 mM substrate, and 100 mM Tris-HCl (pH 8.0), followed by incubation at 37 °C for three minutes. The hydrolysis of the substrate was subsequently determined at 270 nm.

Measurement of NO

To measure the level of nitric oxide (NO) in serum, the Griess method was implemented. Initially, serum deproteinization was performed using 250 μL of serum in the presence of ZnSO_4 and 0.3M NaOH. Conversion of nitrate to nitrite was done with vanadium (III) chloride (VCl_3), followed by Griess reagent (2% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD) in deionized water) added to the mixture. After incubation at 37 °C for 30 min, the absorbance of the serum was quantified at 540 nm (Samareh et al. 2022).

Measurement of MDA

A modified version of the Yagis method (Yagi 1984) was employed to determine serum MDA levels. Shortly, 125 μL of serum was combined with 1.5 mL of phosphoric acid and supplemented with 0.5 mL of thiobarbituric acid. The mixture was then stirred and heated in boiling water for 4 min. Afterward, 1 mL of n-butanol was added, and the test tube was centrifuged at 750 g for 10 min. The ensuing pink phase was isolated and analyzed for MDA level at 532 nm using the tetraethoxypropane standard curve.

Determination of CPs

Levine et al. (1990) were the trailblazers in measuring CPs by covalently reacting them with 2,4-dinitrophenylhydrazine (DNPH). The process entailed adding 125 μL of serum and 500 μL of 2,4-dinitrophenylhydrazine (10 mM in 2 M HCl) to each test tube. Next, 500 μL of 20% trichloroacetic acid was added and centrifuged for 3 min at 11,000 g. The resulting precipitates were washed three times with ethanol-ethyl acetate (1:1), and the final substance was dissolved in 6 M guanidine. The absorption of 2,4-dinitrophenyl (DNP) hydrazone was measured at 370 nm.

Measurement of Serum TAC

Determination of TAC was performed utilizing a commercially available kit (CAT No. NS-15012 Naxifer, Orumi-yeh, Iran) that relies on the ability of antioxidants to reduce the colorless ferric 2,4,6-Tri(2-pyridyl)-s-triazine (Fe (III)-TPTZ) complex to blue ferrous (Fe (II)-TPTZ) at low pH, which was quantified by determining the absorbance at 593 nm (Samareh et al. 2022).

SOD3 Activity Assay

The Ransod kit protocol (UK:Cat No. SD 125) was employed to assess the total activity of SOD3, in which superoxide ions (O_2^-) generated by xanthine oxidase (XOD) along with NitroBlue Tetrazolium (NBT) produce NBT-diformazan with maximum absorbance at 560 nm. The conversion of superoxide radical into hydrogen peroxide (H_2O_2) and oxygen (O_2) was catalyzed by SOD3, and the activity of this enzyme was determined by measuring the degree of reduction in the formation of NBT-diformazan due to the decrease in the concentration of superoxide ions.

GPx3 Activity Assay

GPx3 activity was assessed indirectly through a coupled reaction with glutathione reductase (GR) using the Randox kit (UK:Cat No. RS504). GPx3 is the enzyme that triggers the oxidation of glutathione (GSH) in the presence of cumene hydroperoxide. The oxidized glutathione (GSSG) is rapidly converted to its reduced form by GR, and NADPH is concurrently oxidized to NADP. The reduction in absorbance at 340 nm is then measured, enabling the determination of GPx3 activity.

CAT Activity Assay

The method previously described by Sinha (Hadwan 2016) was applied to determine CAT activity with reagents including 30 mM H_2O_2 , phosphate buffer (50 mM:pH 7.4), dichromate/acetic acid solution (5% aqueous potassium dichromate solution in distilled water, and 150 mL of Glacial (98–100%) acetic acid). In this technique, the reduction of dichromate in acetic acid to chromic acetate occurs on heating with H_2O_2 , and the resulting chromic acetate was measured at 570 nm using a spectrophotometer.

Quality Assurance and Quality Control

Continuous quality assurance and quality control (QA/QC) assessments were performed to ensure accurate quantification of OCPs. Analysis of all samples (including samples, field blanks, and equipment blanks) was performed in triplicate, and the results were presented as the mean of these three values. To construct the calibration curves, a series of pesticide standard solutions with specific concentrations (including 0.05, 0.1, 0.5, 0.75, 1, 2, 4, 8, 16, 25, 50, and 100 $\mu\text{g/L}$) were spiked in the pooled sample. Procedure blanks were also prepared using ethyl acetate, which was analyzed to assess the column, inlet, and contamination detector during injection and extraction, to detect the possibility of the instrument's background contamination, and to investigate cross-contamination.

