



## ANTIOXIDANT BIOCHEMICAL PARAMETERS IN PARKINSON'S DISEASE IN LIBYAN POPULATION

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**ABSTRACT** **Background:** Parkinson's disease, PD is characterized by dopaminergic neuronal loss in the substantia nigra at the central nervous system (CNS), a significant reduction in dopamine levels, and the presence of Lewy bodies. Lewy bodies are composed of abnormal deposits of protein aggregates, particularly synuclein proteins. While the main etiology of the disease has yet to be determined. Environmental, occupational and genetic factors seem to play important roles. Risk factor studies have pinpointed age, gender, occupation, area of residence, smoking, coffee/tea consumption, drinking well-water, and being exposed to herbicides/pesticides. Biochemical studies designated to elucidate the underlying mechanism of P.D pathogenesis and also if there is any correlation between any of the biochemical parameters that might give a key for the association between the environmental and genetic study. **Materials and Methods:** Estimation of the levels of Nonenzymatic antioxidants: Vitamins, C, A, and E and Antioxidant Enzymes: Superoxide Dismutase (SOD) Glutathione peroxidase (GPX) and, Catalase (CAT). A total of 104 subjects, including control, were enrolled in the study and further grouped as 52 clinically examined Idiopathic Parkinson's disease patients (35 males and 17 females) while remaining 52 were taken as age and sex-matched healthy controls. **Results:** The biochemical studies have shown that serum vitamin D concentration was lower among Parkinson's disease cases and it was also associated with age, sex, marital status, education, leisure-time physical activity, smoking, alcohol consumption, body mass index, diabetes, hypertension, and the serum cholesterol. The RR of the disease between the highest and lowest quartiles of vitamin D concentration was 0.35 (95% CI 0.15–0.81, p for trend=0.006). In our study, there is a significant decrease in the activity of Glutathione peroxidase ( $p < 0.001$ ), Superoxide dismutase ( $p < 0.001$ ), and Catalase ( $p < 0.001$ ) in PD patients as compared with controls. Further Vitamin C ( $p < 0.05$ ), Vitamin E ( $p < 0.05$ ) significantly decreased, but Vitamin A and Uric acid levels ( $p > 0.05$ ) remain unchanged and this may be due to the compensatory mechanism of the body against oxidative stress, which not allowed much alteration in other parameters in the PD patients as compared with controls. We found statistically significant differences between cases and control regarding age, and gender ( $p = 0.04$ , and  $p = 0.01$ , resp.). Elevated homocysteine concentrations, homocysteine was  $14.4 \mu\text{mol/L}$  in PD and  $12.6 \mu\text{mol/L}$  in controls ( $P > 0.05$ ). Additionally, there were no statistically significant differences in the levels of B12 and folic acid levels between cases as compared to control. The proportion of cases with coenzyme Q10 deficiency was also significantly greater in cases than in controls (32–36% vs. 8–9%;  $P = 0.0012–0.006$ ). **Conclusion:** Present study concludes that all antioxidants both enzymatic and non enzymatic are showing lower activity in patients suffering from parkinson's disease. There is requirement to know the levels of albumin along with antioxidants.

**KEYWORDS :** Pratishtaya, Pradhman Nasya, Shathi, Chaturjat Churna

### INTRODUCTION

Approximately 190 years passed since James Parkinson wrote his essay entitled "An Essay On The Shaking Palsy" in which he described the clinical symptoms that now carries his name. Although the cardinal motor symptoms of Parkinson's disease (P.D) are well known to include tremor at rest, bradykinesia, increased tone, and loss of postural reflexes. Several non-motor features are also very common including autonomic features, cognitive impairment depression, olfactory deficits, psychosis and sleep disturbances, bladders, and

sexual dysfunctions [1].

P.D results from the progressive death of the dopaminergic neurons in the substantia nigra (SN) pars compacta (PC) [2].

The neuropathological diagnosis of P.D was based traditionally based on the loss of dopaminergic neurons, accompanying gliosis and the presence of Lewy bodies, eosinophilic intracytoplasmic inclusions in the remaining nerve cells of the SN and neurochemically related nuclei, more recently diagnosis taking into the account the alpha-

synuclein (SNCA) burden of the brain [3].

While the main etiology of the disease has yet to be determined, environmental, occupational, and genetic factors seem to play important roles. The prevalence of Parkinson's disease varies among ethnic and geographic groups around the world, being very low in countries of the far east Asia and high in Latin America, and varies in some areas in north Africa and Europe [4].

