The Impact of Pesticides on Parkinson's Disease; A Case-Control Study

Ali Samareh
samarehali@yahoo.com
Kerman University of Medical Sciences

Hossein pourghadamyari
ghadamyarih91@gmail.com
Kerman University of Medical Sciences

Mohammad Hadi Nemtollahi
mh.nematollahi@yahoo.com
Kerman University of Medical Sciences

Hossain-Ali Ebrahimi
ebrahimi.ha@gmail.com
Kerman University of Medical Sciences

Mohammad Erfan Norouzmahani
erfan.norouzmahani@yahoo.com
Kerman University of Medical Sciences

Gholamreza Asadikaram
Gh_asadi@kmu.ac.ir
Kerman University of Medical Sciences

Research Article

Keywords: Organochlorine pesticides, Organophosphorus pesticides, Parkinson's disease, Oxidative stress, Pesticide exposure

DOI: https://doi.org/

Additional Declarations: No competing interests reported.

The Impact of Pesticides on Parkinson's Disease; A Case-Control Study

Ali Samareh[1,2,3], Hossein pourghadamyari[1,3], Mohammad Hadi Nemtollahi[1,3], Hossain-Ali Ebrahimi [4]*, Mohammad Erfan Norouzmahani[3,5], Gholamreza Asadikaram[1,3]*

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

[2]Neuroscience Research Center, Institute of Neuropharmacology, School of Medicine, 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

*Correspondence to:

Gholamreza Asadikaram, Applied Cellular and Molecular Research Center, Kerman University of Medical Sciences, Kerman, Iran. Orcid: https://orcid.org/ 0000-0002-9100-0756, Email: Gh_asadi@kmu.ac.ir.

* Hossain-Ali Ebrahimi, Neurology Research Center, Kerman University of Medical Sciences, Kerman, Iran. Email: ebrahimi.ha@gmail.com

Abstract

Background: 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 organochlorines (OCPs) and organophosphate pesticides (OPPs), recognized as the main environmental elements, in the genesis of PD.

Methods: In this case-control study, 29 PD patients and 51 healthy subjects (controls) were involved. Gas chromatography (GC) 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.

Results: 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 (CAT) activity, and superoxide dismutase 3 (SOD3) activity in PD patients were significantly less than controls. Although the levels of carbonyl protein (CP), total antioxidant capacity (TAC), malondialdehyde (MDA), and nitric oxide (NO) in PD patients were higher than the controls.

Conclusion: 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.

Keywords: Organochlorine pesticides, Organophosphorus pesticides, Parkinson's disease, Oxidative stress, Pesticide exposure.

LIST OF 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.

1. 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 (1). 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 (1). Although the current estimated prevalence of the disease is 0.1-0.2% worldwide (2), 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 (3).

Pesticide exposure is assumed to be one of the main environmental factors leading to PD (4). 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 (5). Indeed, synthetic and organic pesticides are classified into three main groups based on their structure, including organophosphates, organochlorines, and organonitrogens (6). 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 (5, 6). One of the acute effects of these pesticides is neurotoxicity, and the usual target is acetylcholinesterase (AChE) (6). 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 (7). Recent studies have shown that AChE activity and, therefore, acetylcholine levels change in patients with PD (8, 9). These pesticides can also inhibit paraoxonase-1 (PON1) activity (10). PON-1 (EC 3.1.8.1.) is a 355 amino acid glycoprotein with 3 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) (11). 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 (5). 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 (5, 12). In addition, OCPs such as dieldrin may cause adverse consequences in the dopamine system (13).

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) (14). ROS is considered one of the pivotal factors in the progression of PD and other aging-related diseases (15, 16). Indeed, it is widely suggested that oxidative stress can be associated

with a number of neurodegenerative diseases, such as PD, AD, ALS, and Huntington's disease (16). 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. (5, 14). Moreover, total antioxidant capacity (TAC) is one of the most important parameters to measure the capability of cellular non-enzymatic antioxidant defenses (5). 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 (17). 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 (5).

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 (4). Therefore, in order 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, and 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.

2. Materials and Methods

2.1. 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 as case groups and 51 samples from healthy subjects as controls were enrolled in this study. 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.