Statistical Analysis

Depending on the type of variable, mean $\pm$ SEM or percentage was used to represent the data. To check the normality of the data, Kolmogorov–Smirnov test was employed. Qualitative and quantitative variables were compared between the two groups using appropriate statistical tests, such as chi-square/Fisher's exact test or independent sample *t*-test/Mann–Whitney *U* test. All analyses were conducted using SPSS 24, and comparisons were considered statistically significant at the 5% level. It's worth noting that all pesticides were detected above the LOD (limit of detection) determined based on the slope of the calibration curve and the SD of the regression line.

Results

Demographic Profile and Biochemical Parameters

The demographic characteristics and biochemical factors are described in Table 1. In this research, seven individuals were included as new patients, fifteen participants who had been suffering from the condition for less than two years, and seven individuals who had been battling the disease for more than two years. The findings demonstrated that there was no significant difference between the two groups in terms of age, gender, BMI, education level, and history of farming. However, the fact that 62.1% of the PD patients resided in the north of Kerman versus 37.9% in the southern regions of the province demonstrated a significant difference between the two groups in terms of the geographic distribution of the disease. ($P < 0.05$). ALT, AST, total protein, creatinine, triglycerides, TC, HDL-c, and LDL-c were also not significantly different between the two groups ($P > 0.05$).

The Levels of OCPs

A GC method was performed to measure the levels of OCPs. In order to demonstrate the proper operation of the GC method, illustrations depicting the chromatograph of both the control and the patient can be observed in Fig. 1A and B, respectively. Also, the obtained findings showed that the level of all OCPs was significantly higher in the patient group compared to the control group ($P < 0.05$) (Fig. 2).

Enzyme Activity and Oxidative Stress Parameters

The comparison of patient groups with controls revealed that the activity of AChE [5.21 ± 0.37 vs 7.64 ± 0.17 (U/L)], SOD3 [26.74 ± 1.85 vs 38.68 ± 1.78 (U/mL)], CAT [36.59 ± 0.75 vs 130.00 ± 6.33 (KU/mL)], and arylesterase activity of PON-1 [60.92 ± 3.64 vs 80.98 ± 2.53 (U/L)]

Table 1 Demographic, clinical, and other selected baseline characteristics of the patients with Parkinson's disease and the control group

Parameters	Group	Patient (N=29)	Controls (N=51)	P-value
Age (year)	Female	73.80 ± 3.18	61.16 ± 1.26	0.002
	Male	65.11 ± 2.33	65.94 ± 1.66	0.571
	Total	68.10 ± 2.01	64.16 ± 1.18	0.13
Gender	Female	10 (34.5%)	19 (37.3%)	0.804
	Male	19 (65.5%)	32 (62.7%)	
BMI (kg/m ²)	Female	24.19 ± 1.86	25.05 ± 0.43	0.557
	Male	24.92 ± 1.07	24.85 ± 0.3	0.937
	Total	24.67 ± 0.93	24.92 ± 0.25	0.739
Level of education	High school or less	16 (55.2%)	35 (68.6%)	0.229
	Greater than high school	13 (44.8%)	16 (31.4%)	
Agriculture precedent	Yes	9 (31%)	20 (39.2%)	0.464
	No	20 (69%)	31 (60.8%)	
Residence	North of Kerman	18 (62.1%)	16 (31.4%)	0.008*
	South of Kerman	11 (37.9%)	35 (68.6%)	
Biochemical profile	ALT (U/L)	25.45 ± 1.81	27.43 ± 1.30	0.266
	AST (U/L)	32.24 ± 1.75	33.37 ± 0.78	0.37
	TP (g/dl)	5.93 ± 0.07	5.87 ± 0.04	0.467
	Cr (mg/dl)	1.35 ± 0.11	1.29 ± 0.06	0.763
	TG (mg/dl)	96.21 ± 7.54	98.61 ± 3.97	0.26
	TC (mg/dl)	160.6 ± 6.77	157.75 ± 4.06	0.806
	HDL-c (mg/dl)	42.45 ± 0.82	42.73 ± 0.39	0.636
	LDL-c (mg/dl)	98.86 ± 7.02	95.3 ± 4.26	0.76
Family history	Yes	6	N/A	
	No	23		
Duration of PD (month)	Female	30 ± 6.00	N/A	
	Male	18.95 ± 5.65		
	Total	22.76 ± 4.29		
PD medications	No	9	N/A	
	Under 2 years	14		
	More than 2 years	6		
UPDR IV	Female	10.1 ± 0.98	N/A	
	Male	6.74 ± 0.80		
	Total	7.89 ± 0.69		
PDQ39	Female	13.99 ± 1.28	N/A	
	Male	12.3 ± 2.15		
	Total	12.89 ± 1.47		
H&Ym	Female	2.25 ± 0.28	N/A	
	Male	1.76 ± 0.13		
	Total	1.93 ± 0.14		
Beck	Minimal depression	24	N/A	
	Mild depression	3		
	Moderate depression	1		
	Severe depression	1		

