Research Paper: Evaluation of the Association Between Serum Levels of Testosterone and Prolactin With 6-Hydroxydopamine- Induced Parkinsonism in Male Rats

Roghaieh Razaghi[1], Hossein Piri[2] , Hanieh Jafari[1] , Nafiseh Rastgoo[3] , Mohammad Ali Hosseini[3] , Hashem Haghdoost – Yazdi[2*]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Cellular and Molecular Research Center, Research Institute for Prevention of Non- Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran.
3. Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran.

ABSTRACT

Introduction: Parkinson's Disease (PD) associates with changes in sex hormones; however, it remains unknown whether this is either a cause for or a result of the disease. To further evaluate it, we investigated if the development of 6-Hydroxydopamine (6-OHDA)-induced Parkinsonism changes the serum levels of testosterone and prolactin or not.

Methods: 6-OHDA was injected into the medial forebrain bundle using stereotaxic surgery. The development of Parkinsonism was evaluated by apomorphine-induced rotational test and the immunofluorescence labeling of Dopaminergic (DA) neurons in substantia nigra. The necessary blood samples were collected before the toxin and in the third and sixth weeks afterward. The hormones levels were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Results: The severity of rotations was different among 6-OHDA-treated rats; accordingly, they were divided into two subgroups of severe and mild parkinsonian rats. The degeneration of DA neurons was observed in both subgroups; however, it was significantly less in the mild group. In the sixth week after the toxin, testosterone level increased but only in the severe subgroup. Prolactin increased in both subgroups in the third week after the toxin but returned to normal in the sixth week. There was no association between the pre-toxin levels of these hormones and the intensity of Parkinsonism.

Conclusion: Our findings indicated that the development of 6-OHDA-induced Parkinsonism increases the serum levels of testosterone and prolactin. Increased prolactin occurred earlier and was observed in rats with less DA neuronal loss. Therefore, prolactin levels can predict the death of DA neurons before the clinical signs of PD were revealed.

* Corresponding Author:
Hashem Haghdoost – Yazdi, PhD.
Address: Cellular and Molecular Research Center, Research Institute for Prevention of Non- Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran.
Tel: +98 (912) 4188325
E-mail: hhaghdoost@gmail.com

Highlights

- 6-OHDA-induced Parkinsonism is associated with an increase in testosterone and prolactin.

- Testosterone levels increased six weeks after 6-OHDA only in severe parkinsonian rats.

- Prolactin increased in the third week after 6-OHDA but returned to normal in the sixth week.

- Prolactin increased in both mild and severe parkinsonian rats.

- Serum prolactin level can predict PD before its clinical signs are revealed.

Plain Language Summary

Parkinson's Disease (PD) is the most common movement disorder and represents the second most common neurodegenerative disease after Alzheimer's disease. PD affects 1–2 per 1000 of the population at any time. Its prevalence is increasing with age and affects 1% of the people above 60 years. The leading cause of PD is the death of neurons called dopaminergic neurons in the brain stem. Clinical diagnostic of PD relies on motor symptoms of tremor, rigidity, and bradykinesia. However, these signs appear when many dopaminergic neurons are already destroyed, and the disease is in advanced stages. Thus, nonmotor symptoms which frequently precede the onset of motor symptoms, have gained increasing attention. In this regard, biomarkers have more chance. A biomarker is an indicator of a particular disease that can be used to evaluate its progress. Identifying specific biomarkers for neurodegenerative disorders is one of the main goals of the current clinical research. Usually, researchers estimate the change in blood parameters to find a biomarker for a specific disease. In the past decades, researchers have developed PD in rats. Through a certain surgery, a neurotoxin called 6-hydroxydopamine is injected into a particular site in the brain. This toxin gradually destroys dopaminergic neurons in the brain stem and thereby generates PD in rats. Because PD is an age-related disease and blood levels of sex hormones change with age, we assessed changes in blood levels of two sex hormones of testosterone and prolactin in rats with PD. Our findings show that PD development in rats is associated with an increase in serum levels of testosterone and prolactin. An increase in prolactin occurred earlier and was observed in rats with less dopaminergic neuronal loss. Therefore, prolactin level probably can predict PD before its clinical signs have appeared.

1. Introduction

Parkinson's Disease (PD) is the most common age-associated neurodegenerative disease after Alzheimer's disease. The main pathological characteristic of PD is the death of Dopaminergic (DA) neurons located in the Substantia Nigra pars compacta (SNc). The clinical symptoms of PD include tremor, muscle stiffness, bradykinesia, and difficulty with walking which appear after 60%-80% of DA neurons degenerate (Jankovic, 2008; Shulman, De Jager, & Feany, 2011). The etiology of DA neuronal death remains unclear; however, studies suggested that oxidative stress, mitochondrial dysfunction, the accumulation of proteins, and autophagic stress are the main pathophysiological mechanisms underlying the death of DA neurons (Dauer & Przedborski, 2003; Tsang & Chung, 2009; Moon & Paek, 2015; Darabi et al., 2018).