2.2. 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 prior to 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 microliters of which were poured into tubes containing EDTA to assess AChE activity in red blood cells, and the remainder was centrifuged at 750g for 7 minutes after keeping it at room temperature and forming a clot. Separated serum samples were stored in appropriate tubes at -70°C for further analysis.

2.3. 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:

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

2.4. 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 ScharlauChemie (Barcelona, Spain). OCP amounts were evaluated using the method described by Zumbado (18) 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 µL) 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 minutes, 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.

2.5. Erythrocyte Acetylcholine Esterase Activity Assay

To measure erythrocyte AChE activity, the modified Ellman's method (14) 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 20µM 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.

2.6. Measurement of PON-1 arylesterase activity

Sigma (Saint Louis, MO, USA) supplied phenylacetate, which serves as a substrate for the PON-1 enzyme. In order to measure the arylesterase activity of this enzyme in serum, the procedure proposed by Bobin-Dubigeon *et al*. (19) 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.

2.7. Measurement of Nitric Oxide

To measure the level of NO in serum, the Griess method was implemented. Initially, serum deproteinization was performed using 250 µL of serum in the presence of ZnSO4 and 0.3M NaOH. Conversion of nitrate to nitrite was done with vanadium (III) chloride (VCl3), followed by Griess reagent (2% sulphanilamide 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 minutes, the absorbance of the serum was quantified at 540 nm (5).

2.8. Measurement of Malondialdehyde

A modified version of the Yagis method (20) 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 minutes.

Afterward, 1 mL of n-butanol was added, and the test tube was centrifuged at 750g for 10 min. The ensuing pink phase was isolated and analyzed for MDA level at 532 nm using the tetraethoxypropane standard curve.

2.9. Determination of Carbonyl Proteins

Levine *et al*. (21) 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 minutes 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.

2.10. Measurement of serum TAC

Determination of TAC was performed utilizing a commercially available kit (CAT No. NS-15012 Naxifer, Orumiyeh, 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 (5).

2.11. 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.

2.12. 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.

2.13. CAT Activity Assay

The method previously described by Sinha (22) was applied to determine CAT activity with reagents including 30 mM H2O2, 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.

2.14. 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 µg/L) was 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.

2.15. Statistical analysis

Depending on the type of variable, the mean ± SD, or percentage, was used to represent the data. To check the normality of the data, the Kolmogorov-Smirnov test was employed. The qualitative and quantitative variables were compared between the two groups using appropriate statistical tests such as the 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.

3. Results

3.1. 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).

3.2. 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 Figures 1A and 1B, 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) (Figure 2).

3.3. Enzyme Activity and Oxidative Stress Parameters

The comparison of patient groups with controls revealed that the activity of AChE (5.08 ± 1.95 vs 7.64 ± 1.21 (U/L)), SOD3 (27.36 ± 11.37 vs 40.50 ± 16.06 (U/mL)), CAT (36.59 ± 4.04 vs 138.18 ± 64.28 (KU/mL)), and arylesterase activity of PON-1 (63.1 ± 20.25 vs 81.06 ± 21.82 (U/L)) was significantly different (P<0.05, Figure 3). Although the activity of the mentioned enzymes in PD patients was significantly lower than in controls, TAC (405.6 ± 127.0 vs 272.8 ± 65.5 (µM)), NO (30.25 ± 2.76 vs 23.27 ± 12.14 (µM)), MDA (2.25 ± 1.66 vs 1.62 ± 0.24 (µM)), and CP (1.69 ± 0.38 vs 0.78 ± 0.2 (nmol/mg protein)) levels were significantly higher in patients with PD compared to controls (P<0.05, Figure 3). Nevertheless, no notable differences were found between the two groups regarding GPx3 activity (32.78 ± 8.45 vs 31.33 ± 10.32 (U/L)) (P>0.05, Figure 3).

3.4. 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).

5. 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 neuronal loss remains elusive, several genetic and environmental factors are believed to contribute to its development (23). 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 (24). 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 (25). 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 (5, 26). OCPs, as illegal chemicals (despite the fact that the use of OCPs has been banned for many years, but 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 (27).

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 (28). Importantly, in living organisms, the production of dichlorodiphenyldichloroethylene (DDE) is facilitated by the enzyme cytochrome P450 through the reduction reactions on DDT (29). 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 negative correlation between 4,4-DDT and the PDQ39 score that may suggest that further exposure to OCPs accelerates the progression of the disease. Previous studies revealed that levels of OCPs, such as αHCH, 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 (24). Notably, the observed elevation in dieldrin concentrations may potentially be associated with alterations in dopaminergic response (30).

