Iran J Public Health, Vol. 50, No.3, Mar 2021, pp.606-615

Original Article

The Association between Serum Oxidative Stress Indexes and Pathogenesis of Parkinson's Disease in the Northwest of Iran

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(Received 09 Jan 2020; accepted 15 Apr 2020)

Abstract

Background: Parkinson's disease (PD) is a prevalent neurodegenerative disorder. Oxidative stress is a main modulator in the advancement of PD. This investigation aimed to evaluate the relations between serum trace elements, vitamin C, ferritin, transferrin, Nitrite Oxide (NOx) and Peroxynitrite (PrN) concentrations and clinical parameters in patients with PD.

Methods: Serum concentrations of variables were measured in 75 PD patients and 75 healthy subjects from Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran between Feb 2016 and Sep 2018. Receiver Operating Characteristic (ROC) analysis was performed to examine incremental diagnostic value of vitamin C, NOx, and PrN in the study groups.

Results: Mean serum NOx (35.81±5.16 vs. 11.27±3.59 mol/L, $P<0.001$) and PrN (15.78±4.23 vs. 9.62±4.57 mol/L, $P= 0.004$) were markedly higher in patient group versus healthy individuals. Significant differences were also observed in the serum levels of vitamin C ($P<0.001$), copper (Cu) ($P<0.001$), Iron (Fe) ($P=0.003$), and Zinc (Zn) ($P<0.001$) between patients with PD and healthy subjects. Nevertheless, the serum levels of Se ($P=0.515$), ferritin ($P=0.103$), and transferrin ($P=0.372$) were not statistically significant between the study groups. ROC analysis has revealed a diagnostic ability of serum vitamin C levels for PD with an area under ROC curve of ≥0.7 ($P<0.05$) and relatively high sensitivity and specificity.

Conclusion: Serum levels of NOx and PrN are significantly higher in patients with PD. In additions, serum vitamin C levels have a diagnostic value as a biomarker. Further studies are required with larger sample size to provide more detailed information about the cognitive profile of participants and the outcome measures.

Keywords: Parkinson diseases; Trace elements; Oxidative stress; Ferritin; Nitric oxide

Introduction

Parkinson's Disease (PD) is defined as a neurodegenerative disease that is associated with senility. In this disease, dopamine-producing neurons are damaged in the substantia nigra, and the disease progresses with the death of these cells (1). The motor symptoms of PD include bradykinesia, muscle stiffness, tremor, and disturbance in walking and tremor. Non-motor symptoms of PD include depression, swallowing disorders, and anxiety (2).

Oxidative stress is an important factor in the initiation and progression of PD (3). A process in which the production of free radicals is far beyond the antioxidant defense system is taken inside the body as oxidative stress (4). The accumulation of iron in the cells of the body stimulates the production of free radicals from the Fenton reaction pathway (5). On the other hand, ferritin is an important protein in the transportation and storage of iron; studies have shown that the amount of ferritin has been increases in patients with PD (6). There is a direct correlation between iron content and ferritin levels in the body. An increase in the amount of iron in the cells results in the accumulation of ferritin levels in the serum. Increasing the iron stimulates the production of free radicals, which further damages various tissues, including the brain tissue (7). Transferrin is also measured to understand the status of iron metabolism in the body in addition to ferritin. It significantly increases in patients with PD compared to healthy subjects (8). Iron abnormal metabolism plays a role in the pathogenesis of PD. However, the results were different from different researches (9, 10).

Selenium (Se) is known as an important antioxidant in the body. Se has its effects through selenoproteins, such as glutathione reductase and thyroidotoxin reductase (11). This factor can be considered as an important factor in counteracting oxidative stress in the nervous system (12, 13). Trace elements play a vital role in balancing oxidative stress to the cells. This finding was obtained by research about post-mortem autopsy in the brain of patients with PD (14). The serum levels of trace elements have been limited in PD patients and contradictory (15).

Nitrite Oxide (NOx) is produced by nitrate oxide synthase. Increasing the level and long-term exposure of cells NOx promotes the production of malignant inflammatory reactions that can lead to cell death. NOx can react with anion superoxide and produce prooxis nitrite (ONOO-), which is a potent oxidant. Proxynitrite (PrN) can be converted to hydroxyl and nitrogen radicals (16). NOx and PrN play an important role in PD etiology (2).

