Journal of
Kerman University of Medical Sciences

https://jkmu.kmu.ac.ir
10.34172/jkmu.2024.18
Vol. 31, No. 3, 2024, 104-112

Original Article

Open Access
Publish Free

Assessment of Serum Levels of Phosphate and Corticosterone as Possible Biomarkers for Early Diagnosis of Parkinson's Disease in Rats with 6-OHDA-Induced Parkinson

Hashem Haghdoost-Yazdi[1], Sepideh Nigjeh[2], Seyed Amir Hadi Hosseini[2], Sahar Sharifi[2], Fatemeh Kaveh[3], Hossein Piri[1*]

[1]Cellular and Molecular Research Center, Research Institute for Prevention of Non-Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran
[2]Student Research Committee, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[3]Department of Cellular Therapy, Institute for Cancer Research, Oslo University Hospital, Norway

***Corresponding Author:** Hossein Piri, Email: hosseinpiry@gmail.com

Abstract

Background: Early diagnosis of Parkinson›s disease can play an important role in improving its prognosis. Due to controversies about the serum level of corticosterone and phosphate in parkinsonian rats, this study was designed to measure and evaluate the levels of corticosterone and phosphate as possible biomarkers of early diagnosis in rats with 6-OHDA-induced parkinsonism.

Methods: Forty rats were divided into three groups: control (n = 10), sham (n = 10), and 6-OHDA (n = 20). The rats in the 6-OHDA and sham groups underwent stereotaxic surgery to be injected with 6-OHDA and its carrier into their medial forebrain bundle (MFB). Apomorphine-induced rotational and cylinder tests were done to examine parkinsonism progression and sensory-motor function. Corticosterone and phosphate serum levels were measured in the serum and striatum.

Results: Net contralateral rotations and asymmetry scores in the fourth, sixth, and eighth weeks after surgery compared with the second week showed a gradual increase in the 6-OHDA group. The serum levels of corticosterone were 90 ± 13 before surgery, and they declined to 88 ± 36 and 55 ± 9 ng/L in the second and eighth weeks after surgery, respectively. The serum levels of phosphate were 6 ± 0.22 before surgery, and they decreased to 5.2 ± 0.13 and 5 ± 0.12 mg/dl in the second and eighth weeks after surgery, respectively. The serum levels of phosphate and corticosterone remained relatively unchanged in the sham and control groups.

Conclusion: In conclusion, the progressive death of dopaminergic neurons is accompanied by decreased serum corticosterone and phosphate levels. Thus, the serum level of corticosterone can be used as a biomarker in diagnosing Parkinson's disease. In contrast, serum phosphate levels were still within the normal range and cannot be used as a biomarker.

Keywords: Parkinson's disease, Neurodegeneration, Corticosterone, Phosphate

Citation: Haghdoost-Yazdi H, Nigjeh S, Hosseini SAH, Sharifi S, Kaveh F. Assessment of serum levels of phosphate and corticosterone as possible biomarkers for early diagnosis of parkinson's disease in rats with 6-OHDA-induced parkinson. *Journal of Kerman University of Medical Sciences*. 2024;31(3):104–112. doi: 10.34172/jkmu.2024.18

Received: November 6, 2022, **Accepted**: January 15, 2024, **ePublished**: June 29, 2024

Introduction

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease (1). From a pathological point of view, the gradual destruction of dopaminergic neurons in the striatum leads to a drop in dopamine levels in the corpus striatum. Parkinson's disease usually occurs when 60% to 70% of dopaminergic neurons in the striatum are destroyed (2). Not only the dopaminergic neurons but also serotonergic and cholinergic neurons, and even catecholaminergic neurons, can be affected by Parkinson's disease, and their function is usually impaired (3).

The etiology of Parkinson's disease has not been completely understood yet. Some studies consider oxidative stress, mitochondrial dysfunction, and some protein accumulations as contributing factors in its development (4). Parkinson's disease also causes movement disorders, such as akinesia and bradykinesia, rigid muscles, impaired posture and balance, and tremors in the hands or fingers (3).

Early diagnosis of Parkinson's disease may help healthcare providers choose the appropriate treatment to slow the neurodegenerative process and improve outcomes (5). Identifying sensitive and reliable biomarkers for Parkinson's disease would be valuable for early diagnosis. Biomarkers also allow us to have

better patient follow-up and objective measurements in clinical trials (6). However, it should also be noted that research on biomarkers for Parkinson's disease is still in the early stages despite the need to develop newer and more efficient treatment strategies. Biomarkers should be sensitive and reliable to indicate disease progression. Invasiveness, costs, and the patient's response to pharmacological treatments should be considered (7).

Due to contradictions in findings about the level of corticosterone and phosphate in the serum and striatum of parkinsonian patients and the need to find a sensitive and reliable biomarker for early diagnosis of Parkinson's disease diagnosis (2), this study was designed to measure the level of corticosterone and phosphate in the serum and striatum of rats with 6-hydroxy dopamine-induced parkinsonism. It also should be noted that 6-hydroxy dopamine-induced parkinsonism has a pathophysiology similar to Parkinson's disease in humans; thus, it is the most appropriate model to study the early and late stages of Parkinson's disease (8).

Materials and Methods

Animals and ethics

In this study, male Wistar rats weighing from 175 g to 225 g were kept in individual large clean cages with free access to water and food. The rats were kept in a 21 °C environment with a 12-hour day/night cycle (9). All the procedures of the current study followed the ethical guidelines of the Research Council at Qazvin University of Medical Sciences for animal experiments (10).