Bold value indicates the significant difference was observed between the two groups ($P < 0.05$)

Data are expressed as numbers of individuals or means ± SEM and comparisons were made by the chi-square test or Student's sample t test and Mann–Whitney U test, respectively: N: overall participants; *A significant difference ($P < 0.05$); P value demonstrates the difference between the patient group and the control group: BMI body mass index, ALT alanine aminotransferase, AST aspartate aminotransferase, TP total protein, Cr creatinine, TG triglycerides, TC total cholesterol, HDL high-density lipoprotein, LDL low-density lipoprotein, PD Parkinson's disease, UPDR: unified Parkinson's disease rating scale, PDQ39 Parkinson's disease questionnaire, H&Ym modified Hoehn and Yahr scale, Beck Beck depression inventory

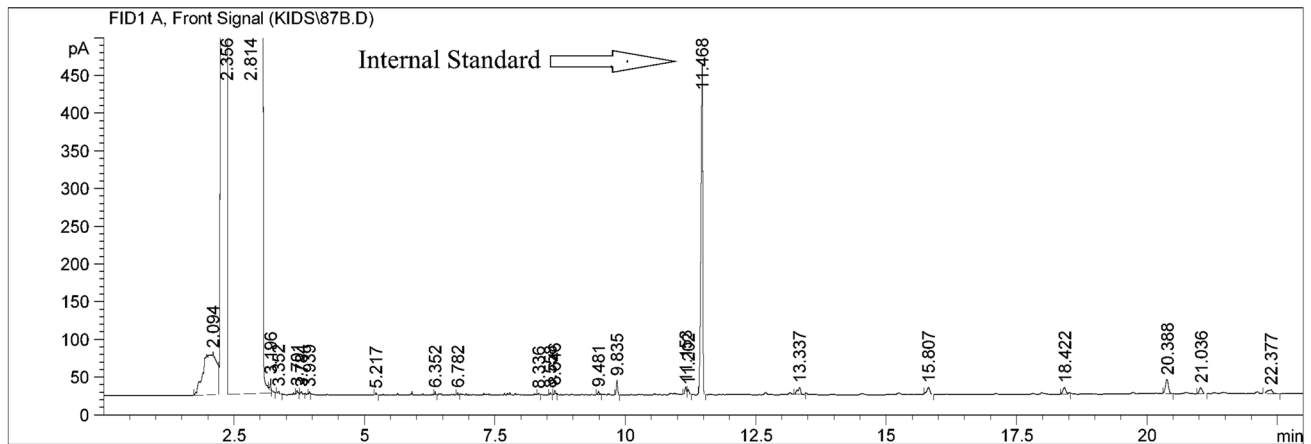
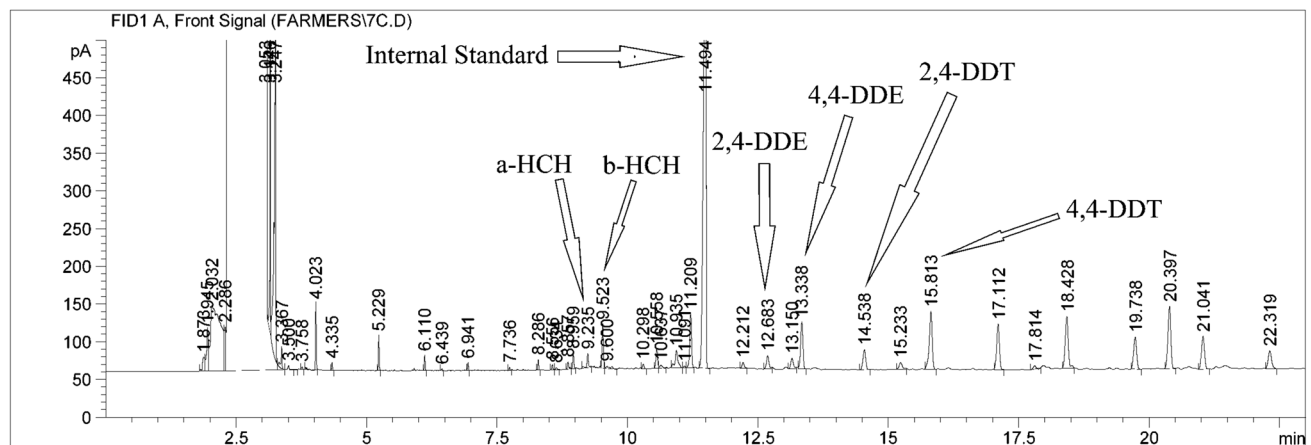
A.**B.**

Fig. 1 The serum levels of OCPs were measured by the GC. The figure demonstrates an example chromatograph of controls (**A**) and patients (**B**) to reveal the appropriate performance of the used GC method, as well as a presentation of higher levels of OCPs in patients with PD. *a-HCH* α -Hexachlorocyclohexane, *b-HCH*