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 (5). 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 (31). 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 (5). 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 (32). 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 (32, 33).

A variety of enzymatic and non-enzymatic oxidative-related indicators were assessed in the current study in order 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 (34, 35). 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 (36).

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 (37). Consistent with previous research (38), the present study found that protein carbonylation is increased in PD, which may have occurred following exposure to pesticides (39). 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 (40). 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 (41) and unchanged (42) 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 (14). It has been documented that exposure

to pesticides can decrease the activity of this enzyme and subsequently lead to induce OS and inflammation (43). 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 (44). 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 (45). CAT, in cooperation with GPx3, functions in mitigating hydrogen peroxide levels and detoxifying a variety of peroxides (14). 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 (46). Moreover, enhanced CAT activity can serve as a protective measure against the detrimental impact of ROS induced by paraquat on the body (47). 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 (PPARγ) transcription activity, is implicated in the diminished expression of CAT as well as the escalated level of OS (48). 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 (42) or decrease (49) in individuals with PD as compared to a control group. Although pesticide exposure could potentially lower enzyme function (14), it's important to note that the gene expression of these enzymes might have heightened as a protective mechanism to offset reduced activity (50).

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 (51). Pesticide exposure can reduce TAC by triggering OS and decreasing antioxidant enzyme activity (14). 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 (52). 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 (53, 54). 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 (32, 55).

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.

Acknowledgments:

The authors thank all the individuals who participated in this study. This study was a Ph.D. thesis and was financially supported by the Kerman University of Medical Sciences through Grant No. KMU.AC.IR. 400001168.

Funding: The current study was financially supported by Kerman University of Medical Sciences through Grant No. KMU.AC.IR. 400001168.

Conflicts of interest: The authors have no relevant financial or non-financial interests to disclose.

Availability of data and material: Data are available upon reasonable request from the corresponding author, Dr. Gholamreza Asadikaram.

Authors' contributions:

Ali Samareh: Investigation, Methodology, Writing- original draft.

Gholamreza Asadikaram: Supervision, Conceptualization, Methodology, Writing-Review and Editing.

Hossain-Ali Ebrahimi: Resources, data curation, and scientific support.

Mohammad Hadi Nemtollahi: Writing-Review and Editing.

Hossein pourghadamyari: Writing-Review and Editing.

Mohammad Erfan Norouzmahani: Investigation

All authors have approved the final version of the manuscript.

Ethics 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.

References

1. Wang D, Gao H, Li Y, Jiang S, Yong Y, Yang X. Genome-scale expression pattern of long non-coding RNAs in Chinese Uyghur patients with Parkinson's disease. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2020;26:e925888-1.
2. Goel A, Viswanathan VK, Purudappa PP, Sakthivelan V, Mounsamy V, Sambandam SN. Cost and early complication analysis following total hip arthroplasty in Parkinson disease patients-A propensity-matched database study. The Archives of Bone and Joint Surgery. 2022.
3. Goldman SM, Marek K, Ottman R, Meng C, Comyns K, Chan P, et al. Concordance for Parkinson's disease in twins: a 20-year update. Annals of Neurology. 2019;85(4):600-5.

4. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Frontiers in cellular neuroscience. 2015;9:124.
5. Samareh A, Asadikaram G, MojtabaAbbasi-Jorjandi, Abdollahdokht D, Abolhassani M, Khanjani N, et al. Occupational exposure to pesticides in farmworkers and the oxidative markers. Toxicology and Industrial Health. 2022;38(8):455-69.
6. Kamboh M, Wan Ibrahim W, Rashidi Nodeh H, Marsin Sanagi M, Sherazi S. Removal of selected organophosphorus pesticides from water using newly fabricated amino-substituted calixarene-based magnetic sporopollenin. New J Chem. 2016;40:3130-8.
7. Camacho-Pérez MR, Covantes-Rosales CE, Toledo-Ibarra GA, Mercado-Salgado U, Ponce-Regalado MD, Díaz-Resendiz KJG, et al. Organophosphorus pesticides as modulating substances of inflammation through the cholinergic pathway. International Journal of Molecular Sciences. 2022;23(9):4523.
8. Baik D, Yu YM, Jung S-Y, Kang H-Y. Prevalence and patterns of the concurrent use of anticholinergics for the motor symptoms of Parkinson's disease and acetylcholinesterase inhibitors in Parkinson's disease patients with dementia: a cross-sectional study using Korea National Health Insurance claims data. BMC geriatrics. 2022;22(1):1-10.
9. Shim KH, Go HG, Bae H, Jeong D-E, Kim D, Youn YC, et al. Decreased exosomal acetylcholinesterase activity in the plasma of patients with Parkinson's disease. Frontiers in Aging Neuroscience. 2021;13:665400.
10. Hofmann JN, Keifer MC, Furlong CE, De Roos AJ, Farin FM, Fenske RA, et al. Serum cholinesterase inhibition in relation to paraoxonase-1 (PON1) status among organophosphate-exposed agricultural pesticide handlers. Environmental health perspectives. 2009;117(9):1402-8.
11. Menini T, Gugliucci A. Paraoxonase 1 in neurological disorders. Redox Report. 2014;19(2):49-58.
12. Paydar P, Asadikaram G, Fallah H, Zeynali Nejad H, Akbari H, Abolhassani M, et al. Serum levels of organochlorine pesticides and breast cancer risk in Iranian women. Archives of environmental contamination and toxicology. 2019;77:480-9.
13. Richardson JR, Caudle WM, Wang M, Dean ED, Pennell KD, Miller GW, et al. Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. The FASEB journal. 2006;20(10):1695-7.
14. Kiani Z, Asadikaram G, Faramarz S, Salimi F, Ebrahimi H. Pesticide Exposure and Alzheimer's Disease: A Case-control Study. Current Alzheimer Research. 2022;19(13):892-903.
15. Sule RO, Condon L, Gomes AV. A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. Oxidative Medicine and Cellular Longevity. 2022;2022.
16. Li J, O W, Li W, Jiang Z-G, Ghanbari HA. Oxidative stress and neurodegenerative disorders. International journal of molecular sciences. 2013;14(12):24438-75.
17. Picklo Sr MJ, Montine TJ, Amarnath V, Neely MD. Carbonyl toxicology and Alzheimer's disease. Toxicology and applied pharmacology. 2002;184(3):187-97.
18. Zumbado M, Goethals M, Álvarez-León EE, Luzardo OP, Cabrera F, Serra-Majem L, et al. Inadvertent exposure to organochlorine pesticides DDT and derivatives in people from the Canary Islands (Spain). Science of the Total Environment. 2005;339(1-3):49-62.