The rats in this study were divided into three groups: 6-OHDA (n = 20), sham, and control (each n = 10). Neither surgery nor injection was performed on the control group rats, but the sham and 6-OHDA groups underwent surgery for injection of 6-hydroxy dopamine and ascorbic acid solution as a neurotoxin carrier, respectively (11).

Apomorphine-induced rotational test

The incidence and severity of Parkinson's disease in rats were examined by the apomorphine-induced rotation test. In this test, more than 30 spins in 30 minutes after injection of apomorphine indicated that the rats had Parkinson's disease (12). At first, the animals were allowed to adapt to the new environment for 5 minutes; apomorphine hydrochloride (0.5 mg/kg) was injected intraperitoneally. After one minute, the number of complete rotations in 30 minutes was counted. The net number of rotations was calculated by subtraction of the contralateral and ipsilateral rotations. The experiment design can be seen in Figure 1. Apomorphine-induced rotational tests were performed before and in the second, fourth, sixth, and eighth weeks after the experiment (13). Only rats with less than ten spins in thirty minutes in the rotational test were included in this study.

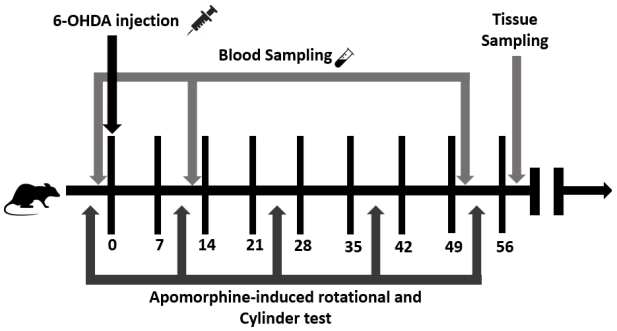

Figure 1. The general design of the study

Cylinder test

The cylinder test measures spontaneous forelimb use to assess the sensory-motor function in rodents with 6.OHDA-induced parkinsonism (14). In this test, a rat is placed in a cylindrical container; the number of times it touches the wall is counted. After scoring based on the number of times the rat touches the right or left side or both sides, the results are expressed as asymmetry scores. The positive and negative asymmetry scores indicate the dominance of touching movements on the damaged and undamaged sides, respectively. Therefore, a severe hemi-parkinsonian lesion results in a high positive asymmetry score.

Blood sampling

Blood samples were taken from the tail vein before the surgery and in the second and eighth weeks after stereotaxic surgery. In the eighth week blood samples were taken directly from the heart. Blood samples were centrifuged at 4000 rpm for 5 minutes to separate the serum from the precipitate, and serum samples were frozen at -80 °C.

Injection and stereotaxic surgery

The rats were completely anesthetized with ketamine and xylazine (70 mg/kg and 6 mg/kg, respectively) by intraperitoneal injection. The hair on the skull was shaved, and the site of surgery was entirely disinfected. The exact surgery location was identified using the Paxinos and Watson rat brain atlas. The incisor bar was set at -3.3 below the interaural line. The skull surface was exposed with an incision in the skin, and the bregma position was determined. Using a dental drill, two holes in the skull with the following coordinates were made: Anterior-Posterior (AP): -4, Lateral (L): -1.8, Dorso-Ventral (DV): 9, and AP: -4.4, L: -2, DV: 8.8. AP and L were measured from the bregma while DV was measured from the surface of the skull. Then, 4 µL of 6-OHDA dissolved in isotonic NaCl solution, containing 0.2% ascorbic acid, was slowly injected into the medial forebrain bundle (MFB) of the right hemisphere area for 8 minutes using a 10-µl syringe. The animals' skull was then sutured and disinfected.

Tissue sampling

Tissue sampling of the brain was performed after the rats were decapitated under deep anesthesia. After removing the animals' brains, the brains were weighed, and the striatal tissues were isolated; then for the preparation of the striatal supernatant, the striata were homogenized in a solution of 100 mM hydrochloric acid (pH = 7.4), 750 mM NaCl, 10 mM EDTA, 5 mM EGTA, and protease inhibitors. Finally, the samples were centrifuged at 2000 rpm for 10 minutes, the solid particles precipitated and the supernatant was used for further analysis.

Statistical analysis

The collected data were expressed as mean ± standard error of mean (SEM). First, data were analyzed using the Kolmogorov-Smirnov test to determine their normality. Behavioral tests and biochemical data were compared using a one-way analysis of variance followed by the Newman-Keuls test. The software used for statistical analysis was SPSS version 20 and a P value ≤ 0.05 was considered as the level of significance.

Variable measurement

Corticosterone in both serum and striatal supernatant was measured according to the instructions of the ELISA kit (Eastbiopharm, USA). Also, corticosterone was measured based on readings at a wavelength of 450 nm and a sensitivity of 2.51 ng/mL. The colorimetric method was employed using a Biowave spectrophotometer (Model F2100) and Zist Chem phosphate kits to measure serum and striatal supernatant phosphate.