Vitamin C has an anti-oxidant property measured in blood (17). Vitamin C plays a role in the brain as an antioxidant. It also acts as a neurotransmitter in dopamine-producing neurons (18).

In the current investigation, we evaluated serum levels of Se, Cu, Zn, iron, ferritin, transferrin, vitamin C, NOx, and PrN in both patients with PD and healthy subjects. Then, we studied the possible relationship between these variables and clinical characteristics of patients with PD. Receiver Operating Characteristic (ROC) curve analysis was also conducted to determine the diagnostic value of vitamin C, NOx, and PrN in the patient group compared to healthy individuals.

Materials and Methods

Subject recruitment

The present study consisted of 75 patients with PD and 75 healthy subjects. Patients with PD were employed from Imam Reza Hospital, Tabriz University of Medical Sciences, Tabriz, Iran between Feb 2016 and Sep 2018. The diagnosis was in match with the UK Parkinson's Disease Society Brain Bank Research criteria. The diagnostic standards were reviewed by a neurologist with investigation in movement symptoms (19). Based on Hoeh and Yahr (H & Y) classification all PD patients were at stages< 4. (1). Moreover, patients with PD and control individuals were completed the informed consent form.

The study was approved by the Committee of Clinical Research Ethical in Tabriz University of Medical Sciences, Iran (Ethical code: IR.TBZMED.REC.1395.823).
Patients with secondary Parkinsonism, gout, migraine, multiple sclerosis, epilepsy, chronic inflammation, hepatic, hematologic diseases and patients with a history of cancer and severe systemic disease, and those who were taking antioxidant drugs were excluded. The control group included age matched subjects with similarly dietary habit. Demographic characteristics were recorded at the time of sampling.

Sample preparation

Early-morning blood specimens (5 mL) were gathered from peripheral vein of candidates who were fast for 12 hours. After the clot formation, the serum was isolated by centrifugation at 3000 rpm for 15 min at room temperature. To assess the biochemical variables, the serum transferred into micro tubes and saved at −70 °C.

Measurement of variables

The serum level of NOx was assessed via nitrate/nitrite colorimetric assay kit (Cayman, Ann Arbor, MI, USA). The level of NOx in the samples was determined at a wavelength of 540 ± 2 nm by comparing the O.D. of the samples to the standard curve.
Serum concentration of PrN was measurement by a method for determining Reactive Oxygen Species (ROS) via Hydroxyphenyl Fluorescein (HPF) probe (20). The serum concentration of PrN was assessed by hydroxyl radical (OH), and peroxynitrite (ONOO–) detection Kit (Cell Technology, Mountain View, CA, USA). The fluorescence power was then determined by an ELISA reader (Stat FAX 2100, USA) at a wavelength of 488 nm. The serum PrN levels were calculated by comparing the O.D. of the samples to the standard curve(21).
After the serum samples centrifuged, the specimens were ready for assessing the levels of vitamin C, transferrin, and ferritin. The analysis of transferrin and ferritin were performed on cobas mira using modified methods (Roche Diagnostics, Basel, Switzerland). Vitamin C analysis was evaluated via the standard method (22).
Conventional wet acid digestion method was used to digest serum as it has been described before with slight modification (11). The serum concentrations of iron, Cu, Se, and Zn were performed by Atomic absorption using graphite furnace (novAA 400, Analytic Jena, Germany). Each specimen was analyzed in triplicate and the average results were reported.

Statistical analysis

Statistics analysis was performed via SPSS (ver. 16, Chicago, IL, USA), graphPad Prism (ver. 6.07) software. Kolmogorov-Smirnov test was used to evaluate the normality of the quantitative variables. Quantitative and qualitative variables were presented as mean ± SD and frequency (percent), respectively. Chi-Square and Independent Sample t-test were used for statistics analysis. Moreover, a binary logistic regression was established to examine the combination of the studied tests. Significant differences were shown as $P<0.05$ between study groups.

Results

Patients and samples

Overall, 150 serum specimens were wnrolled in this study which were counting 75 samples from patients with PD and 75 samples from healthy persons. There were no statically significant differences in age, gender, hypertension, diabetes mellitus, BMI, and smoking between two groups (Table 1).