19. Bobin-Dubigeon C, Jaffré I, Joalland M-P, Classe J-M, Campone M, Hervé M, et al. Paraoxonase 1 (PON1) as a marker of short term death in breast cancer recurrence. Clinical biochemistry. 2012;45(16-17):1503-5.
20. Yagi K. [39] Assay for blood plasma or serum. Methods in enzymology. 105: Elsevier; 1984. p. 328-31.
21. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A-G, et al. [49] Determination of carbonyl content in oxidatively modified proteins. Methods in enzymology. 186: Elsevier; 1990. p. 464-78.
22. Hadwan MH. New method for assessment of serum catalase activity. Indian Journal of science and technology. 2016;9(4):1-5.
23. Chen X-Y, Liu C, Xue Y, Chen L. Changed firing activity of nigra dopaminergic neurons in Parkinson's disease. Neurochemistry International. 2022:105465.
24. Xu S, Yang X, Qian Y, Luo Q, Song Y, Xiao Q. Analysis of serum levels of organochlorine pesticides and related factors in Parkinson's disease. Neurotoxicology. 2022;88:216-23.
25. Tudi M, Li H, Li H, Wang L, Lyu J, Yang L, et al. Exposure routes and health risks associated with pesticide application. Toxics. 2022;10(6):335.
26. Zago AM, Faria NM, Favero JL, Meucci RD, Woskie S, Fassa AG. Pesticide exposure and risk of cardiovascular disease: A systematic review. Global Public Health. 2022;17(12):3944-66.
27. Bhatt P, Zhou X, Huang Y, Zhang W, Chen S. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. Journal of hazardous materials. 2021;411:125026.
28. Fénichel P, Coquillard P, Brucker-Davis F, Marchand P, Cano-Sancho G, Boda M, et al. Sustained bloodstream release of persistent organic pollutants induced by extensive weight loss after bariatric surgery: Implications for women of childbearing age. Environment International. 2021;151:106400.
29. Cárdenas-González M, Gaspar-Ramírez O, Pérez-Vázquez FJ, Alegría-Torres JA, González-Amaro R, Pérez-Maldonado IN. p, p′-DDE, a DDT metabolite, induces proinflammatory molecules in human peripheral blood mononuclear cells "in vitro". Experimental and Toxicologic Pathology. 2013;65(5):661-5.
30. Boyd SL, Kuhn NC, Patterson JR, Stoll AC, Zimmerman SA, Kolanowski MR, et al. Developmental exposure to the Parkinson's disease-associated organochlorine pesticide dieldrin alters dopamine neurotransmission in α-synuclein pre-formed fibril (PFF)-injected mice. bioRxiv. 2023:2023.06. 21.545967.
31. Kumar D, Sinha SN, Rajendra S, Sharma K. Assessing farmer's exposure to pesticides and the risk for non-communicable diseases: A biomonitoring study. Science of The Total Environment. 2023;891:164429.
32. Mota A, Hemati-Dinarvand M, Taheraghdam AA, Nejabati HR, Ahmadi R, Ghasemnejad T, et al. Association of Paraoxonse1 (PON1) Genotypes with the Activity of PON1 in Patients with Parkinson's Disease. Acta Neurol Taiwan. 2019;28(3):66-74.
33. Ikeda K, Nakamura Y, Kiyozuka T, Aoyagi J, Hirayama T, Nagata R, et al. Serological profiles of urate, paraoxonase-1, ferritin and lipid in Parkinson's disease: changes linked to disease progression. Neurodegenerative diseases. 2011;8(4):252-8.
34. Barmaki H, Morovati A, Eydivandi Z, Naleshkenani FJ, Saedi S, Musavi H, et al. The association between serum oxidative stress indexes and pathogenesis of Parkinson's disease in the northwest of Iran. Iranian journal of public health. 2021;50(3):606.

35. Rathnayake D, Chang T, Udagama P. Selected serum cytokines and nitric oxide as potential multi-marker biosignature panels for Parkinson disease of varying durations: a case-control study. BMC neurology. 2019;19:1-10.
36. Santos-Lobato BL, Bortolanza M, Pinheiro LC, Batalhão ME, Pimentel ÂV, Capellari-Carnio E, et al. Levodopa-induced dyskinesias in Parkinson's disease increase cerebrospinal fluid nitric oxide metabolites' levels. Journal of Neural Transmission. 2022:1-9.
37. Solana-Manrique C, Munoz-Soriano V, Sanz FJ, Paricio N. Oxidative modification impairs SERCA activity in Drosophila and human cell models of Parkinson's disease. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2021;1867(7):166152.
38. Chiaradia E, Renzone G, Scaloni A, Caputo M, Costanzi E, Gambelunghe A, et al. Protein carbonylation in dopaminergic cells exposed to rotenone. Toxicology Letters. 2019;309:20-32.
39. Márquez-Lázaro J, Díaz-Pineda K, Méndez-Cuadro D, Rodríguez-Cavallo E. Fluoroquinolone antibiotics and organophosphate pesticides induce carbonylation on Eisenia fetida muscle proteins. Science of The Total Environment. 2021;758:143954.
40. Rafeeinia A, Asadikaram G, Karimi-Darabi M, Abolhassani M, Abbasi-Jorjandi M, Moazed V. Organochlorine pesticides, oxidative stress biomarkers, and leukemia: a case-control study. Journal of Investigative Medicine. 2022;70(8):1736-45.
41. de Farias CC, Maes M, Bonifácio KL, Bortolasci CC, de Souza Nogueira A, Brinholi FF, et al. Highly specific changes in antioxidant levels and lipid peroxidation in Parkinson's disease and its progression: Disease and staging biomarkers and new drug targets. Neuroscience Letters. 2016;617:66-71.
42. Gökçe Çokal B, Yurtdaş M, Keskin Güler S, Güneş HN, Ataç Uçar C, Aytaç B, et al. Serum glutathione peroxidase, xanthine oxidase, and superoxide dismutase activities and malondialdehyde levels in patients with Parkinson's disease. Neurological sciences. 2017;38:425-31.
43. Paluzar H, Sagiroglu A. Effects of Organophosphorus and Pyrethroid pesticides on antioxidant enzymes and reactivation effects of Pralidoxime: In vitro studies. Kuwait Journal of Science. 2022;49(3).
44. Li D-n, Lian T-h, Zhang W-J, Zhang Y-n, Guo P, Guan H-y, et al. Potential roles of oxidative distress on neurodegeneration in Parkinson's disease with neuropsychiatric symptoms. Frontiers in Aging Neuroscience. 2022;14:875059.
45. Zhang M-S, Liang J-H, Yang M-J, Ren Y-R, Cheng D-H, Wu Q-H, et al. Low serum superoxide dismutase is associated with a high risk of cognitive impairment after mild acute ischemic stroke. Frontiers in Aging Neuroscience. 2022;14:834114.
46. Lu S, Liu S, Cui J, Liu X, Zhao C, Fan L, et al. Combination of patulin and chlorpyrifos synergistically induces hepatotoxicity via inhibition of catalase activity and generation of reactive oxygen species. Journal of agricultural and food chemistry. 2019;67(41):11474-80.
47. Joguchi A, Fujii M, Ayusawa D. Increased catalase activity in mouse cell mutants resistant to paraquat. Biogerontology. 2004;5:193-200.
48. Yakunin E, Kisos H, Kulik W, Grigoletto J, Wanders RJ, Sharon R. The regulation of catalase activity by PPAR γ is affected by α-synuclein. Annals of Clinical and Translational Neurology. 2014;1(3):145-59.
49. Baillet A, Chanteperdrix V, Trocmé C, Casez P, Garrel C, Besson G. The role of oxidative stress in amyotrophic lateral sclerosis and Parkinson's disease. Neurochemical research. 2010;35:1530-7.