Results

Behavioral tests

It is a well-established fact that an apomorphine-induced rotational test can evaluate the progression and severity

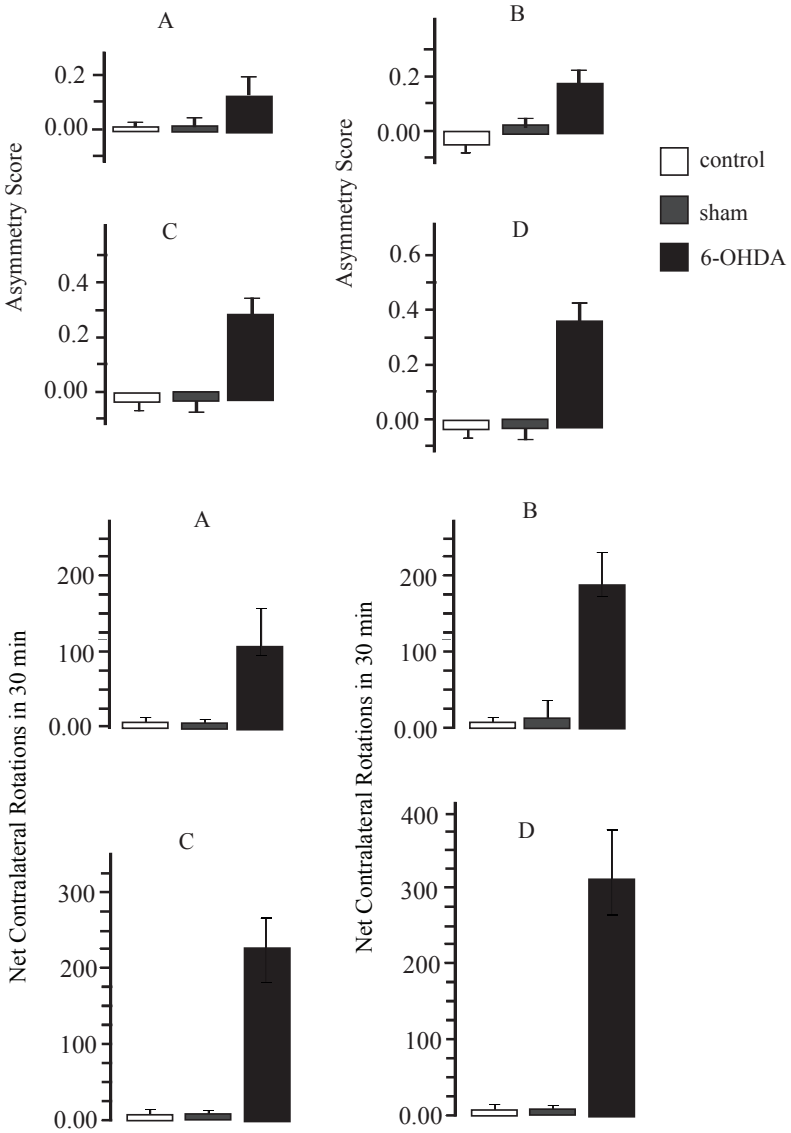

Figure 2. Apomorphine-induced rotation test (net contralateral rotations in 30 minutes in different study groups) and cylinder test data (asymmetry score); A, B, C, and D present the data of the second, fourth, sixth, and eighth week after stereotaxic surgery, respectively

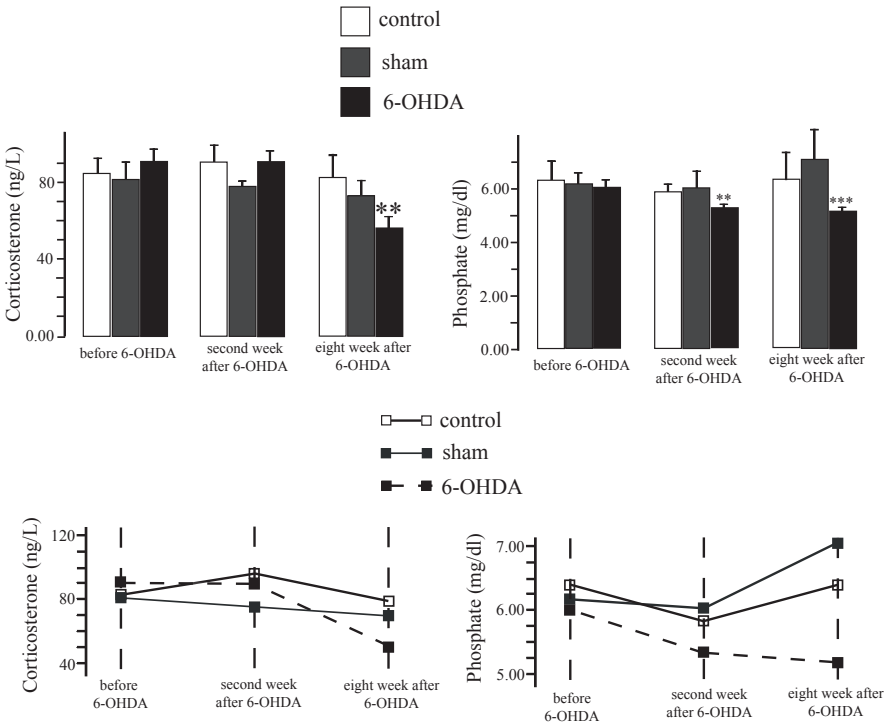

Figure 3. Serum corticosterone and phosphate levels in the study groups based on severity (sham, control, and 6-OHDA) (** and *** are significant $P<0.05$)

of parkinsonism (15). Intraperitoneal injection of apomorphine as an agonist of dopaminergic neurons leads to asymmetrical rotations in rats (contralateral to the hemisphere). The net number of asymmetrical rotations is a quantitative tool for evaluating 6-OHDA-induced parkinsonism.