Biochemical studies designated to elucidate the underlying mechanism of P.D pathogenesis and also if there is any correlation between any of the biochemical parameters that might give a key for the association between the environmental and genetic study.

Estimation of the levels of Non-enzymatic antioxidants: Vitamins, C, A, and E and Antioxidant Enzymes: Superoxide Dismutase (SOD) Glutathione peroxidase (GPX) and, Catalase (CAT).

Oxidants and antioxidants related substances may contribute to the pathogenesis and the progression of Parkinson's disease. The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost[5]. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules, commonly called "free radicals." Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and functions. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants[6].

Parkinson's disease (PD) is one of the major progressive neurological disorders, characterized by the loss of dopaminergic neurons in pars compacta of the substantia nigra. The causes for this are the interactions between external toxins (which arise from environmental, dietary, and lifestyle factors) and internal toxins arising from normal metabolism, genetic and epigenetic (mitochondria, membranes, and proteins) components [7]. Oxidative stress is one of the intermediary risk factors that could initiate and promote the degeneration of neurons. Even though oxidative stress in the brain is an important factor in the neuropathology of PD, yet the role of systemic oxidative stress is inconclusive [8].

Research can make great progress in understanding and further treating the PD. This study demonstrates a significant variation of oxidants-antioxidants status in Parkinson's disease. Oxidative stress plays a crucial role in the progression of PD; however, oxidative stress is a cause or the consequence of PD is debatable. Aabha Sharma *et. al.* concluded from the study carried out on Parkinson's disease patients that, increased lipid peroxidation, decreased Glutathione levels and increased Superoxide Dismutase (SOD) activity are due to the deteriorative action of various reactive oxygen species (ROS) which have been generated during the state of oxidative stress present in PD patients [9]. Shashikant *et.al* observed in Parkinson's disease patients that, Plasma thiobarbituric SOD, GPX, Catalase (CAT), vitamin-E, vitamin-C, vitamin A, copper, zinc and selenium levels were significantly low in Parkinson's disease when compared with control subjects, and concludes that the elevated oxidative stress may be playing a role in dopaminergic neuronal loss in substantia nigra pars compacta and involved in the pathogenesis of the Parkinson's disease [10].

Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralizing oxidants in the aqueous phase before lipid peroxidation is initiated. Vitamin C acts as a prooxidant at low concentration, including lipid peroxidation in the presence of metal ions such as Fe<sup>3+</sup> or Cu<sup>2+</sup>. However, high concentration, it is a potent antioxidant, and is capable of reacting with superoxide and hydroxyl radicals to prevent their toxic action.[11]. As an antioxidant, vitamin E acts as a peroxy radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state. As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage. Uric acid is a natural antioxidant, accounting for up to 60% of the free radical scavenging activity in human blood. Uric acid can scavenge superoxide, the hydroxyl radical, and singlet oxygen. Uric acid may assist in the removal of superoxide by preventing against the degradation of SOD, the enzyme that is responsible for clearing superoxide from the cell. Uric acid also can bind iron and inhibit iron-dependent ascorbate

oxidation, preventing an increased production of free radicals that can further contribute to oxidative damage. As reported by researchers that, Vitamin C and Vitamin E levels significantly fell in PD patients as compared with controls [12].

Homocysteine, a sulfur-containing amino acid formed by demethylation of methionine, is involved in numerous processes of methyl group transfer, all playing pivotal roles in the biochemistry of the human body. Increased levels of plasma homocysteine (hyperhomocysteinemia) - which may result from a deficiency of folate, vitamin B6 or B12 or mutations in enzymes regulating the catabolism of homocysteine - are associated with a wide range of clinical manifestations, mostly affecting the central nervous system. Recent evidence suggests that changes in the metabolic fate of homocysteine, leading to hyperhomocysteinemia, may also play a role in the pathophysiology of neurodegenerative disorders, particularly Parkinson's disease (PD) [13]. An increase in plasma Hcy levels has been reported in Parkinson's disease (PD) patients who were using levodopa. Total Hcy concentrations in the cerebrospinal fluid were also higher following levodopa therapy than before treatment and than in controls.

The catalysis of levodopa with the catechol-O-methyltransferase (COMT) enzyme results in the formation of S-adenosylhomocysteine (SAH), which hydrolyzes to form homocysteine (Hcy). Experimental studies have demonstrated that Hcy can be neurotoxic and excitotoxic to the substantia nigra. Furthermore, Hcy may be associated with dyskinesia, which is an indicator of possible neurodegeneration due to the disruption of the balance of striatal activity [14].