50. Han Y, Song S, Wu H, Zhang J, Ma E. Antioxidant enzymes and their role in phoxim and carbaryl stress in Caenorhabditis elegans. Pesticide Biochemistry and Physiology. 2017;138:43-50.
51. Li J, Li D, Guo J, Wang D, Zhang X. Age of Onset Moderates the Association between Total Antioxidant Capacity and Cognitive Deficits in Patients with Drug-Naïve Schizophrenia. Antioxidants. 2023;12(6):1259.
52. Bernieri T, Rodrigues D, Randon Barbosa I, Perassolo MS, Grolli Ardenghi P, Basso da Silva L. Effect of pesticide exposure on total antioxidant capacity and biochemical parameters in Brazilian soybean farmers. Drug and Chemical Toxicology. 2021;44(2):170-6.
53. Arabuli L, Lovecka P, Jezek R, Viktorova J, Macek T, Junkova P, et al. AChE inhibitory effect, anti-oxidant and anti-inflammatory properties of cyclen and L-Dopa related compounds: Targeting in neurodegenerative disease. Journal of Molecular Structure. 2023;1287:135665.
54. Duarte-Jurado AP, Gopar-Cuevas Y, Saucedo-Cardenas O, Loera-Arias MdJ, Montes-de-Oca-Luna R, Garcia-Garcia A, et al. Antioxidant therapeutics in Parkinson's disease: Current challenges and opportunities. Antioxidants. 2021;10(3):453.
55. Oderinu KA, Ajose OA, Salawu L, Komolafe MA, Oseni FA, Smith OS, et al. Serum cytokines are related to oxidative stress in Nigerian patients with Parkinson's disease.