As shown in Figure 2, the rats in the sham and control groups did not show significant rotational behavior after injections. In contrast, the rats who received 6-hydroxy dopamine showed a higher number of net collateral rotations to the left side as a sign of parkinsonism progression. This indicated that the model had worked well and parkinsonism had been induced. The net collateral rotations gradually increased after stereotaxic surgery in rats in the 6-OHDA group and increased by 63%, 88%, and 163% in the fourth, sixth, and eighth weeks after surgery, respectively, in comparison with the second week after stereotaxic surgery.

The cylinder test evaluates motor impairments in rodents especially rats with 6-OHDA-induced parkinsonism (14). The cylinder test results showed that before stereotaxic surgery, the asymmetry score in all study groups was almost zero and negligible. After stereotaxic surgery, the asymmetry score was close to zero in the control and sham group, but in the 6.OHDA group, the asymmetry scores gradually increased to 0.11 ± 0.09, 0.19 ± 0.03, 0.29 ± 0.06, and 0.38 ± 0.04 in the second, fourth, sixth, and eighth weeks after receiving 6.OHDA, respectively.

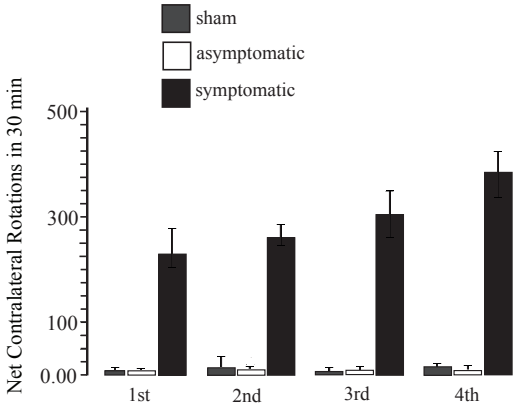

Figure 4. Apomorphine-induced rotation test data (net contralateral rotations in 30 minutes in the sham, symptomatic, and asymptomatic groups); A, B, C, and D show the data for the second, fourth, sixth, and eighth week after stereotaxic surgery, respectively

Serum levels of corticosterone and phosphate

Figure 3 and Table 1 show the serum levels of corticosterone and phosphate in different study groups before and in the second and eighth weeks after stereotaxic surgery. There were no significant differences in corticosterone levels between the study groups in the second week after stereotaxic surgery. In the eighth week after stereotaxic surgery, although the serum level of corticosterone in the 6-hydroxy dopamine group was 21% lower than in the control group, statistical analyses did not show a significant difference. However, corticosterone levels in the 6-hydroxy dopamine group were lower than those before stereotaxic surgery ($P<0.05$). Before stereotaxic surgery, there were no significant differences in phosphate

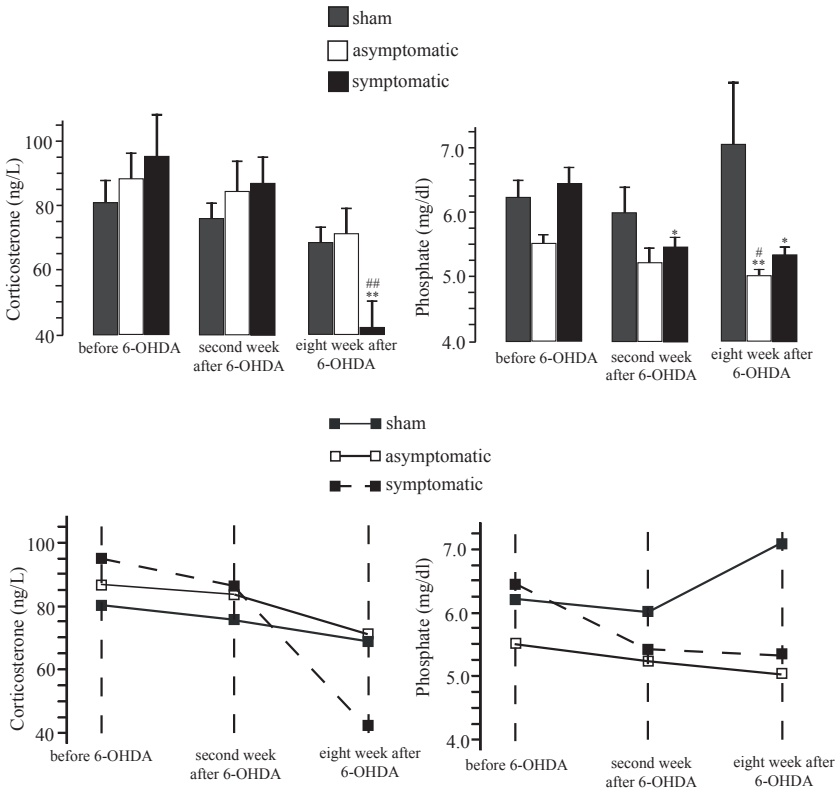

Figure 5. Serum corticosterone and phosphate levels in the study groups based on severity (sham, asymptomatic, and symptomatic rats) (*, **, and # are significant, $P<0.05$)

levels between different study groups. Serum phosphate levels in the group receiving 6-hydroxy dopamine decreased by 13% ($P<0.01$) and 17% ($P<0.001$) in the second and eighth weeks after stereotaxic surgery, respectively, which were statistically significant.