β -Hexachlorocyclohexane, *2,4-DDE* 2,4-Dichlorodiphenyldichloroethylene, *4,4-DDE* 4,4-Dichlorodiphenyldichloroethylene, *2,4-DDT* 2,4-Dichlorodiphenyltrichloroethane, *4,4-DDT* 4,4-dichlorodiphenyltrichloroethane

was significantly different ($P < 0.0001$, Fig. 3). Although the activity of the mentioned enzymes in PD patients was significantly lower than in controls, TAC [371.3 ± 13.78 vs 271.3 ± 8.54 (μM)], NO [30.25 ± 0.51 vs 18.95 ± 0.85 (μM)], MDA [1.85 ± 0.13 vs 1.59 ± 0.03 (μM)], and CP [1.61 ± 0.06 vs 0.78 ± 0.03 (nmol/mg protein)] levels were significantly higher in patients with PD compared to controls ($P < 0.05$, Fig. 3). Nevertheless, no notable differences were found between the two groups regarding GPx3 activity [30.86 ± 0.88 vs 30.76 ± 1.31 (U/L)] ($P > 0.05$, Fig. 3).

The Correlation Between Serum Levels of OCPs and Biochemical Factors

The current study analyzed the correlation between OCPs levels and studied biochemical indices, including MDA, SOD3,

PON1, and GPx3. The findings indicated that SOD3 exhibits a negative association with 4,4 DDE ($r = -0.486$ $P = 0.008$), 2,4 DDT ($r = -0.529$ $P = 0.003$), and NO ($r = -0.616$ $P < 0.001$). In addition, 2,4 DDE demonstrated a positive correlation with the PDQ39 score ($r = 0.443$ $P = 0.016$). Furthermore, NO represented a direct correlation with 2,4 DDT ($r = 0.412$ $P = 0.026$) and a negative correlation with TAC ($r = -0.436$; $P = 0.018$). Additionally, 4,4 DDT was inversely related to PON1 ($r = -0.417$ $P = 0.025$) (Table 2).

Discussion

PD is a chronic neurodegenerative disease characterized by the degeneration of dopaminergic neurons within the substantia nigra pars compacta. While the exact cause of

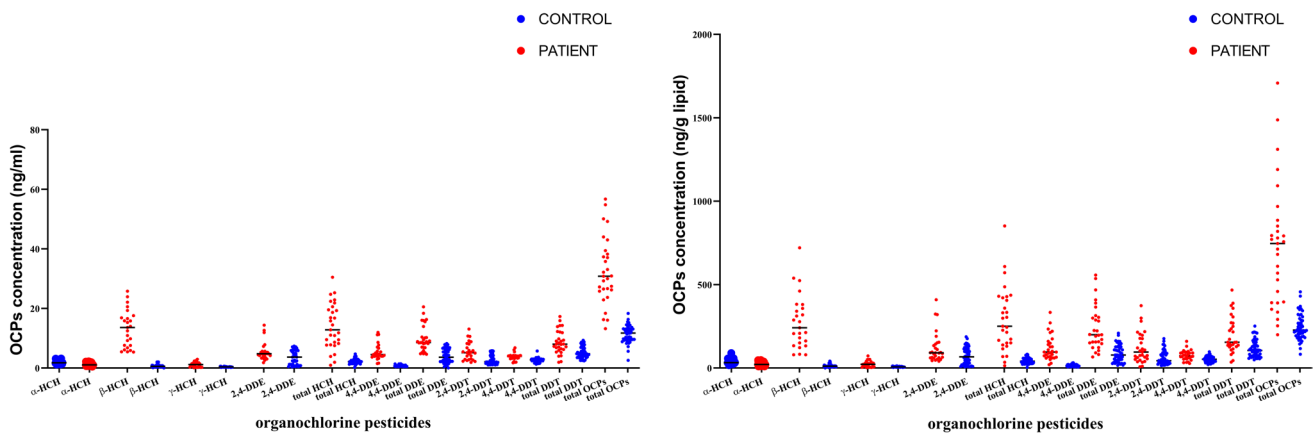


Fig. 2 The serum levels of the studied OCPs in patients with PD compared to controls. The scatter chart compares the serum levels of OCPs in patients to the control group (C). As the figure represents, all seven OCPs were significantly higher in the patients compared to the control group. *OCPs* organochlorine pesticides, *alpha-HCH*

alpha-Hexachlorocyclohexane, *beta-HCH* β -Hexachlorocyclohexane, *gamma-HCH* γ -Hexachlorocyclohexane, *2,4-DDE* 2,4-Dichlorodiphenyldichloroethylene, *4,4-DDE* 4,4-Dichlorodiphenyldichloroethylene, *2,4-DDT* 2,4-Dichlorodiphenyltrichloroethane, *4,4-DDT* 4,4-Dichlorodiphenyltrichloroethane