Epidemiological studies revealed that age is among the major risk factors for neurodegenerative diseases (Dauer & Przedborski, 2003; Wirdefeldt, Adami, Cole, Trichopoulos, & Mandel, 2011; Tysnes & Storstein, 2017; Marinus, Zhu, Marras, Aarsland, & van Hilten, 2018). Aging is associated with reduced levels of sex steroid hormones. A study demonstrated that age-related reduction in sex hormones significantly increases the risk of Alzheimer's disease in both genders (Vest & Pike, 2013). Regarding PD, it has been suggested that the incidence of this disease in men is higher than in women (Marinus et al., 2018; Nitkowska, Tomasiuk, Czyżyk, & Friedman, 2015). Epidemiological studies also revealed that the lower levels of testosterone in aged men enhance the risk of PD (Nitkowska et al., 2015). Furthermore, testosterone deficiency is more common in men with PD than healthy men (Zolbanin, Zolali, & Mohajjel Nayebi, 2014); treatment with testosterone improves the non-motor symptoms of this condition (Ready, Friedman, Grace, & Fernandez, 2004). Moreover, age-related testosterone deficiency can increase brain vulnerability, leading to Parkinson's or Alzheimer's diseases (Al-Sweidi, Morissette, Bourque, & Di Paolo, 2011). In rats, rotenone reduces the activity of
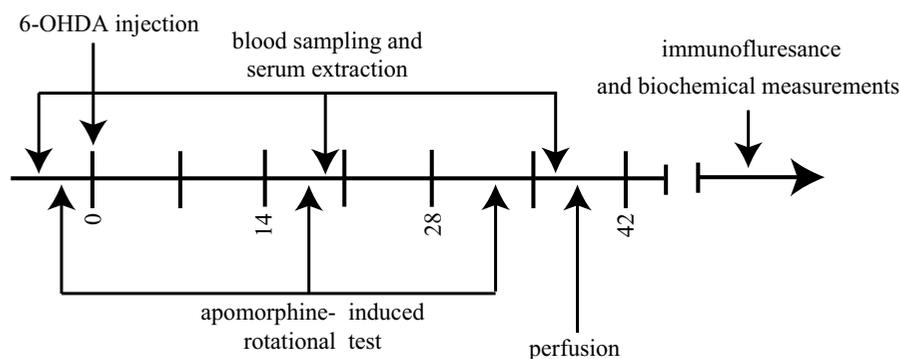
Figure 1. Experiment design

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complex I mitochondria, which in turn, induces the selective degeneration of SNc DA neurons. Additionally, in rotenone-treated rats, the testosterone level decreases in peripheral blood (Alam & Schmidt, 2004). Other studies reported that prolactin level in patients with PD is significantly higher than that in the control-matched group (Nitkowska et al., 2015). Besides, there is a negative correlation between prolactin level and sex steroids concentrations (Nitkowska et al., 2015).

Epidemiological studies revealed an association between PD and sex hormones; however, these studies cannot discriminate if a change in these hormones underlies the DA neuronal death or death of DA neurons causes this change in the sex hormones. This discrimination is critical because in the first case, the treatment of sex hormones disorders could present a preventative effect; however, in the second case, measuring these hormones can be used for the early diagnosis of PD. The latter case is also useful to identify individuals who are at risk of PD or the patient's follow-up to evaluate the progress of disease and its treatment efficiency. Besides, human studies are limited by the following factors: first of all, the interfering factors, such as age, race, health, nutrition, comorbidity (having comorbid multiple diseases), and treatment with various medications confuse the data. Secondly, the levels of sex hormones in patients with PD were measured after developing clinical signs when the most of SNc DA neurons are lost. In this study, we examined the serum levels of testosterone and prolactin in the 6-OHDA-induced animal model of PD to find if the development of Parkinsonism in male rats changes the serum levels of these hormones. In the 6-OHDA model of PD, the toxin is injected into the specific sites in the rat's brain to selectively destroy the SNc DA neurons and DA terminals in the striatum during a few days to weeks. Therefore, mechanistically, this model is very similar to PD in human beings. Additionally, this model has a major advantage; the development and intensity of

Parkinsonism can examine quantitatively by evaluating a circling behavior, i.e., induced by the administration of dopamine agonists.