The severity of parkinsonism and serum levels of corticosterone and phosphate

Based on the net number of rotations in the apomorphine-induced rotation test results, the rats in the 6.OHDA group had a wide range in the net number of rotations. Some showed more rotations while others had fewer or no rotations. The rats of the 6.OHDA group were divided into two subgroups to be evaluated in a more detailed way: 1) symptomatic rats ($n=7$) with > 200 rotations in 30 minutes and 2) asymptomatic rats ($n=6$) with < 30 or non-significant number of rotations in 30 minutes.

Figure 4 shows that the intensity of rotational behavior gradually increased in the weeks after stereotaxic surgery only in the symptomatic subgroup. The intensity of rotational behavior gradually increased in the weeks after stereotaxic surgery in the symptomatic subgroup and reached 230 ± 50, 262 ± 25, 305 ± 45, and 380 ± 45 in the second, fourth, sixth, and eighth weeks after surgery, respectively.

As shown in Figure 5, in the eighth week, serum corticosterone levels in the asymptomatic subgroup were lower than those before stereotaxic surgery, although

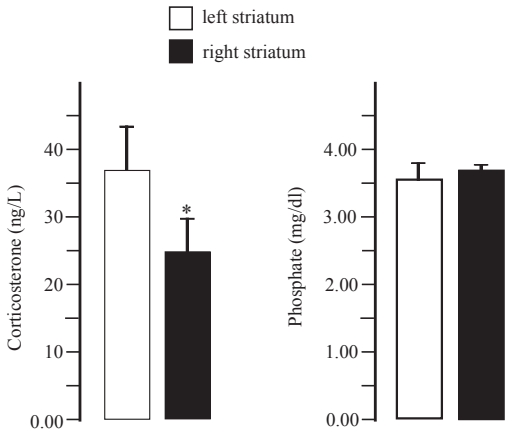

Figure 6. Levels of corticosterone and phosphate in the 6.OHDA group in both left and right striata (* is significant, $P<0.05$)

this difference was not statistically significant. There was also no significant difference in serum corticosterone levels in the eighth week between the asymptomatic and sham subgroups. A progressive decrease in serum corticosterone level was observed in the symptomatic subgroup from 90 ± 13 before surgery to 36 ± 88 and 43 ± 9 ng/L in the second and eighth weeks after stereotaxic surgery, respectively. Also, both of these changes, in comparison with former measurements, were statistically significant ($P<0.01$).

After stereotaxic surgery, a decrease in serum phosphate levels occurred in both asymptomatic and

Table 1. Serum level of phosphate and corticosterone in different study groups

Groups	Before surgery		second week after surgery		eighth week after	
	Corticosterone (ng/L)	Phosphate (mg/dL)	Corticosterone (ng/L)	Phosphate (mg/dl)	Corticosterone (ng/L)	Phosphate (mg/dL)
Control	84 ± 12	6.4 ± 0.76	90 ± 22	5.9 ± 0.23	82 ± 11	6.3 ± 1
Sham	81 ± 12	6.2 ± 0.32	87 ± 17	6 ± 0.8	72 ± 9	7.1 ± 0.17
6-OHDA	90 ± 13	6 ± 0.22	88 ± 36	5.2 ± 0.13	55 ± 9	5 ± 0.12

All data are expressed as mean ± SEM.

symptomatic subgroups. The decrease in phosphate level was more evident in the asymptomatic compared to the symptomatic subgroup. The serum phosphate level increased from 5.54 ± 0.15 before surgery to 5.2 ± 0.23 in the second week and 4.96 ± 0.06 mg/dL in the eighth week after surgery. The difference between the phosphate level in the eighth week compared to its level before stereotaxic surgery was significant ($P < 0.05$), while, the phosphate level in the eighth week was significantly lower in the asymptomatic compared to the sham group ($P < 0.01$). In the symptomatic subgroup, the phosphate level increased from 6.43 ± 0.3 before surgery to 5.45 ± 0.15 in the second week and 5.35 ± 0.12 in the eighth week after surgery. The decrease was statistically significant in both the second and eighth weeks compared to phosphate levels before surgery ($P < 0.05$).

Striatal levels of corticosterone and phosphate in symptomatic rats

Measurement of corticosterone and phosphate in the left and right striata of the symptomatic rats of the 6.OHDA group revealed that phosphate levels in the left and right striata were 3.5 ± 0.2 and 3.7 ± 0.2, respectively. Corticosterone levels in the left and right striata were 37 ± 8 and 25 ± 6, respectively (Figure 6). Statistical analysis also showed that corticosterone levels in the right striatum were significantly ($P < 0.05$) lower than in the left striatum. Statistical analyses also revealed that phosphate levels in the left and right striata were not significantly different ($P > 0.05$).

Discussion

Regarding the pathophysiology of Parkinson's disease, it has been found that inflammation and oxidative stress are among the leading factors in the incidence and progression of Parkinson's disease (16). By regulating cortisol secretion, the HPE axis can play an essential role in controlling inflammation in patients with Parkinson's disease by its regulatory role.

In Parkinson's disease, the body typically experiences long-term stress; the body needs to adapt to this chronic stress, and the HPA axis secretes corticosterone to help the body adapt to the conditions. There is no consensus on the effects of glucocorticoids such as corticosterone on the central nervous system (17). As we know, neuronal inflammation and oxidative stress cause neuronal damage and apoptosis; some studies have suggested that short-term glucocorticoid exposure inhibits inflammation and has a protective effect (18,19). On the other hand, other studies have suggested that long-term exposure to glucocorticoids can be harmful, increase inflammation and oxidative stress, and induce neurodegeneration (16,20,21).