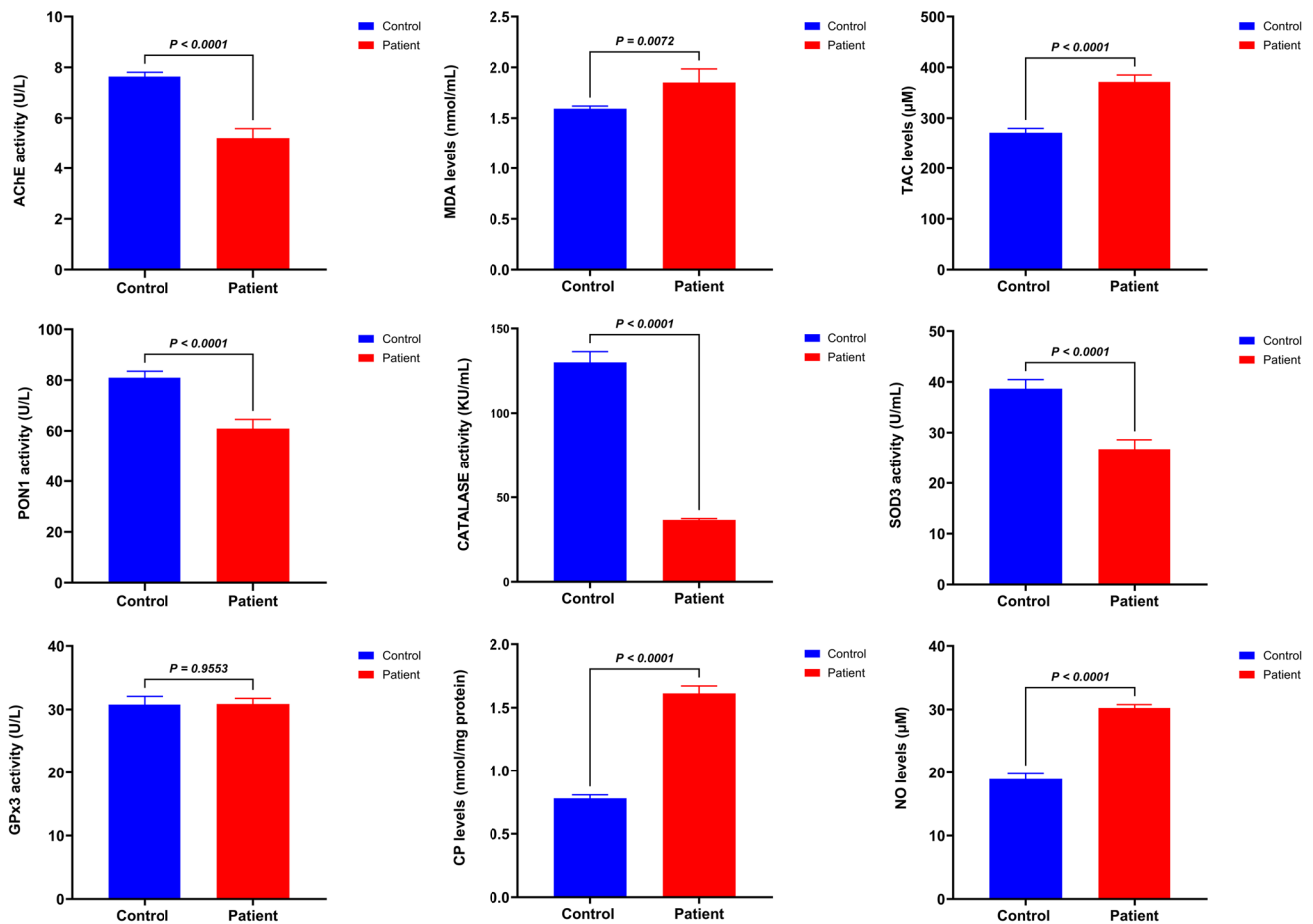


Fig. 3 The comparison of biochemical factors between patients with PD and controls. The charts compare some oxidative stress factors between patients and controls. The activity of AChE, PON-1, CAT, and SOD3 enzymes in patients was significantly lower than in controls, whereas the levels of MDA, TAC, CP, and NO in patients were

remarkably higher when compared to controls. There was no difference between the two groups regarding GPx3 activity. *AChE* acetylcholinesterase, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *PON-1* paraoxonase-1, *CAT* catalase, *SOD* superoxide dismutase, *GPx* glutathione peroxidase, *CP* carbonyl protein, *NO* nitric oxide

Table 2 The overall correlation between the studied parameters in the patient group