2. Methods

Adult male Wistar rats (Razi Institute, Karaj, Iran), with the weight range of 250-300 g were housed in large cages (38×59×20 cm) in a temperature-controlled room with 12:12h light/dark cycle and free access to water and pellet food. All the procedures of the current study followed the guidelines of ethics for animal experiments of the Research Council at Qazvin University of Medical Sciences. The obtained rats were divided into 3 experimental groups of control (con, n=8), which did not receive anything; sham (n=8), which Intracerebrally (IC) received the solvent of 6-OHDA, and 6-OHDA (n=13), which intracerebrally received 6-OHDA.

Figure 1 illustrates the timing schedule of different experiments in this study. Apomorphineinduced rotational tests were performed before the toxin and at the third and fifth weeks afterward. Blood sampling and serum extractions were conducted before the toxin and at the third and sixth weeks after that. Within the Sixth week after the toxin, the study animals were perfused through the heart and the brain was removed from the skull. The immunofluorescence and biochemical measurements of hormones were performed, accordingly. Before the toxin, the apomorphine-induced rotational test was initially conducted and the examined rats revealed <10 rotations in one hour, i.e., chose to continue studies. A rotational test was also performed at the third and fifth weeks after the toxin. Blood sampling and serum extractions were performed before the toxin and at the third and sixth weeks after that. In the sixth week, the brain of three rats in each group was perfused through the heart, and immunofluorescence was conducted on the midbrain sections. Numbers indicated the days after the toxin injection.

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

455

6-OHDA was injected into the Medial Forebrain Bundle (MFB) of the right hemisphere using stereotaxic surgery and through a 10-μl Hamilton syringe. The study rats were initially anesthetized with a combination of ketamine and xylazine [70 mg/kg & 6 mg/kg, respectively; Intraperitoneal (IP)]. Then, they fixed at the frames of a stereotaxic instrument (stoleting) and the incisor bar was set at -3.3 below to interaural line. Consequently, with an incision in the skin, the skull surface was exposed and the bregma position was determined. Using a dental drill two holes at 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 bregma, while (DV) was measured from the surface of the skull according to the atlas of Paxinos and Watson (2007). Finally, 4 μL of 6-OHDA (Sigma, 4 μg/μL) dissolved in isotonic NaCl solution, containing 0.2% of ascorbic acid was injected into the brain through these holes. The injection time was 5 minutes and the syringe needle was withdrawn slowly in 1 minute.

Apomorphine (APO)-induced rotational test was performed according to the method we have previously described (Haghdoost-Yazdi et al., 2010; Sarukhani, Haghdoost-Yazdi, & Khandan-Chelarci, 2018). Briefly, the explored animals were initially allowed to acclimate for 5 minutes; apomorphine hydrochloride (0.5 mg/ kg) was then injected IP. One minute later, the number of full rotations was counted for 30 minutes in a cylindrical container (diameter: 28 cm; height: 38 cm). Positive and negative scores were assigned to contralateral (away from the injection side) and ipsilateral rotations respectively. Furthermore, the net number of rotations was calculated by subtraction of the negative scores from the positive ones.

The immunofluorescence labeling of SNc DA cells was conducted on the brain sections of 3 rats from each experimental group. The studied rats were transcardially perfused and under deep anesthesia with Phosphate-Buffered Saline (PBS) and 4% paraformaldehyde. Subsequently, the brain was removed and postfixed overnight in paraformaldehyde, then, floated in 30% sucrose in PBS at 4oC. The midbrain portion of the brain was separated and frozen in a cryostat embedding medium (Bio-Optica, Italy) at -22 °C. The coronal sections (8-μm thickness) were cut using a cryostat (Histo-Line Laboratories, Italy). One out of every 3 serial sections was permeabilized with 0.2% Triton X-100 and blocked with 10% normal goat serum for one hour. The sections were incubated overnight at 4 °C with an anti-Tyrosine Hydroxylase (TH) antibody (1:250; Abcam) and proper fluorescently labeled rabbit secondary antibodies. Sub-

sequently, using a mounting medium (sc-24941; Santa Cruz), which contained 4',6-Diamidino-2-Phenylindole (DAPI) as the nuclear stain, the sections were cover-slipped. Sections were observed by an Olympus microscope at 10× magnification, and those including the SN (AP: -4.8 to -5.2 relative to the bregma) were selected. Next, 5 sections from each animal were selected from a total of 50 sections, i.e., rostrocaudally divided into 5 series. There was≥5 sections interval between the two selected sections. TH-positive cells (DA neurons) were visualized and counted manually at 400× magnification.