The electron transport chain (ETC) generates most of the cellular ATP and is comprised of five multi-subunit enzyme complexes. Two electron carriers, coenzyme Q (CoQ) and cytochrome c, are vital for the mitochondrial synthesis of ATP. Mutations in genes of either genome may cause mitochondrial diseases, which are common among inherited metabolic and neurological disorders [15].

CoQ is a lipid-soluble component of virtually all cell membranes. It is composed of a benzoquinone ring with a polyprenyl side chain, the number of isoprene units being a characteristic of given specie, e.g., 10 in humans (CoQ10). CoQ10 transports electrons from Complexes I and II to complex III. These electrons come from either NADH or succinate,] although CoQ10 can be alternatively reduced with electrons provided by different redox reactions in mitochondria [16].

Consequently, CoQ10 is essential for ATP production inside mitochondria, although it is also an indispensable antioxidant in extramitochondrial membranes and a key factor for pyrimidine nucleotide synthesis. The level of CoQ is highly regulated inside cells and tissues but its concentration is different in each tissue and organ and depends on dietary conditions and age. CoQ also varies greatly in human diseases such as Alzheimer's disease, Parkinson's disease, cardiomyopathy, Niemann-Pick and diabetes. Reactive oxygen species (ROS) are well known to contribute to the pathophysiology of Parkinson's disease (PD),[17].

## MATERIALS AND METHODS

Information on socioeconomic background, diseases, medications, and lifestyle was collected via questionnaires and interviews.

History including age, sex, education, community population, occupation, coffee consumption, body mass index (BMI) {height and weight were measured, and the body mass index (kg/m<sup>2</sup>) was calculated

The neurologist stating that all the diagnostic criteria for Parkinson's disease are met. This must include symptom history and reports of clinical findings, including the presence of resting tremor, bradykinesia, and/or muscle rigidity.

Estimation of the levels of Nonenzymatic antioxidants: Vitamins, C, A, and E and Antioxidant Enzymes: Superoxide Dismutase (SOD) Glutathione peroxidase (GPX) and, Catalase (CAT).

A total of 104 subjects, including control, were enrolled in the study and further grouped as 52 clinically examined Idiopathic Parkinson's disease patients (35 males and 17 females) while remaining 52 were taken as age and sex-matched healthy controls.

## Inclusion criteria:

- 1) Male and female patients diagnosed as Idiopathic Parkinson's disease aged between 50 to 70 years. Without any drug therapy.
- 2) Control group included healthy volunteers who were consistent with the patients according to age, sex, and body mass index.

**Exclusion Criteria:**

- 1) Patients having blood disorders, obvious malignancy, hepatic, renal or cardiac disease and additional history of alcohol or smoking will be excluded from the study.
- 2) Patients with co-existing neurological disorder like Alzheimer's disease, stroke or any kind of neural deficit was also excluded.
- 3) Patients on any concomitant medication such as Lipid-lowering drug, antioxidants, vitamins, minerals, herbal treatment, or the substance which may alter our study parameters excluded from the study.

10 ml venous fasting blood samples from patients and controls were collected. 4 to 5ml blood was collected in the heparinized vacutainers and the remaining 5 to 6 ml blood was collected in plain vacutainers. Heparinized whole blood was used for the estimation of SOD and GPX activity and hemoglobin concentration. Plasma was used for the estimation of Vitamin C and Vitamin E levels. Serum separated from plain blood used for the estimation of Uric acid and the activity of CAT. All the spectral analyses were carried out on a fully automated UV-Visible spectrophotometer and for reagent preparation glass distilled water used throughout the study [19,20]. So, the present work was designed with a specific aim to study the following biochemical parameters in the blood of PD patients, and the same we have to compare with the blood levels of healthy controls. To study the activity of antioxidant enzymes involved in free radical scavenging in PD and controls we have to analyze, the activity of SOD, GPX, and CAT. To study the status of nonenzymatic antioxidants involved in free radical scavenging in PD and controls we have to analyze levels of Plasma Vitamin E, Vitamin C, and serum Uric acid.

For this prospective study, we enrolled one hundred patients (54 men, 54%) diagnosed. The average age of the patients was 70 years (range 40–89). For all patients, blood samples were taken from the peripheral vein after 12 hours of fasting and at 12-hour drug-free periods. Plasma Hcy levels, B12, and folic acid levels were analyzed by automated electrochemiluminescent immunoassay (Elecsys 2010, Roche Diagnostics, Basel, Switzerland) [21,22].