Parameter	TAC	CAT	SOD3	GPx3	CP	MDA	NO	PON-1	AChE	α -HCH	β -HCH	γ -HCH	2,4-DDE	4,4-DDE	2,4-DDT	4,4-DDT	PDQ39
TAC	1	0.106	0.313	-0.197	-0.051	-0.124	-0.436*	-0.033	0.101	-0.127	0.216	0.233	0.132	0.027	-0.035	0.215	0.037
CAT		1	-0.074	-0.298	-0.299	-0.055	-0.104	-0.407	0.124	-0.305	0.218	-0.043	-0.326	-0.028	-0.222	0.056	0.107
SOD3			1	-0.166	0.243	0.076	-0.616**	0.047	-0.126	0.110	0.059	0.121	-0.239	-0.486**	-0.529**	0.024	-0.253
GPx3				1	0.176	0.081	0.119	0.155	-0.107	-0.006	-0.139	0.041	0.078	0.279	0.307	-0.082	-0.312
CP					1	-0.313	-0.288	-0.222	0.070	0.092	0.332	0.297	-0.160	0.137	-0.106	0.158	-0.272
MDA						1	0.060	0.209	-0.011	0.341	-0.193	-0.132	0.283	-0.326	-0.088	-0.365	-0.019
NO							1	0.286	0.233	0.073	-0.253	-0.514	0.275	0.344	0.412*	-0.392	0.170
PON-1								1	0.106	0.309	-0.200	-0.343	0.079	-0.282	-0.016	-0.417*	-0.042
AChE									1	0.083	0.088	-0.266	-0.161	-0.248	-0.247	-0.282	0.016
α -HCH										1	0.073	0.042	-0.135	-0.294	-0.266	-0.445	-0.319
β -HCH											1	.458	-0.064	0.163	-0.118	0.115	-0.068
γ -HCH												1	0.123	0.143	0.064	.457	-0.221
2,4-DDE													1	0.286	.438	0.094	0.443*
4,4-DDE														1	.820	0.331	0.056
2,4-DDT															1	0.290	0.195
4,4-DDT																1	0.210
PDQ39																	1

*Spearman correlation is significant at the 0.05 level (2-tailed)

**Spearman correlation is significant at the 0.01 level (2-tailed)

Eta co-efficient was used nominal variable. Adjusted for, AChE, PON1, SOD3, GPx3, CAT, MDA, TAC, NO, PC, α -HCH, β -HCH, γ -HCH, 2,4 DDE, 4, 4 DDE, 2, 4 DDT, 4, 4 DDT, PDQ39

neuronal loss remains elusive, several genetic and environmental factors are believed to contribute to its development (Chen et al. 2022). Among the environmental factors, pesticide exposure has emerged as a potential risk to PD progression. These chemicals are widely utilized to mitigate pests, combat crop diseases, and enhance agricultural yield (Xu et al. 2022). Exposure to pesticides and subsequent possible damage to human health may occur through various routes, such as inhalation (the act of breathing in air contaminated with pesticides), dermal contact (direct interaction with pesticide-treated surfaces), oral ingestion (consuming contaminated food, and water, or improper hygiene practices), occupational exposure, and environmental exposure (Tudi et al. 2022). It is widely accepted that pesticide exposure is crucially related to chronic and acute disorders, such as cardiovascular disease, type 2 diabetes, and neuro-related diseases (Samareh et al. 2022; Zago et al. 2022). OCPs, as illegal chemicals (even though the use of OCPs has been banned for many years, they are still one of the most problematic poisons in many societies due to their high stability in the environment and possible abuses), and OPPs (as pesticides that are widely used in various cases all over the world) are among the most common pesticides that cause various toxicities to humans and other organisms despite their high efficiency (Bhatt et al. 2021).

OCPs are lipophilic compounds with a long half-life and slow metabolism; hence, they can accumulate for a long time in adipose tissues and move between these tissues and body fluids such as plasma (Fénichel et al. 2021). Two primary categories of OCPs exist, encompassing DDT-like compounds and chlorinated alicyclic compounds (Ensley 2018) that operate through distinct mechanisms of action. DDT-like pesticides primarily target the peripheral nervous system by inhibiting sodium channel closure at axonal terminals. This leads to sodium leakage from nerve cells, causing hyperexcitability (Costa 2015). Chlorinated alicyclic compounds affect the central nervous system by disrupting gamma-aminobutyric acid function, hindering chloride entry into neurons (Costa 2015). These substances interfere with neural pathways, potentially impairing the central nervous system's functionality and leading to various physiological consequences. Long-term exposure can result in chronic effects, including oxidative stress, which shares similarities with organophosphate pesticide toxicity and may contribute to Parkinson's disease pathogenesis (Chang and Chen 2020; Lee et al. 2017; Yu et al. 2021). Importantly, in living organisms, the production of dichlorodiphenyldichloroethylene (DDE) is facilitated by the enzyme cytochrome P450 through the reduction reactions on DDT (Cárdenas-González et al. 2013). The results of the current study exhibited that individuals diagnosed with PD had elevated concentrations of OCPs, including 2,4-DDT, 4,4-DDT, 2,4-DDE, 4,4-DDE, α -HCH, β -HCH,