The first and second blood specimens were collected from the tail's caudal vein and the third sample was collected from the heart of animals under deep anesthesia. Blood was allowed to coagulate and then serum was separated and kept at -80°C until measurement time. Testosterone and prolactin levels were measured using specific ELISA kits (HAZNGZHOUEASTBIOPHARM, USA) and its manufacturer's instruction. First, 40 microliters of the serum were added to the test wells, and specific antibodies and Streptavidin-HRP were added, accordingly. Then, the sealing membrane was sealed, gently shaking, and incubated for 60 minutes at 37 °C. The absorbance was read at 450 nm for testosterone and prolactin.

The collected data were expressed as mean±Standard Error of Mean (SE). They were initially analyzed by the Kolmogorov-Smirnov test to define their normality. Then, between-group data were subjected to Analysis of Variance (ANOVA, Single Factor) followed by Newman–Keuls test. Within-Group data were analyzed using Paired Samples t-test. Significant differences were considered at P≤0.05.

3. Results

The development and intensity of Parkinsonism were evaluated by an apomorphine-induced rotational test. In this test, systemic injection of DA agonists, like apomorphine leads to the asymmetrical rotations of the animal contralateral to the toxin-injected hemisphere. The number of rotations is a useful quantitative parameter for evaluating Parkinsonism's intensity. Plot A in Figure 2 demonstrates the findings of this test in different experimental groups. Rats in the control and sham groups presented no significant rotational behavior; however, 6-OHDA-treated rats demonstrated numerous net rotations to the left side, indicating the model was well established. In 30 minutes, the Mean±SD number of rotations in the 6-OHDA group was measured as 197±40 and 340±70 in the first and second tests after the toxin, respectively.
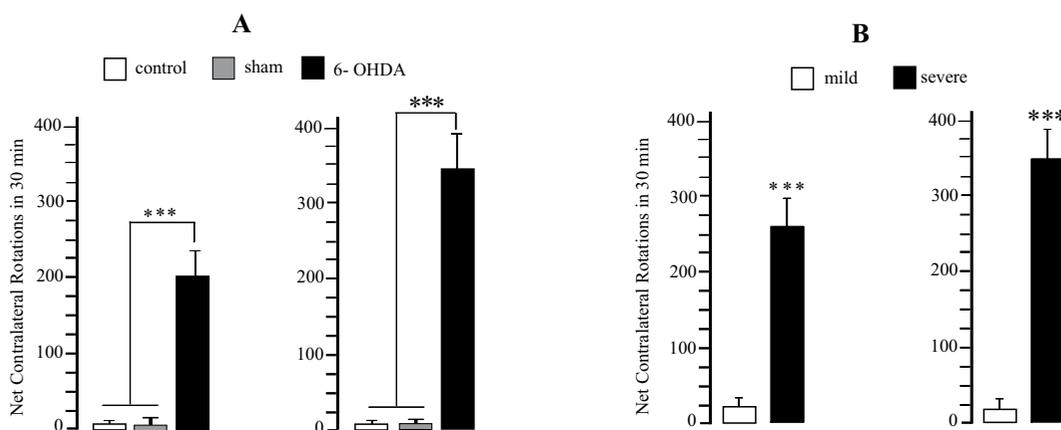
Figure 2. Plots illustrate the number of a pomorphine induced net contralateral rotations in the third (left plots) and the sixth (right plots weeks after the toxin As shown, number r of rotations was significantly higher in the sixth week, Plot A: Compared to the third week in 6 OHDA group; and Plot B: The severe subgroup

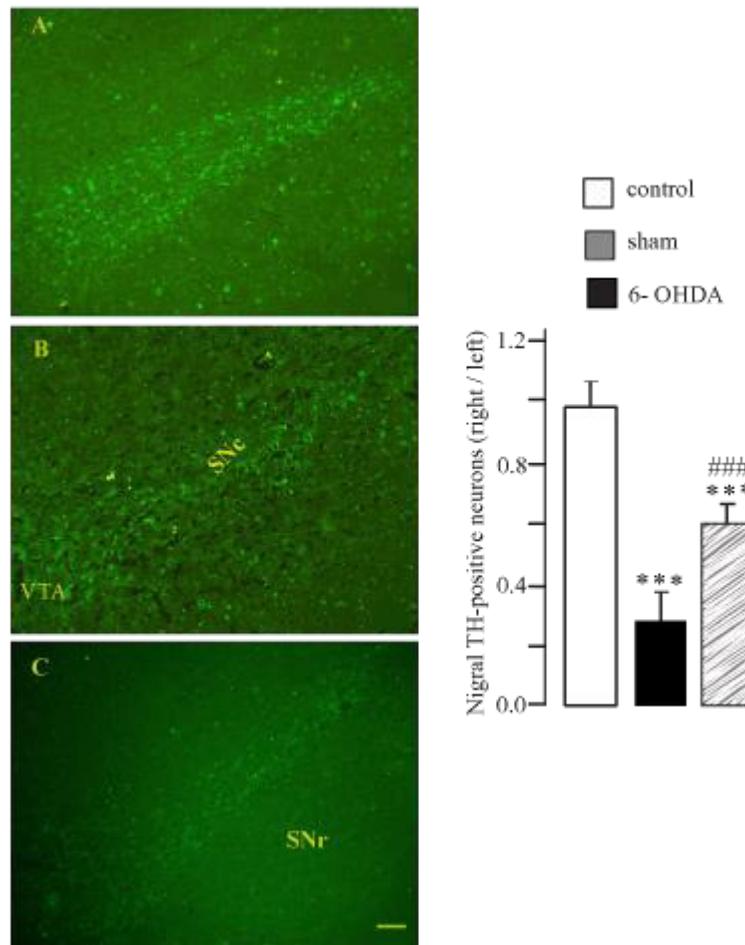***P<0.001 compared to the control, sham groups and the mild Subgroup.