Assessment of Se, Zn, Cu, and iron levels

The serum concentrations of Se, Zn, Cu, and iron in the patients with PD were evaluated and compared to healthy subjects. Se levels in patients (101.35±7.42 µg/L) were not markedly different compared to controls (97.68±6.11µg/L) ($P=0.515$) (Fig. 1A). Zn concentrations in PD patients (725.29±19.23 µg/L) were statically significant compared to healthy subjects (973.61±15.37 µg/L) ($P=<0.001$) (Fig. 1B). The

serum levels of Cu in patients with PD (935.14±12.30 µg/L) were significantly different versus controls (1089.58±15.08 µg/L) (*P*=<0.001) (Fig. 1C). Furthermore, iron concentrations in PD patients (1458.21±23 µg/L) were significantly different compared to control group (1125.75±19.64 µg/L) (*P*= 0.003) (Fig. 1D).

Table 1: Demographic data of the patients with PD and healthy individuals

Characteristics	*PD subjects (n = 40)*	*Controls (n = 40)*	*P-values*
Gender			
Male n (%)	27 (67.5)	22 (55)	-
Female n (%)	13 (32.5)	18 (45)	
Age (yr)	65.70 ± 6.32	64.35 ± 3.75	0.249*
DD (yr)	3.78 ± 2.25	0	-
Hypertension n (%)	9 (32.5)	8 (20)	0.785**
Smoking n (%)	5 (12.5)	3 (7.5)	0.456**
DM n (%)	5 (12.5)	3 (7.5)	0.456**
BMI (kg/m²)	26.39 ± 1.65	25.98 ± 1.26	0.181*

**P*-value was reported based on Independent Sample T test. *** P*-value was reported based on Chi-Square test. DM = Diabetes mellitus; DD = disease duration; PD = Parkinson disease; BMI = Body mass index.

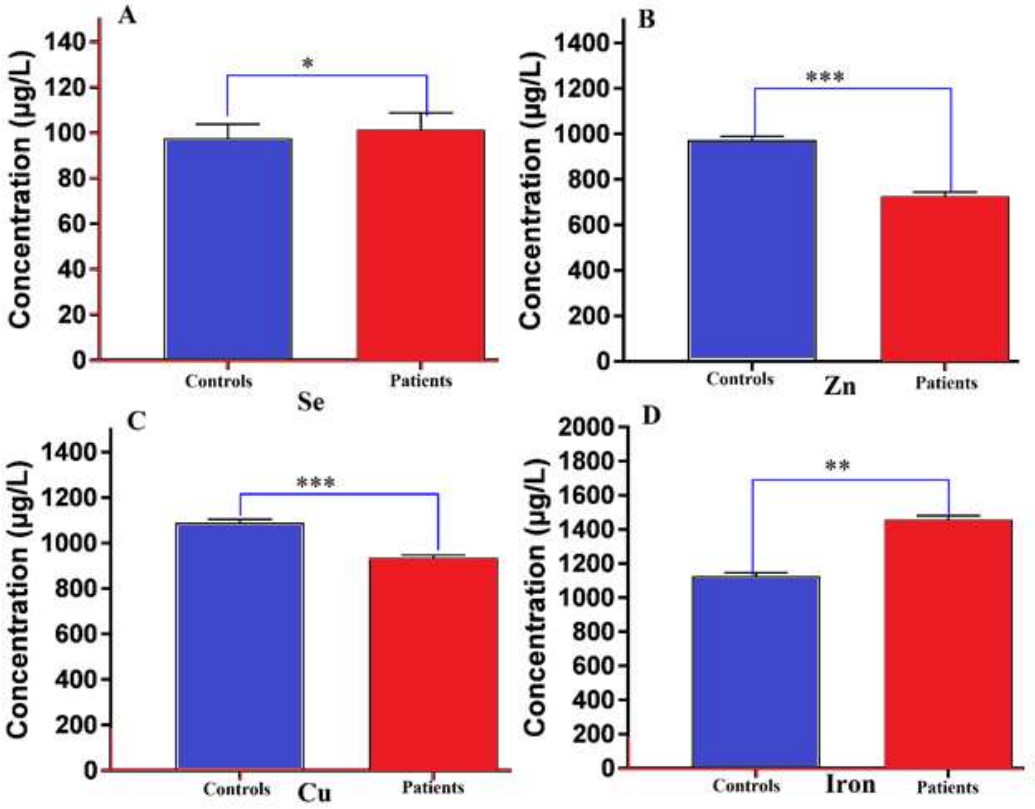

Fig. 1: Comparison of the serum concentrations of (A) Se, (B) Zn, (C) Cu and (D) Iron between patients with PD and healthy subjects. **P*>0.05. ***P*=0.003. **** P*<0.001. Statistical analysis was done by Independent Sample *t*-test