and γ -HCH, in comparison to the control group. Moreover, there was a positive correlation between 2,4-DDE and the PDQ39 score which may suggest that further exposure to OCPs accelerates the progression of the disease. Previous studies revealed that levels of OCPs, such as α -HCH, β -HCH, γ -HCH, δ -HCH, propanil, heptachlor, dieldrin, hexachlorobenzene, 4,4 DDT, and o,p'-dichloro-diphenyl-trichloroethane, were higher in PD patients than in the control group (Xu et al. 2022). Notably, the observed elevation in dieldrin concentrations may potentially be associated with alterations in dopaminergic response (Boyd et al. 2023).

The evaluation of AChE and PON-1 enzyme activity unveiled a significant decrease in the activity of these enzymes in PD patients when compared to the control group. AChE serves as an enzymatic catalyst for the degradation of acetylcholine at nerve terminals. The presence of OPPs can irreversibly impede enzyme action, resulting in the accumulation of acetylcholine, interference with neural networks, and subsequent consequences (Samareh et al. 2022). The decline in AChE activity indicated in the current study strongly implies an elevated exposure to OPPs within the aforementioned population. Similarly, Kumar et al. have made a noteworthy discovery regarding the reduced activity of this particular enzyme and the development of non-communicable diseases, such as PD, obesity, and AD (Kumar et al. 2023). PON-1 represents antioxidative properties and is a calcium-dependent enzyme that circulates in the bloodstream, predominantly bound to HDL. It exhibits various activities, including arylesterase, paraoxonase, and lactonase (Samareh et al. 2022). The current findings revealed reduced activity of PON-1 in patients with PD, and this reduction was correlated to exposure to OPPs and 4,4 DDT, which in turn may indicate the mixed exposure of PD patients to OCPs and OPPs. Concordantly, it has been suggested that decreased activity of the aforementioned enzyme in patients with PD could serve as a valuable biomarker to assess the state of the disease and predict the prognosis (Mota et al. 2019). Moreover, a reduction in enzyme activity can lead to the accumulation of OPPs, exacerbating OS and ultimately elevating the susceptibility to neurodegenerative disorders like PD (Ikeda et al. 2011; Mota et al. 2019).

A variety of enzymatic and non-enzymatic oxidative-related indicators were assessed in the current study to elucidate the oxidative status of patients with PD. The present findings revealed that NO levels increased considerably in patients with PD compared to the control group. Moreover, NO showed a positive correlation with 2,4 DDT. These findings are consistent with prior research indicating that serum NO levels and inflammatory response are elevated in PD patients (Barmaki et al. 2021; Rathnayake et al. 2019). It is also noteworthy that the results of Santos-Lobato's study revealed an increase in the NO ratio in CSF/plasma, which

could indicate that the brain generates even more NO than peripheral tissues (Santos-Lobato et al. 2022).

In addition, this study showed that MDA and CP levels were significantly higher in the patient group than in the control group. Carbonylation is an irreversible process and one of the most common post-translational modifications of proteins, which can be induced by both non-oxidative and oxidative agents. The increment in protein carbonylation is a sign of OS and can interfere with the normal function of proteins (Solana-Manrique et al. 2021). Consistent with previous research (Chiaradia et al. 2019), the present study found that protein carbonylation is increased in PD, which may have occurred following exposure to pesticides (Márquez-Lázaro et al. 2021). The escalation of OS could also lead to lipid peroxidation and an elevate in its end-products, such as MDA. It has been established that exposure to pesticides can augment MDA levels by triggering OS (Rafeeina et al. 2022). The present study found that MDA levels were significantly higher in the patient group than in the control, suggesting that these patients may have been exposed to pesticides. Concerning this matter, previous studies have reported both increased (de Farias et al. 2016) and unchanged (Gökçe Çokal et al. 2017) MDA levels in people with PD compared to controls.