Tables

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	73.8 ± 10.6	61.16 ± 5.5	0.002
	Male	65.11 ± 10.16	65.94 ± 9.4	0.571
	Total	68.10 ± 10.8	64.16 ± 8.4	0.13
Gender	Female	10 (34.5%)	19 (37.3%)	0.804
	Male	19 (65.5%)	32 (62.7%)	
BMI (kg/m²)	Female	24.19 ± 5.88	25.05 ± 1.91	0.557
	Male	24.92 ± 4.65	24.85 ± 1.7	0.937
	Total	24.67 ± 5.02	24.92 ± 1.76	0.739
Level of education	High school or less	16 (55.2%)	35 (68.6%)	0.229
	Greater than highschool	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 ± 9.75	27.43 ± 9.29	0.266
	AST (U/L)	32.24 ± 9.45	33.37 ± 5.59	0.37
	TP (g/dl)	5.93 ± 0.4	5.87 ± 0.34	0.467
	Cr (mg/dl)	1.35 ± 0.57	1.29 ± 0.41	0.763
	TG (mg/dl)	96.21 ± 40.65	98.61 ± 28.32	0.26
	TC (mg/dl)	160.55 ± 36.5	157.75 ± 29.2	0.806
	HDL-c (mg/dl)	42.45 ± 4.43	42.7 ± 2.89	0.636
	LDL-c (mg/dl)	98.86 ± 37.81	95.3 ± 30.44	0.76
Family history	Yes	6	N/A	
	No	23		
Duration of PD (month)	Female	30 ± 18.97	N/A	
	Male	18.95 ± 24.63		
	Total	22.76 ± 23.11		
PD medications	No	9	N/A	
	Under 2 years	14		
	More than 2 years	6		
UPDR IV	Female	10.1 ± 3.11	N/A	
	Male	6.74 ± 3.5		
	Total	7.89 ± 3.69		
PDQ39	Female	14 ± 4.1	N/A	
	Male	12.3 ± 9.4		

	Total	12.89 ± 7.92	
H&Ym	Female	2.25 ± 0.9	N/A
	Male	1.76 ± 0.61	
	Total	1.93 ± 0.74	
Beck	Minimal depression	24	N/A
	Mild depression	3	
	Moderate depression	1	
	Severe depression	1	

Data are expressed as numbers of individuals or means ± SD 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.

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

Param	***TAC***	***CAT***	***SO***	***GP***	***CP***	***MDA***	***NO***	***PO***	***AC***	***α-H***	***β-H***	***γ-H***	***2,4-D***	***4,4-D***	***2,4-D***	***4,4-D***	***PDQ***

eter			***D3***	***x3***				***N-1***	***hE***	***CH***	***CH***	***CH***	***DE***	***DE***	***DT***	***DT***	***39***
TAC	1	0.1106	0.3113	-0.1197	-0.0551	-0.1124	**-.4366***	-0.0033	0.1011	-0.1127	0.2116	0.2333	0.1132	0.0027	-0.0035	0.2115	0.037
CAT		1	-0.0074	-0.2988	-0.2999	-0.0555	-0.1104	-.4077	0.1124	-0.3055	0.2118	-0.0433	-0.3226	-0.0028	-0.2222	0.0056	0.107
SOD3			1	-0.1666	0.2433	0.0076	**-.6166****	0.0047	-0.1126	0.1110	0.0559	0.1121	-0.2239	**-.4886****	**-.5299****	0.0024	-0.253
GPx3				1	0.1766	0.0081	0.1119	0.1555	-0.1077	-0.0006	-0.1399	0.0411	0.0078	0.2799	0.3077	-0.0082	-0.312
CP					1	-0.3133	-0.2888	-0.2222	0.0700	0.0922	0.3332	0.2977	-0.1600	0.1137	-0.1066	0.1558	-0.2722
MDA						1	0.0600	0.2009	-0.0111	0.3411	-0.1933	-0.1132	0.2833	-0.3266	-0.0888	-0.3655	-0.0199
NO							1	0.2866	0.2333	0.0073	-0.2533	-.5144	0.2755	0.3444	**.4122***	-.3922	0.1700
PON-1								1	0.1006	0.3099	-0.2000	-0.3433	0.0079	-0.2822	-0.0016	**-.4177***	-0.0422
AChE									1	0.0833	0.0888	-0.2666	-0.1611	-0.2488	-0.2477	-0.2822	0.0166

α-HCH										1	0.073	0.042	-0.135	-0.294	-0.266	-.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	**.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.

Figure legends

Figure 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.

Figure 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: α-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.

Figure 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. A: AChE activity. B: MDA serum levels. C: Serum levels of TAC. D: PON-1 arylesterase activity. E: CAT activity. F: SOD3 activity. G: GPx3 activity. H: CP serum levels. I: NO serum levels. 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.

Figures

Figure 1

Figure 2

Figure 3