Additionally, the intensity of rotational behavior in 6-OHDA- treated rats was not equal and some responded to apomorphine with the high number of rotations; however, others revealed low number or no rotation. The most number of recorded rotations was equal to 860 and the lowest number was calculated as 6. For more precise evaluations, 6- OHDA-treated rats were further divided into two subgroups of severe (n=7) with >200 rotations in 30 minutes and mild (n=6) with rotations <30 rotations. Plot B in Figure 2 illustrates the severity of rotations in these subgroups. Accordingly, in the mild group, there was no significant difference in the number of rotations between the two tests after the toxin. Besides, the number of rotations was much higher in the second test, compared to the first test in the severe group.

For further evaluation, the number of survived DA neurons in SNc was examined using the Tyrosine-Hydroxylase (TH)- immunofluorescence. As per Figure 3, in the severe group, the lesion was very intensive, and only <30% of DA neurons survived in the lesioned hemisphere. The lesion in the mild group was significantly less than the severe group; however, the number of survived DA cells (~50%) was significantly less than that in the control group (plot in Figure 3).

Serum testosterone level was measured one week before the toxin and in the third and sixth weeks after that. Figure 4 illustrates serum testosterone levels at these times. As shown in plot A, there was no significant difference in serum testosterone concentrations between the experimental groups before the toxin. In the control

group, the Mean±SD testosterone level was computed as 7.29±0.9 μg/L before the toxin and 7.3±0.7 μg/L and 6.1±0.4 μg/L in the samples collected at the third and sixth weeks after the toxin, respectively (Plots B & C). In the 6-OHDA group, the Mean±SD serum testosterone concentrations were measured to be 7.2±0.4 μg/L, 7.7±0.6 μg/L, and 8.1±05 μg/L before, and within the third, and sixth weeks after the toxin, respectively. Furthermore, in the 6-OHDA group, the testosterone level in the sixth week was significantly higher than that in the control and sham groups (Plot C).

To further investigate the association between testosterone and the intensity of Parkinsonism, the testosterone concentrations were further evaluated in severe and mild subgroups. Plots D to F in Figure 4 demonstrate a comparison between these two subgroups. Accordingly, there was no significant difference between them in the rum concentrations of testosterone before the toxin and three weeks after that. However, in the samples collected in the sixth week after the toxin, the level of testosterone in the severe subgroup was significantly higher than that in the mild group. Diagrams G and H in Figure 4 illustrate the change in the testosterone concentration in the experimental groups. Testosterone concentration in the control and sham groups decreased as a function of time. Moreover, this decrease reached a statistically significant level in the sixth week after the toxin. Additionally, the concentration of testosterone in the 6-OHDA group increased after the toxin. Again, an increase in concentration was statistically significant in the sixth week. This increase was observed in both subgroups; however, it was only significant in the severe subgroup.