Determination of ferritin, transferrin, and Vitamin C levels

The serum concentrations of ferritin, transferrin, and Vitamin C in the patient's individuals and controls were assessed and the findings were compared together. Ferritin concentrations in PD patients (127.89±13.48 ng/mL) were not statically significantly in healthy individuals (141.34±9.26 ng/ mL) (*P*=0.103) (Fig. 2A). Also, transferrin levels in patient subjects (261.74±16.89 mg/L) significantly differ in controls (248.36±13.21 mg/L) (*P*=0.372) (Fig. 2B). However, the serum levels of Vitamin C in patients with PD (20.12±3.10 µmol/L) were lower than healthy subjects (38.77±5.19 µmol/L) (*P*=<0.001) (Fig. 2C).

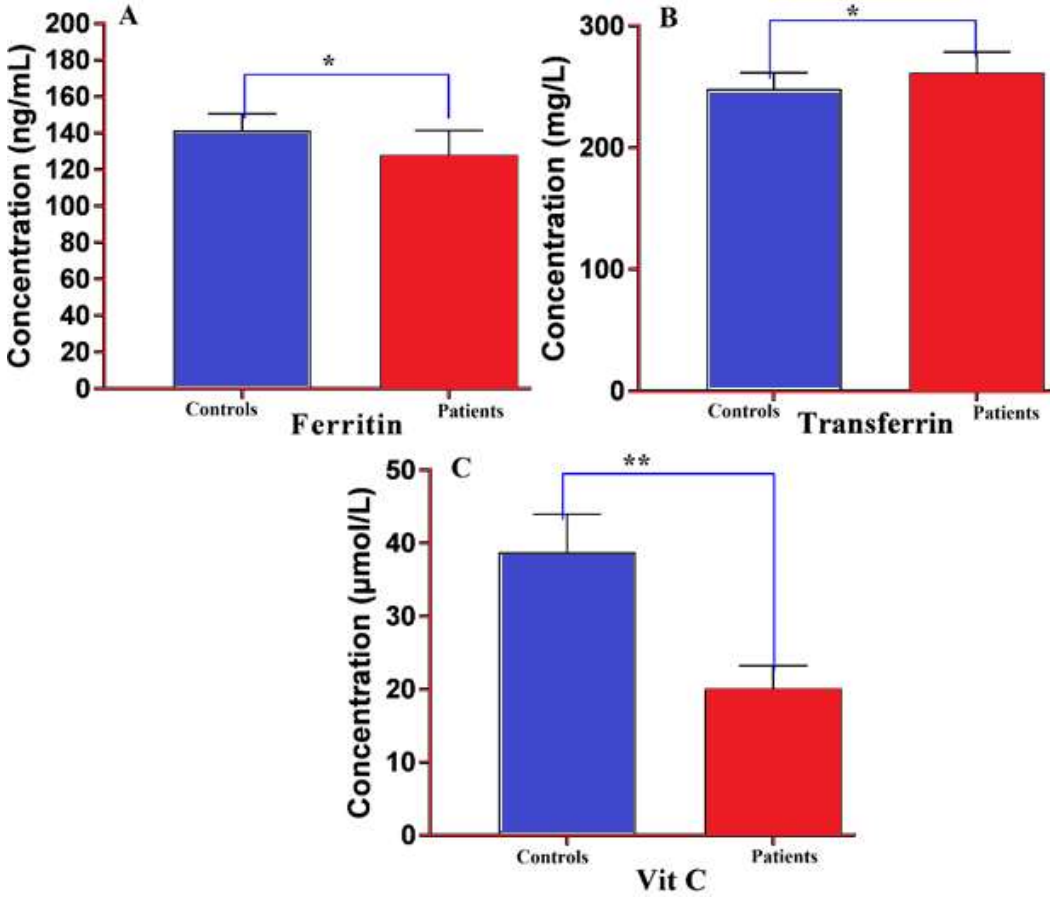

Fig. 2: Comparison of the serum concentrations of (A) Ferritin, (B) Transferrin and, (C) Vit C between patients with PD and healthy subjects. *P>0.05. ** P<0.001. Statistical analysis was done by Independent Sample *t*-test