Twenty patients with PD elected to have an antioxidant assessment (Functional Intracellular Assay (FIA), Spectra Cell Lab, Nottingham, UK) performed. Biochemical analysis (ELISA) was created. The proportion of cases with functional deficiency was also compared to that for controls (18 patients) by chi2 test.

**RESULTS**

Table 1 Depicts the clinical background of subjects who participated in the present study. As from Table 1 there is no significant difference in the age of Parkinson's disease patients as compared with control (p > 0.05).

In this study, as from Table 2, there is a significant decrease in the activity of Glutathione peroxidase (p < 0.001), Superoxide dismutase (p < 0.001), and Catalase (p < 0.001) in PD patients as compared with controls. Further Vitamin C (p < 0.05),

Vitamin E (p < 0.05) significantly decreased, but Vitamin A and Uric acid levels (p > 0.05) remain unchanged and this may be due to compensatory mechanism of the body against oxidative stress, which not allowed much alteration in other parameters in the PD patients as compared with controls.

**Table 1. The difference in age of Parkinson's disease patients as compared with controls.**

	Control (n=52)	P.D cases (n=52)
Men	35	35
Women	17	17
Mean age ± S.D.	60.05 ± 5.4	59.97 ± 5.4 #
# p > 0.05 No significant difference in age of Parkinson's disease patients as compared with controls.		

**Table 2. Comparison of antioxidants enzymes and non-antioxidant parameters between Controls and Cases.**

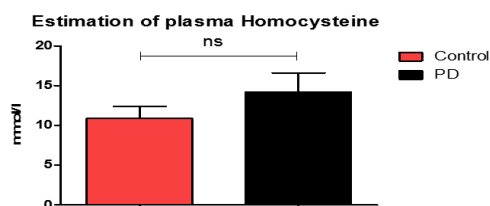
No	Parameter	Controls	Cases
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1	Glutathione peroxidase U/g	63.73 ± 4.1	43.73 ± 2.3**
2	Superoxide dismutase U/g	1463 ± 131	1004 ± 120**
3	Serum Catalase kU/l	45.61 ± 14.2	23.25 ± 5.8**
4	Plasma Vitamin C mg/dl	0.90 ± 0.1	0.82 ± 0.1*
5	Plasma Vitamin E mg/l	11.25 ± 1.7	10.51 ± 2.0*
6	Serum Vitamin A U/g	0.57 +/- 0.03	0.59 +/- 0.03#
7	Serum Uric Acid mg/dl	4.47 ± 1.0	4.45 ± 1.0 #
Values are expressed as Mean ± S.D, * p < 0.05 as compared with control, ** p < 0.001 as compared with control, and # p > 0.05as compared with control.			

In this study we found there is statistically significant differences between cases and control (table 3) regarding age, and gender (p = 0.04, and p = 0.01, resp.). Elevated homocysteine (Hcy) concentrations, Hcy was 14.4 μmol/L in PD and 12.6 μmol/L in controls (P > 0.05). Additionally, there were no statistically significant differences in the levels of B12 and folic acid levels between cases as compared to control.

**Table 3. Demographic data, clinical characteristics, and serum vitamin B12, folic acid, and Hcy levels are shown.**

	Control	Cases
Age (years)	65.5 (42–84)	70 (40–89)
Male/female	54 (54%)/46 (46%)	58(58%) 42(42)%
Hcy (μmol/L)	12.6	14.45
Vitamin B12 (pg/mL)	268.1	263.5
Folic acid (ng/mL)	9.38	9.29



**Figure 1. Elevated plasma homocysteine level in P.D. as compares to control.**

**DISCUSSION**

Under normal conditions, the continuous production of free radicals is compensated by the powerful action of protective enzymes. SOD, CAT, and GPX are the major antioxidant enzymes present in the human body that protect against oxygen toxicity [18]. Oxidative stress may be a consequence of reduced efficiency of these endogenous antioxidants that may render PD patients more vulnerable to oxidative stress [24,25].

As reported by researchers that, Vitamin C and Vitamin E levels significantly fell in PD patients as compared with controls, our results regarding this non-enzymatic antioxidant consistent with them table 2. Further, we did not find any change in levels of serum Vitamin A and Uric acid of PD patients as compared to control, and this may be because, the compensatory mechanism of the body against oxidative stress not allowed much alteration in other parameters [25,26]. In light of our findings and additional data from literature we emphasize that along with clinical examination, they said biochemical investigations in our research project, will be a supportive marker for prognosis and diagnosis of PD. This could help physicians, neurologists to find out the risk of initiation and progression of PD. It also might allow them to find treatments that will halt the disease process in the early stages.