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

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Figure 3. Pho to micrographs display the degree of the lesion in Tyrosine Hydroxylase (TH)-positive (light green cells in sub-stantia nigra pars compacta (SNc) of the lesioned hemisphe re in control group
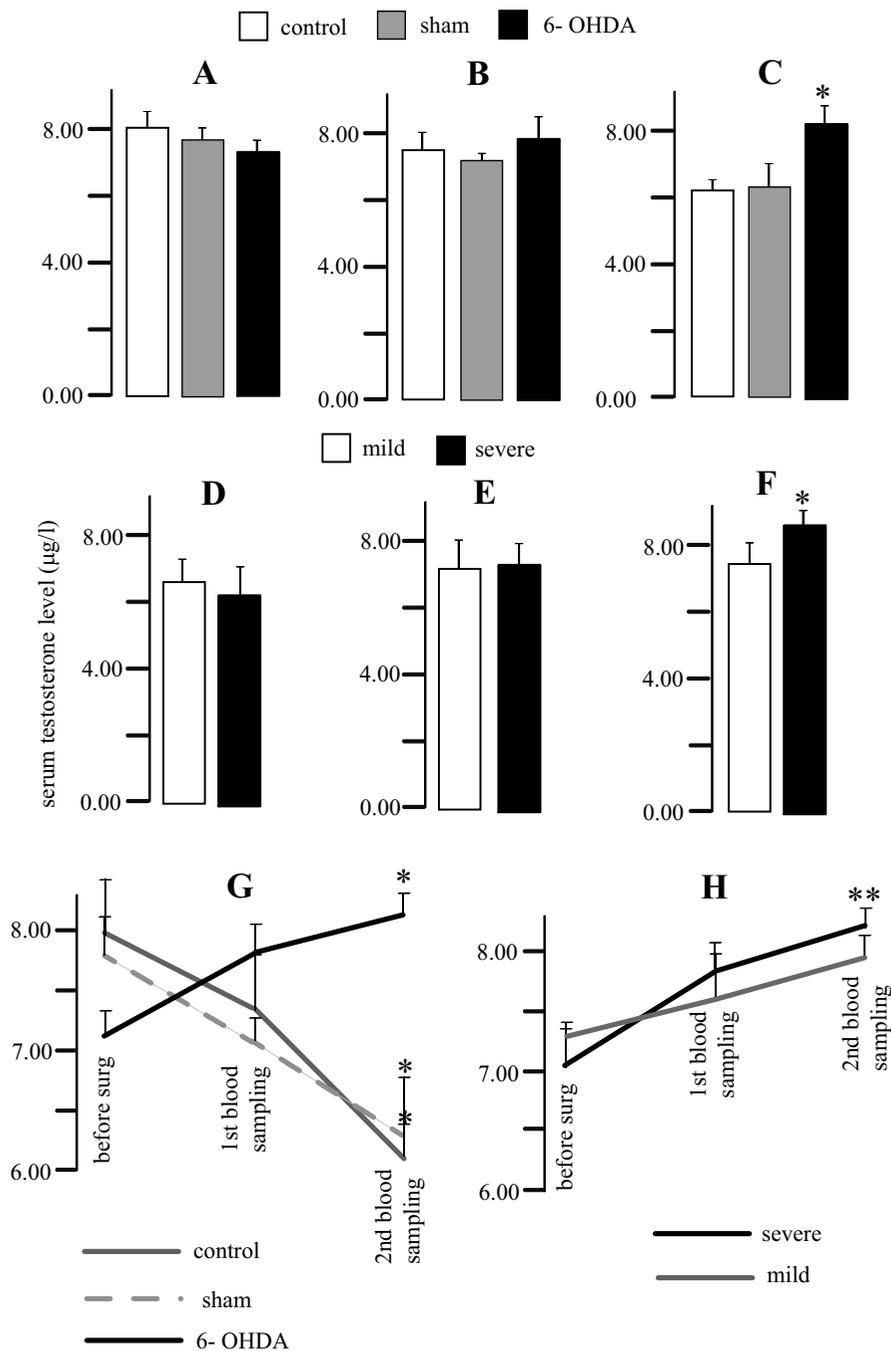
A: Severe; B: Mild; C: Subgroups

The plot shows the rat io of these cells in the lesioned hemisphere to the he intact hemisphere SNr: substantia nigra pars reticu-late, VTA: ventral tegmental area. Scale Bar= 100 µm.

***P<0.001 in compared to control group.

###P<0.001 in compared to severe SubGroup.