Evaluation of NOx and peroxynitrit levels

The serum concentrations of NOx in patients with PD were measured and compared with those concentrations in controls. NOx levels in PD patients (35.81±5.16 mol/L) were markedly significant compared to healthy subjects (11.27±3.59 mol/L) (*P*<0.001) (Fig. 3A). As well as, peroxynitrit concentrations in patient with PD (15.78±4.23 mol/L) were statically different compared to controls (9.62±4.57 mol/L) (*P*= 0.004) (Fig. 3B).

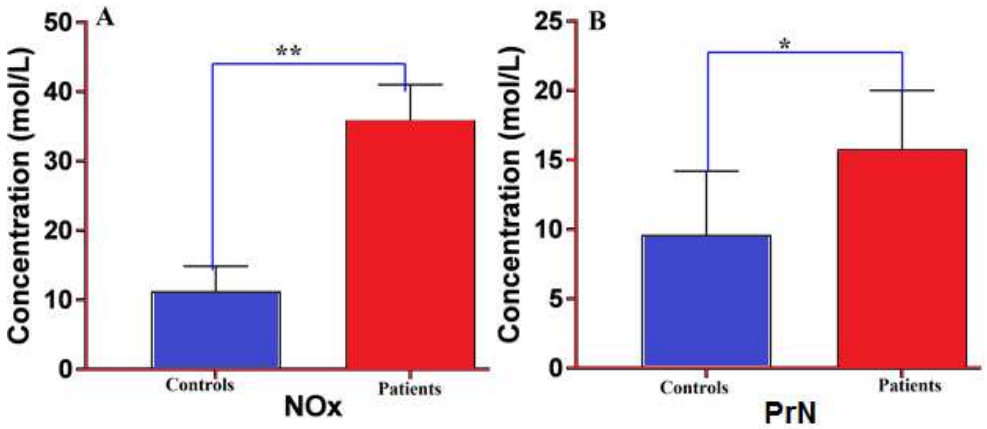

Fig. 3: Comparison of the serum concentrations of (A) NOx and (B) PrN between patients with PD and healthy subjects. * *P*=0.004. ** *P*<0.001. Statistical analysis was done by Independent Sample *t*- test.
NOx, Nitric oxide; PrN, Proxynitrite

Subgroup evaluation of serum levels for trace elements, ferritin, transferrin, vitamin C, NOx, and PrN in patients with PD

We also investigated the association of serum levels of trace elements, ferritin, transferrin, vitamin C, NOx, and PrN in PD patients with age, gender and disease duration. There was a slight increase in NOX1 and ferritin levels in patients over 65 yr old and ferritin levels in males but the associations were not significant (Table 2).

Table 2: Subgroup analysis of serum NOX1, Se, UA and ferritin in patients with PD

Variables	*Age (yr)*			*Gender*			*Disease duration*		
	≤65	*>65*	*P-value*	*Male*	*Female*	*P-value*	*≤3 years*	*> 3 years*	*P-value*
Se (µg/L)	95.73±7.26	97.61±5.47	0.312	99.32±4.84	96.78±5.32	0.249	100.38±6.8	98.15±4.56	0.341
Cu (µg/L)	976.34±14.68	951.53±13.07	0.097	915.45±16.14	1014.12±10.03	0.073	851.89±15.27	919.67±12.54	0.157
Zn (µg/L)	714.19±19.26	745.87±14.46	0.412	925.14±7.12	932.62±11.4	0.739	896.15±14.35	908.81±10.48	0.541
Iron (µg/L)	1324.41±13.45	1303.11±15.23	0.854	1458.84±21.05	1367.12±26.34	0.694	1258.35±19.69	1213.82±19.40	0.783
Ferritin (ng/mL)	121.17±12.36	119.94±10.31	0.836	115.36±12.15	96.17±12.54	0.208	118.65±11.46	109.28±13.01	0.726
Transferrin (mg/L)	245.16±14.87	255.33±12.57	0.812	275.29±13.89	259.38±9.64	0.169	239.86±14.97	245.41±13.48	0.637
Vit C (µmol/L)	17.69±3.45	15.98±4.05	0.671	15.23±3.49	13.58±3.67	0.679	18.28±2.08	18.76±2.59	0.876
NOx (mol/L)	29.11±4.67	31.44±3.57	0.758	25.92±4.39	22.36±5.18	0.803	33.86±5.79	31.64±5.31	0.716
Peroxynitrit (mol/L)	14.29±2.57	16.38±2.31	0.116	16.71±2.59	16.49±3.60	0.815	16.79±2.95	15.21±3.14	0.279