In 1995, Allain et al. were the first to determine that Hcy levels were higher in PD patients than those in healthy subjects Following this, Agil *et al.* showed that the Hcy levels of patients treated with long-term levodopa were higher than those in patients who had never used levodopa [24]. In PD patients, plasma Hcy levels may increase because of chronic levodopa therapy. The catabolism of levodopa with the COMT enzyme results in SAH, which rapidly hydrolyzes and eventually forms Hcy [26,27]. In our study, vitamin B12 and folic acid values were normal in all the three groups and there was no difference between groups regarding vitamin B12 and folic acid values.

The proportion of cases with coenzyme Q10 deficiency was



significantly greater in cases than in controls (32–36% vs. 8–9%;  $P=0.0012-0.006$ ). CoQ is a lipid-soluble component of virtually all cell membranes. It is composed of a benzoquinone ring with a polyprenyl side chain, the number of isoprene units being a characteristic of given specie, e.g., 10 in humans (CoQ10). CoQ10 transports electrons from Complexes I and II to complex III. These electrons come from either NADH or succinate, although CoQ10 can be alternatively reduced with electrons provided by different redox reactions in mitochondria [23,29].

Consequently, CoQ10 is essential for ATP production inside mitochondria, although it is also an indispensable antioxidant in extramitochondrial membranes and a key factor for pyrimidine nucleotide synthesis. The level of CoQ is highly regulated inside cells and tissues but its concentration is different in each tissue and organ and depends on dietary conditions and age. CoQ also varies greatly in human diseases such as Alzheimer's disease, Parkinson's disease, cardiomyopathy, Niemann-Pick and diabetes. Reactive oxygen species (ROS) are well known to contribute to the pathophysiology of Parkinson's disease (PD) [26].

The deficiency of coenzyme Q10 assessed via FIA should be explored as a potential peripheral biomarker of antioxidant status in PD.

There is oxidative stress in PD which leads to an increase in the consumption of vitamin C and E. Decreased vitamin C and E levels may cause oxyradical mediated injury thereby causing nigral neurodegeneration [30]. Vitamin E traps free radicals by interrupting the chain reaction involved in apoptosis [31].

Enzymatic antioxidant status can be estimated by the estimation of erythrocyte SOD, GSHPx, catalase activity, and ceruloplasmin. Serum zinc levels and SOD activity is decreased in PD [30]. The structure of SOD is stabilized by zinc hence decreased zinc levels lead to loss of SOD activity [32]. This says that if there is an increased superoxide radical concentration it leads to an increase in the SOD activity. These superoxide radicals combine with Nitric oxide and elevates the oxidative stress. (a) Some studies have reported an increase in antioxidant enzymes like SOD and glutathione peroxidase [33,34].

Glutathione peroxidase and selenium levels are also decreased in PD. Selenium is present in the form of single selenocysteine residue in Glutathione peroxidase [32]. Decreased selenium concentration decreases the activity of Glutathione peroxidase in PD patients compared to controls. Increased oxidative stress may lead to the oxidation of the hemoprotein subunit of catalase. This oxidation may lead to the dissociation of the tetrameric hemoprotein molecule resulting in the loss of catalase activity. Thus the activity of catalase is decreased in increased oxidative stress [30].

Plasma proteins mainly contribute to 10 to 50% of the total radical trapping capacity of the antioxidants. Compared to the normal controls albumin a major plasma protein is decreased in parkinsonism due to the swallowing difficulty, decreased movement indirectly decreasing the food consumption, etc. [35]. There is blockage of absorption of amino acids like tyrosine, phenylalanine and tryptophan by levodopa which indirectly leads to the decreased albumin levels [36,37,38].

Some studies proved that there is a significant decrease in albumin levels and plasma proteins containing SH groups in PD [39,40]. Therefore it is concluded that the reduction of antioxidant activity in parkinsonism is due to the decreased plasma albumin levels. Thus Protein-rich diet supplementation in PD patients may increase the albumin levels indirectly increasing the antioxidant activity [35].

## CONCLUSION

Present study concludes that all antioxidants both enzymatic and non enzymatic are showing lower activity in patients suffering from parkinson's disease. There is requirement to know the levels of albumin along with antioxidants. Hence it is suggested that patients who are suffering from Parkinson's disease should be supplemented with antioxidants along with regular therapy. Its highly advantageous for elderly people who takes antioxidant may reduce risk of getting parkinsonism.

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