Serum prolactin levels in different experimental groups: As testosterone, serum prolactin level was mea-sured one week before the toxin and in the third and sixth weeks afterward. Figure 5 illustrates serum testosterone levels at these times. As shown in plot A, there was no significant difference in serum prolactin levels between experimental groups before the toxin. In control group, prolactin level was 247±12 ng/L before the toxin and 249±11 and 238±12 ng/L in samples collected at the third (plot B) and sixth (plot C) weeks after the toxin, respectively. In 6-OHDA group, the concentrations were 253±14, 286±14, and 245±11 ng/L, respectively. As shown in plot B, in the third week prolactin level in
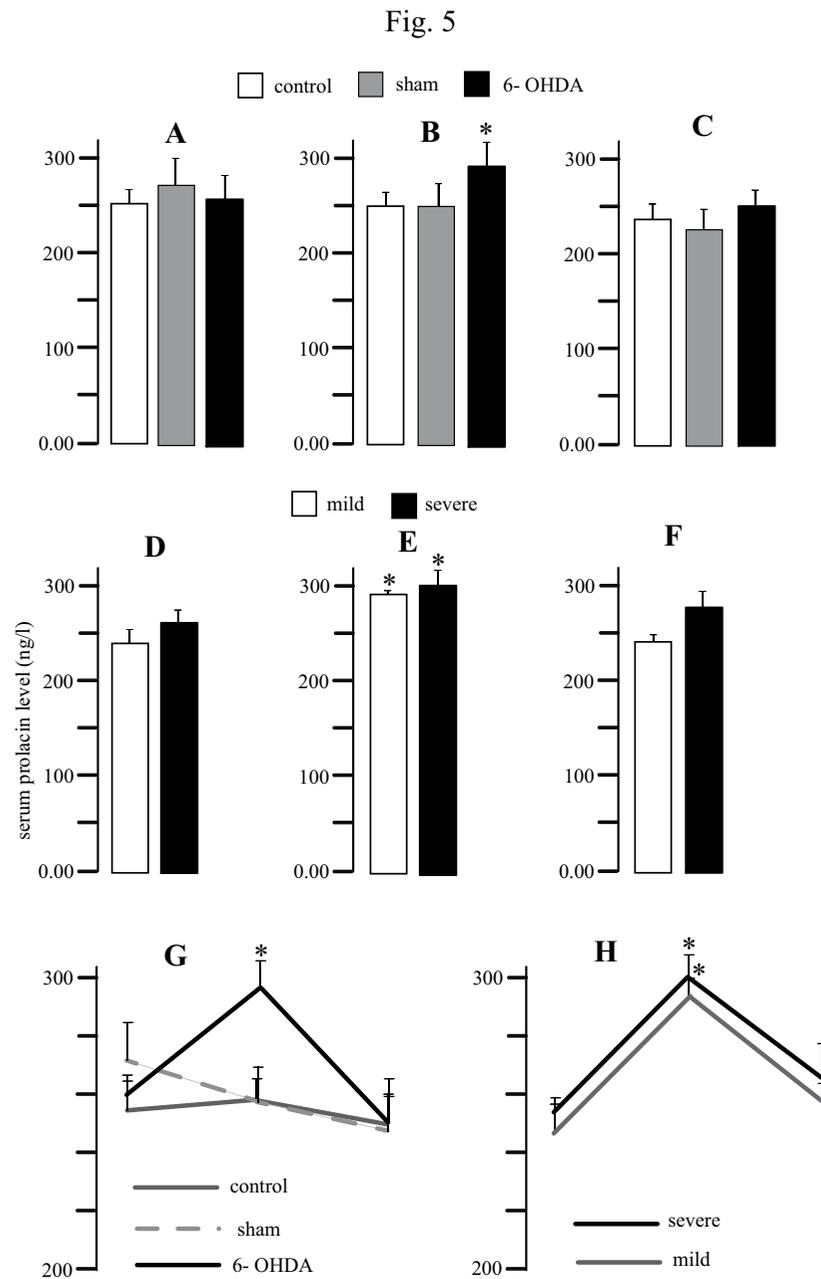
6-OHDA- treated rats was significantly higher than that in sham and control groups. Plots D to F illustrate pro-lactin concentrations in the severe and mild subgroups. Both of them had significantly higher prolactin level in compare to control group. Also, although prolactin level was higher in the severe subgroup in compare to mild subgroup but the difference was not significant. Dia-grams of G and H illustrate change in the serum prolactin concentrations in experimental groups. As shown, serum prolactin level significantly increased in 6-OHDA group in the third week after the toxin but returned to normal level in the sixth week. Such increase was also observed in both severe and mild subgroups.

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

Figure 4. Plots A to C illustrates the serum testosterone concentrations in the control, sham, and 6-OHDA groups

Before the toxin (A) and in the third (B) and sixth (C) weeks after that. Plots D to F show the testosterone concentrations in severe (with more than 200 rotations in 30 minutes) and mild (with less than 30 rotations in 30 minutes) subgroups before the toxin (D) and in the third (E) and sixth (F) weeks after that. Diagrams of G and H show the change in the serum testosterone concentrations in experimental groups (G) and 6- OHDA- treated subgroups (H) before the toxin (before Surg) and in the third (1st blood sampling) and sixth (2nd blood sampling) weeks after that.

*P<0.05, and **P<0.01 in comparison to control or sham groups (plots A to C) or mild subgroup (plots D to F) or before the toxin (diagrams G and H).