Various studies that have examined the levels of cortisol in patients with Parkinson's disease have reported contradictory results. Some studies show that cortisol levels increase, and others have shown that it does not change significantly. Several reasons can be considered for the contradictory data of different studies: 1) differences in Parkinson's induction methods in animal models, 2) different interventions and drug therapies in different studies (22), and 3) diagnosis methods and corticosterone levels over time (short-term and long-term) (23,24).

The clinical signs and symptoms of Parkinson's disease usually occur when most dopaminergic neurons are destroyed, and the level of dopamine in the striatum is low. Thus it is difficult to diagnose Parkinson's disease in the early stages (25). There is almost no suitable diagnostic tool; some symptoms like REM sleep disorders and olfactory dysfunction can be seen, but none of these symptoms are enough to diagnose Parkinson's disease.

Grigoruță et al showed that wild-type rats in the acute and chronic phases of Parkinson's disease had higher corticosterone levels in the symptomatic group than in the asymptomatic group, which contradicts the results of the present study. Also, another part of this study, performed on parkinsonian rats with the PINK knock-out gene, showed that corticosterone levels in symptomatic rats in the chronic phase were lower than in asymptomatic rats (2), which confirms the results of the present study.

In another study on Parkinson's patients, Djamshidian et al found that lower corticosterone levels played an essential role in the progression of Parkinson's disease and the development of antisocial behaviors (26). The results of their study verify the results of the present study.

Hartmann and colleagues' study showed that patients with Parkinson's usually have higher cortisol levels and lower expression of glucocorticoid receptors, in particular (27), which verifies the role of alterations in the HPA axis in the development of Parkinson's disease and contradicts the results of the present study. Mizoguchi et al also showed that by removing the internal source

of corticosterone using adrenalectomy, parkinsonian rats had more memory and movement disorders than the control group. On the other hand, corticosterone replacement therapy relatively improved the cognitive and motor function of the rats after some time. However, it should be noted that dopamine levels also decreased in rats with adrenalectomy (28). Tentillier et al showed that injected liposomal glucocorticoids improved motor function compared with the control group, controlled Parkinson's disease, and increased the survival of dopaminergic neurons in the striatum; they also showed that glucocorticoids protect dopaminergic neurons in the striatum by modifying macrophages, microglial cells, and inflammatory responses (29-31). In the study of Luan et al., it was shown that in the early stages of Parkinson's disease, the level of corticosterone increased relatively but decreased in the middle stages. However, it was also observed that in the advanced stages of the disease, the level of corticosterone increased, showing a fluctuation in corticosterone levels (32).

Another factor examined in the present study was serum phosphate levels. It was found that in symptomatic parkinsonian rats, phosphate levels were significantly reduced. It should be noted that several previous studies have shown that due to its phosphoric properties, corticosterone reduces serum phosphate levels and is inversely related to it (33). Samavarchi Tehrani et al showed that patients with Parkinson's had lower phosphate levels than the control group, and this observation confirms the present study findings (34). Abou-Raya et al. also showed that bone density in patients with Parkinson's disease was lower than in the control group and that serum phosphorus and calcium levels were usually lower than in normal control groups ($P<0.001$), indicating a disturbance in phosphorus levels in these patients (35). Håglin also illustrated that in smokers, increased serum levels of phosphate and triglycerides, which are associated with decreased levels of bone phosphate and bone density, significantly reduce the incidence of Parkinson's disease in these people, which is due to adequate phosphate supply in the brain and prevention of mitochondrial dysfunction due to lack of sufficient phosphate. The results of their study additionally confirm the observations of the present study (36). In a study conducted by Meamar et al on the serum biomarkers of people with Parkinson's disease, the patients were found to have lower phosphate levels than controls. Although they had lower phosphate levels, their phosphate was within the normal range (37).

Conclusion

In conclusion, our study revealed that progressive death of dopaminergic neurons in the striatum is associated with decreased serum levels of corticosterone. Parkinsonian patients can be expected to show a decrease in serum corticosterone levels. Thus, we can conclude that serum levels of glucocorticoids can be used as a biomarker in diagnosing Parkinson's disease. Although serum phosphate levels decrease in Parkinson's patients, the decreased phosphate levels are still within the normal range, so serum phosphate cannot be used as a biomarker to diagnose Parkinson's disease.

Acknowledgements

We are grateful to the biochemistry department of Qazvin University of Medical Sciences for the technical assistance they provided for this project.

Authors' Contribution

Conceptualization: Hashem Haghdoost-Yazdi, Hossein Piri.
Data curation: Hashem Haghdoost-Yazdi, Hossein Piri.
Formal Analysis: Hashem Haghdoost-Yazdi, Hossein Piri, Fatemeh Kaveh.
Funding acquisition: Hossein Piri.
Investigation: Sepideh Nigjeh, Sahar Sharifi.
Methodology: Hashem Haghdoost-Yazdi, Hossein Piri, Sepideh Nigjeh, Sahar Sharifi.
Project administration: Hashem Haghdoost-Yazdi, Hossein Piri.
Resources: Hashem Haghdoost-Yazdi, Hossein Piri, Sepideh Nigjeh, Sahar Sharifi.
Software: Hashem Haghdoost-Yazdi, Hossein Piri.
Supervision: Hashem Haghdoost-Yazdi, Hossein Piri.
Validation: Hashem Haghdoost-Yazdi.
Visualization: Hashem Haghdoost-Yazdi, Hossein Piri.
Writing–original draft: Hossein Piri, Seyed Amir Hadi Hosseini.
Writing–review & editing: Hossein Piri, Hashem Haghdoost-Yazdi, Fatemeh Kaveh.

Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

Ethics Committee of Qazvin University of Medical Sciences (project No. IR.QUMS.REC.1399.084)

Funding

This research project was financially supported by the Deputy for the Research and Technology Division of Qazvin University of Medical Sciences.

References

1. Weller J, Budson A. Current understanding of Alzheimer's disease diagnosis and treatment. F1000Res. 2018;7:F1000 Faculty Rev-1161. doi: 10.12688/f1000research.14506.1.
2. Grigoruță M, Martínez-Martínez A, Dagda RY, Dagda RK. Psychological stress phenocopies brain mitochondrial dysfunction and motor deficits as observed in a parkinsonian rat model. Mol Neurobiol. 2020;57(4):1781-98. doi: 10.1007/s12035-019-01838-9.
3. Willard AM, Bouchard RS, Gittis AH. Differential degradation of motor deficits during gradual dopamine depletion with 6-hydroxydopamine in mice. Neuroscience. 2015;301:254-67. doi: 10.1016/j.neuroscience.2015.05.068.
4. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. Nat Rev Dis Primers. 2017;3:17013. doi: 10.1038/nrdp.2017.13.
5. Pagan FL. Improving outcomes through early diagnosis of Parkinson's disease. Am J Manag Care. 2012;18(7

Suppl):S176-82.
6. Calvani R, Picca A, Landi G, Marini F, Biancolillo A, Coelho-Junior HJ, et al. A novel multi-marker discovery approach identifies new serum biomarkers for Parkinson's disease in older people: an exosomes in Parkinson disease (EXPAND) ancillary study. Geroscience. 2020;42(5):1323-34. doi: 10.1007/s11357-020-00192-2.
7. Cagney DN, Sul J, Huang RY, Ligon KL, Wen PY, Alexander BM. The FDA NIH biomarkers, endpoints, and other tools (BEST) resource in neuro-oncology. Neuro Oncol. 2018;20(9):1162-72. doi: 10.1093/neuonc/nox242.
8. Deumens R, Blokland A, Prickaerts J. Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol. 2002;175(2):303-17. doi: 10.1006/exnr.2002.7891.
9. Jin F, Wu Q, Lu YF, Gong QH, Shi JS. Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats. Eur J Pharmacol. 2008;600(1-3):78-82. doi: 10.1016/j.ejphar.2008.10.005.
10. Haghdoost-Yazdi H, Piri H, Faraji A, Fraidouni N, Dargahi T, Mahmudi M, et al. Pretreatment with potassium channel blockers of 4-aminopyridine and tetraethylammonium attenuates behavioural symptoms of parkinsonism induced by intrastriatal injection of 6-hydroxydopamine; the role of lipid peroxidation. Neurol Res. 2016;38(4):294-300. doi: 10.1080/01616412.2015.1114290.
11. Razaghi R, Piri H, Jafari H, Rastgoo N, Hosseini MA, Haghdoost-Yazdi H. Evaluation of the association between serum levels of testosterone and prolactin with 6- hydroxydopamine-induced parkinsonism in male rats. Basic Clin Neurosci. 2021;12(4):453-64. doi: 10.32598/bcn.2021.671.3.
12. Sofiabadi M, Haghdoost-Yazdi H, Piri H, Esmaeili M. B vitamins supplement potentiates antiparkinsonian effect of flunarizine: the behavioral and biochemical evidences from 6-hydroxydopamine animal model. J Kerman Univ Med Sci. 2018;25(5):414-27. [Persian].
13. Minaei A, Sarookhani MR, Haghdoost-Yazdi H, Rajaei F. Hydrogen sulfide attenuates induction and prevents progress of the 6-hydroxydopamine-induced parkinsonism in rat through activation of ATP-sensitive potassium channels and suppression of ER stress. Toxicol Appl Pharmacol. 2021;423:115558. doi: 10.1016/j.taap.2021.115558.
14. Magno LAV, Collodetti M, Tenza-Ferrer H, Romano-Silva MA. Cylinder test to assess sensory-motor function in a mouse model of Parkinson's disease. Bio Protoc. 2019;9(16):e3337. doi: 10.21769/BioProtoc.3337.
15. Haghdoost-Yazdi H, Faraji A, Fraidouni N, Movahedi M, Hadibeygi E, Vaezi F. Significant effects of 4-aminopyridine and tetraethylammonium in the treatment of 6-hydroxydopamine-induced Parkinson's disease. Behav Brain Res. 2011;223(1):70-4. doi: 10.1016/j.bbr.2011.04.021.
16. Subramaniam SR, Chesselet MF. Mitochondrial dysfunction and oxidative stress in Parkinson's disease. Prog Neurobiol. 2013;106-107:17-32. doi: 10.1016/j.pneurobio.2013.04.004.
17. Herman JP, Seroogy K. Hypothalamic-pituitary-adrenal axis, glucocorticoids, and neurologic disease. Neurol Clin. 2006;24(3):461-81i. doi: 10.1016/j.ncl.2006.03.006.
18. Henchcliffe C, Beal MF. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. Nat Clin Pract Neurol. 2008;4(11):600-9. doi: 10.1038/ncpneuro0924.