* *P*-value was reported based on Independent Sample T test.

ROC curve analysis

The ROC curve analysis was performed to assess the potential predictive value for the serum variables for PD. The prognostic power of serum vitamin C concentrations was revealed an Area Under the Curve (AUC) of 0.86 (95% confidence interval (95% CI): 0.83 to 0.95, *P*<0.001) with a sensitivity of 82% and specificity of 95% (Fig. 4A). Moreover, the AUC of 0.73 (95% CI: 0.62 to 0.84, *P*=0.001) with a sensitivity of 88% and

specificity of 48% was obtained for the serum level of NOx (Fig. 4B). The serum concentrations of PrN exhibited a sensitivity of 55%, specificity of 85%, and the AUC of 0.68 (95% CI: 0.55 to 0.80, *P*=0.008) (Fig. 4C).

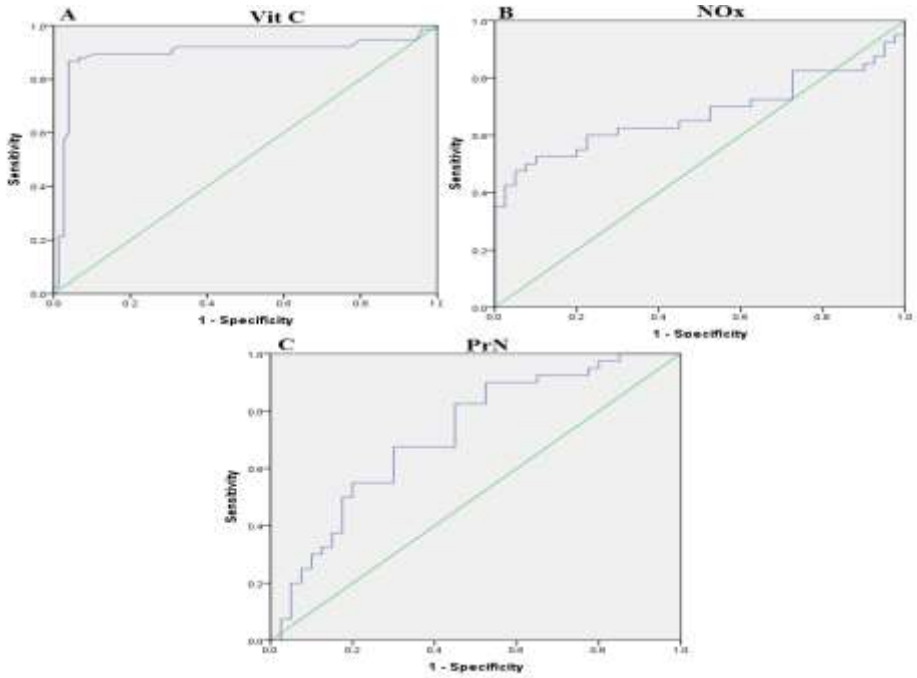

Fig. 4: ROC curve analysis for serum concentrations of (A) Vit C, (B) NOx, and (C) PrN. NOx, Nitric oxide; PrN, Proxynitrite

Discussion

Today, the development of knowledge in the field of oxidative stress has been shown that oxidative stress is an important factor in the initiation and progression of PD (23). The neurons, which are producing dopamine in the substantia nigra area located in the midbrain, are significantly vulnerable to excessive oxidative stress that it can be attributed to the loss of strength of the antioxidant system, increased serum iron levels, and the desire to oxidize dopamine (24).