Assessment of antioxidant enzyme activity demonstrated a notable decrease in SOD3 and CAT activity in the patient group compared to the control group; however, GPx3 activity did not differ significantly when the two groups were compared. SOD3 catalyzes superoxide radicals, thereby contributing to the reduction of OS and inflammation (Kiani et al. 2022). It has been documented that exposure to pesticides can decrease the activity of this enzyme and subsequently lead to induce OS and inflammation (Paluzar and Sagiroglu 2022). As the results of this study showed, there is an inverse correlation between SOD3 activity and the levels of 2,4 DDT and 4,4 DDE, which represents that the level of enzyme activity has decreased with increasing exposure to these pesticides. Consistently, it has been established that SOD activity is significantly decreased in PD patients (Li et al. 2022). Interestingly, Zhang's study found that decreased SOD activity after a mild acute ischemic stroke can lead to cognitive impairment, which underscores the critical role of this enzyme in the functioning of the nervous system (Zhang et al. 2022). CAT, in cooperation with GPx3, functions in mitigating hydrogen peroxide levels and detoxifying a variety of peroxides (Kiani et al. 2022). Several studies have investigated the impact of pesticides on CAT activity and gene expression. Some of these studies have shown that exposure to certain pesticides can decrease CAT activity and even expression (Lu et al. 2019). Moreover, enhanced CAT activity can serve as a protective measure against the detrimental impact of ROS induced by paraquat on the body (Joguchi et al. 2004). Moreover, a

reduction in CAT activity has been observed in diseases such as PD. However, there is a divergence of views on the mechanism by which CAT activity decreases in PD. For instance, Yakunin et al. suggested that the increased accumulation of α -synuclein in PD, through the suppression of peroxisome proliferator-activated receptor γ (PPAR γ) transcription activity, is implicated in the diminished expression of CAT as well as the escalated level of OS (Yakunin et al. 2014). Clarifying this issue and obtaining a definitive result requires more research in this field to correctly show the cause of the decrease in CAT activity. Regarding GPx3, a variety of findings have emerged, suggesting that this enzyme's function may either increase (Gökçe Çokal et al. 2017) or decrease (Baillet et al. 2010) in individuals with PD as compared to a control group. Although pesticide exposure could potentially lower enzyme function (Kiani et al. 2022), it's important to note that the gene expression of these enzymes might have heightened as a protective mechanism to offset reduced activity (Han et al. 2017).

TAC denotes the serum's ability to neutralize oxidants and free radicals, which are influenced by a range of factors, such as thiols, vitamin C, vitamin E, uric acid, and bilirubin (Li et al. 2023). Pesticide exposure can reduce TAC by triggering OS and decreasing antioxidant enzyme activity (Kiani et al. 2022). Conversely, a study conducted in 2018 showed that prolonged exposure of farmers to a combination of pesticides might augment TAC as a compensatory or adaptive mechanism (Bernieri et al. 2021). The findings of this investigation demonstrated that despite the escalation of pesticide exposure and the decline of certain antioxidant enzymes, the level of TAC in patients with PD increased significantly compared to the control group. This may suggest that the administration of medications, such as L-dopa and supplements, such as vitamin C, E, etc., which are commonly prescribed to PD patients, contributes to the increase in TAC levels (Arabuli et al. 2023; Duarte-Jurado et al. 2021). Nevertheless, it is imperative to note that a comprehensive understanding of this issue necessitates further research, as some studies have reported reduced TAC levels in PD (Mota et al. 2019; Oderinu et al. 2019).

Conclusion

The findings from this investigation revealed a marked increase in the average concentrations of all examined OCPs among PD patients, as compared to the control group consisting of healthy individuals. Moreover, it has been observed that the presence of OPPs significantly reduces the activities of AChE and PON-1 enzymes. In the context of this matter, exposure to OPPs and OCPs leads to detrimental impacts on OS parameters. Overall, the research findings suggest that exposure to pesticides could potentially

contribute to the escalating risk of developing Parkinson's disease.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval The present case–control study was approved by the Ethics Committee of Kerman University of Medical Sciences (Ethics code: IR.KMU.REC.1401.508). All participants signed a consent form before enrolling in the study.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Consent for Publication Consent for publication was obtained.

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Authors and Affiliations

Ali Samareh^{1,2,3}  · Hossein Pourghadamyari^{1,3}  · Mohammad Hadi Nemtollahi^{1,3}  · Hossein Ali Ebrahimi Meimand⁴  · Mohammad Erfan Norouzmahani^{3,5} · Gholamreza Asadikaram^{1,3} 

✉ Hossein Ali Ebrahimi Meimand
Hebrahimi@kmu.ac.ir

✉ Gholamreza Asadikaram
Gh_asadi@kmu.ac.ir

Ali Samareh
a.samareh@kmu.ac.ir

Hossein Pourghadamyari
h.pourghadamyari@kmu.ac.ir

Mohammad Hadi Nemtollahi
m.nematollahi@kmu.ac.ir

Mohammad Erfan Norouzmahani
erfan.norouzmahani@yahoo.com

¹ Applied Cellular and Molecular Research Center, Kerman University of Medical Sciences, Kerman, Iran

² Neuroscience Research Center, School of Medicine, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

³ Department of Clinical Biochemistry, Afzalipour Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran

⁴ Neurology Research Center, Kerman University of Medical Sciences, Kerman, Iran

⁵ Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University Medical Sciences, Kerman, Iran