19. Pacosz J. The Effects of Short-Term Glucocorticoid Exposure on Hippocampal Neuronal Structure and Signaling Pathways in Female Rats [dissertation]. University of Guelph; 2021.
20. Sobesky JL, D'Angelo HM, Weber MD, Anderson ND, Frank MG, Watkins LR, et al. Glucocorticoids mediate short-term high-fat diet induction of neuroinflammatory priming, the NLRP3 inflammasome, and the danger signal HMGB1. eNeuro. 2016;3(4):ENEURO.0113-16.2016. doi: 10.1523/eneuro.0113-16.2016.
21. Vyas S, Rodrigues AJ, Silva JM, Tronche F, Almeida OF, Sousa N, et al. Chronic stress and glucocorticoids: from neuronal plasticity to neurodegeneration. Neural Plast. 2016;2016:6391686. doi: 10.1155/2016/6391686.
22. Soares NM, Pereira GM, Altmann V, de Almeida RM, Rieder CR. Cortisol levels, motor, cognitive and behavioral symptoms in Parkinson's disease: a systematic review. J Neural Transm (Vienna). 2019;126(3):219-32. doi: 10.1007/s00702-018-1947-4.
23. Sullivan RM, Gratton A. Relationships between stress-induced increases in medial prefrontal cortical dopamine and plasma corticosterone levels in rats: role of cerebral laterality. Neuroscience. 1998;83(1):81-91. doi: 10.1016/s0306-4522(97)00370-9.
24. Törnhage CJ, Skogar Ö, Borg A, Larsson B, Robertsson L, Andersson L, et al. Short- and long-term effects of tactile massage on salivary cortisol concentrations in Parkinson's disease: a randomised controlled pilot study. BMC Complement Altern Med. 2013;13:357. doi: 10.1186/1472-6882-13-357.
25. Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurol. 2021;20(5):385-97. doi: 10.1016/s1474-4422(21)00030-2.
26. Djamshidian A, O'Sullivan SS, Papadopoulos A, Bassett P, Shaw K, Averbeck BB, et al. Salivary cortisol levels in Parkinson's disease and its correlation to risk behaviour. J Neurol Neurosurg Psychiatry. 2011;82(10):1107-11. doi: 10.1136/jnnp.2011.245746.
27. Hartmann A, Veldhuis JD, Deuschle M, Standhardt H, Heuser I. Twenty-four hour cortisol release profiles in patients with Alzheimer's and Parkinson's disease compared to normal controls: ultradian secretory pulsatility and diurnal variation. Neurobiol Aging. 1997;18(3):285-9. doi: 10.1016/s0197-4580(97)80309-0.
28. Mizoguchi K, Ishige A, Takeda S, Aburada M, Tabira T. Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. J Neurosci. 2004;24(24):5492-9. doi: 10.1523/jneurosci.0086-04.2004.
29. Barnum CJ, Eskow KL, Dupre K, Blandino P Jr, Deak T, Bishop C. Exogenous corticosterone reduces L-DOPA-induced dyskinesia in the hemi-parkinsonian rat: role for interleukin-1beta. Neuroscience. 2008;156(1):30-41. doi: 10.1016/j.neuroscience.2008.07.016.
30. Sugama S, Takenouchi T, Kitani H, Fujita M, Hashimoto M. Microglial activation is inhibited by corticosterone in dopaminergic neurodegeneration. J Neuroimmunol. 2009;208(1-2):104-14. doi: 10.1016/j.jneuroim.2009.01.016.
31. Tentillier N, Etzerodt A, Olesen MN, Rizalar FS, Jacobsen J, Bender D, et al. Anti-inflammatory modulation of microglia via CD163-targeted glucocorticoids protects dopaminergic neurons in the 6-OHDA Parkinson's disease model. J Neurosci. 2016;36(36):9375-90. doi: 10.1523/jneurosci.1636-16.2016.
32. Luan H, Liu LF, Tang Z, Zhang M, Chua KK, Song JX, et al. Comprehensive urinary metabolomic profiling and identification of potential noninvasive marker for idiopathic Parkinson's disease. Sci Rep. 2015;5:13888. doi: 10.1038/

srep13888.
33. Håglin L, Bäckman L. Covariation between plasma phosphate and daytime cortisol in early Parkinson's disease. Brain Behav. 2016;6(12):e00556. doi: 10.1002/brb3.556.
34. Samavarchi Tehrani S, Sarfi M, Yousefi T, Ahmadi Ahangar A, Gholinia H, Mohseni Ahangar R, et al. Comparison of the calcium-related factors in Parkinson's disease patients with healthy individuals. Caspian J Intern Med. 2020;11(1):28-33. doi: 10.22088/cjim.11.1.28.
35. Abou-Raya S, Helmii M, Abou-Raya A. Bone and mineral metabolism in older adults with Parkinson's disease. Age Ageing. 2009;38(6):675-80. doi: 10.1093/ageing/afp137.
36. Håglin L. High serum phosphate concentration as the result of smoking might underlie the lower risk of Parkinson's disease. Med Hypotheses. 2015;85(3):287-90. doi: 10.1016/j.mehy.2015.05.017.
37. Meamar R, Maracy M, Chitsaz A, Aghaye-Ghazvini MR, Izadi M, Tanhaei AP. Association between serum biochemical levels, related to bone metabolism and Parkinson's disease. J Res Med Sci. 2013;18(Suppl 1):S39-42.