Our results, in line with previous studies, showed that serum Se concentrations in patients with PD were higher than the control group, but this difference was not statistically significant. However, in our previous study (1), this difference was statistically significant. The reason for this difference is due to the effect of the diet on serum levels of Se (25). Serum Se is higher in patients with PD than that in the control group. Moreover, this difference was not statistically significant (15, 26). Our study also showed that Cu and Zn concentrations were significantly higher in healthy subjects compared to those with PD. Cu plays a prominent role in applications that require redox reactions. Disturbance in the concentration of Cu in the body can result in the release of radicals that may play a role in pathogenesis of neurological diseases such as PD (26). Serum Cu concentrations are higher in healthy subjects than in patients with PD (15, 27).

Cu and iron are important elements that play a role in redox reactions. Increasing the oxidative stress of the body can lead to the imbalance of these elements in the body (28). Cu, Zn and Se are the most important elements, which have been involved in antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (15). It has a functional role in many

brain functions, such as protecting the structure of Superoxide dismutase and inhibiting nitric oxide synthase (29). In the present study, serum Zn concentration was significantly higher among patients with PD compared to healthy subjects. These results are in line with past studies (11, 15). Changes in iron concentrations in the body may play a role in Parkinson's pathogenicity (30). Therefore, the full recognition of iron homeostasis and its relationship with oxidative stress can be useful in finding a reliable factor that can be associated with the death of dopamine, which is producing cells in the PD process (31).

Our results indicated that iron concentrations in patients with PD were higher than the control group, and this difference was statistically significant. In addition, we also evaluated serum ferritin and transferrin concentrations. Our results showed that serum ferritin concentration in the control group was higher than that in PD patients, but none of them was statistically significant. Ferritin can protect cells against the harmful effects of iron accumulation. The presence of ferritin in the cytoplasmic space between cytoplasmic as well as the increase in iron in the brain tissue may be related to neurological diseases (7). Serum ferritin concentrations in patients with PD were lower than that in healthy subjects, but this difference was not statistically significant (32).

Vitamin C is a non-enzymatic antioxidant that is associated with PD (31). In a study done on blood and cerebrospinal fluid samples, there is a correlation between these two samples (33). In addition, the concentration of vitamin C in patients with PD is significantly lower than healthy subjects (18). Our study is consistent with the results shown that serum vitamin C concentration in subjects with PD was lower than the control group, and this difference was statistically significant.

We also evaluated serum NOx and PrN concentrations between the two groups. The results of this study showed that serum NOx level in patients with PD was more than three-fold in healthy subjects, and this difference was statistically significant. Moreover, serum levels of PrN in patients with PD were significantly higher than those in control group.

The amount of NOx was higher in the CSF of patients with PD compared to the control group, but this difference was not significant (34). In addition, a significant increase was observed in serum NOx and PrN in a study with NOx and PrN concentrations in patients with PD were significantly higher than that in the control group. The results of their research showed that high levels of NOx and PrN are associated with the progression of PD. Even they reported that concentrations of NOx and PrN could be used as a biomarker in PD for future (2).

In our study, the variables in Parkinson's patients were compared in three subgroups including age (≤65 and> 65) sex (female, male) and duration of the disease (≤3 and> 3), and their results were reported. The results did not show significant differences in any of the subgroups (Table 2), but some studies reported different results (1, 11, 35, 36).

The ROC curve analysis showed that serum levels of vitamin C could be a diagnostic value as a biomarker with proper sensitivity and specificity. The results of the ROC curve analysis did not endorse this finding for NOx and PrN.

Conclusion

Serum levels of Iron, NOx, and PrN in patients with PD were higher than those in control group. Moreover, those serum concentrations of Cu, Zn, and vitamin C in healthy subjects were higher than those in PD patients. Besides, serum vitamin C levels have a diagnostic value as a biomarker. Oxidative stress can be involved in the death of dopamine-producing cells. In addition, oxidative stress indexes reflect the state of the body, it can be suggested that by enhancing the body's antioxidant system, it prevents the onset or progression of PD.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission,

redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This work was financially supported by the Drug Applied Research Center at Tabriz University of Medical Sciences. The authors also gratefully acknowledge Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences for technical support.

Conflict of interest

The authors declare that there is no conflict of interests.

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