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

Basic and Clinical
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Fig. 5

Figure 5. Plots A to C illustrate the serum prolactin concentrations in the control, sham and 6   **NEUR⊗SCIENCE**

OHDA groups before the toxin (A) and in the third (B) and sixth (C) weeks after that. Plots D to F illustrate the prolactin concentrations in the severe and mild subgroups before the toxin (D) and the third (E) and six (F) weeks after that Diagrams of G and H show the change in the serum prolactin concentrations in experimental groups (G) and 6 OHDA treated subgroups (H) before the toxin (before Surg) and in the third (1 blood sampling) and sixth (2nd blood sampling) weeks after that.

*$P<0.05$, in comparison to control or sham groups plots to C or before the toxin (diagrams G and H).

4. Discussion

Several studies revealed that the plasma level of testosterone and prolactin is associated with PD (Ready et al., 2004; Mitchell, Thomas, & Burnet, 2006; Kenangil, Orken, Ur, Forta, & Celik, 2009; Bronner, 2011; Zol-banin et al., 2014; Nitkowska et al., 2015). In this study, we evaluated changes in the serum levels of testosterone and prolactin after the development of 6-OHDA- induced Parkinsonism in male rats. We aimed to find the possible association between these hormones and Parkinsonism. Our data indicated that in 6 weeks after the toxin, the se-

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

rum testosterone level gradually increased in the 6-OH-DA-treated rats; however, it decreased in the control and sham groups in this period. This increase was statistically significant only in 6-OHDA-treated rats with severe behavioral symptoms and intensive lesions in SNc DA neurons. In different patterns, the serum prolactin level in 6-OHDA-treated rats significantly increased in the third week after the toxin; however, it returned to normal value in the sixth week after that. This changing pattern was observed in the severe and mild subgroups. Additionally, our data revealed no association between the serum level of testosterone and prolactin before the toxin and the intensity of Parkinsonism.

Considerable behavioral and immunohistochemical data suggested that the development and severity of apomorphine-induced rotational behavior reflect the DA neuronal loss in SNc and its severity (Borlongan, Randall, Cahill, & Sanberg, 1995; Iancu, Mohapel, Brundin, & Paul, 2005; Yuan, Sarre, Ebinger, & Michotte, 2005). Yuan et al. (2005) reported that following the injection of 6-OHDA into the MFB, a severe and extensive lesion in DA neurons of SNc occurs. They also argued that almost 80% of SNc DA neurons were lost within 3 weeks after the toxin injection. Moreover, the severity of rotational behavior does not necessarily indicate the severity of neuronal death in SNc. For instance, Yuan et al. (2005) stated that only 1% of SNc DA neurons remain alive 5 weeks after the toxin. In other words, neuronal death progressively increases from 80% to 99% from the third week to the fifth week after the toxin. However, the number of rotations increases more than twice during this time. Similarly, it was reported that 6-OHDA-treated rats presented a significant number of rotations when the most number of SNc DA neurons were lost; this behavior cannot discriminate mild to a moderate lesion in these neurons (Abrous et al., 1998; Iancu et al., 2005; Yuan et al., 2005). Our immunofluorescence data supported these reports. Moreover, extensive DA neuronal loss was observed in rats with severe behavioral symptoms. However, in 6- OHDA-treated rats, that generated mild rotational behavior (mild subgroup), neuronal death was also considerable (~50%). Overall, our findings indicated that the high serum testosterone level can propose a severe lesion in SNc DA neurons. However, mild to moderate lesions are not necessarily associated with an increase in serum testosterone level. In the human being, severe and intensive DA neuronal death in SNc leads to clinical symptoms of PD are appeared. Thus, the serum testosterone level has little value for the early diagnosis of PD. Furthermore, serum prolactin level may provide a better biochemical sign for the prediction of DA neuronal loss. Our results revealed that

serum prolactin level increases within the 3 weeks after the toxin. Furthermore, this increase was also observed in rats with mild or moderate neuronal death and without remarkable behavioral symptoms. Therefore, a sudden increase in the serum prolactin level could reflect the onset of neuronal death in SNc DA neurons.

The cause of different degrees of Parkinsonism among 6-OHDA-treated rats remains unclear. Technical concerns, such as mistakes in surgery and toxin injection, inappropriate toxin, error in calculating the toxin injection site, or error in the preparation of apomorphine might be involved in this respect. However, it is unlikely that these concerns are involved. This is because the surgeries and behavioral tests were concurrently performed by an expert with the same toxin solution. Additionally, rats might have different susceptibility to toxins due to the genetic and phenotypic variations. The race of rats was similar; therefore, it is difficult to consider this hypothesis, although it is noticeable. The differences in the severity of DA neuronal loss and behavioral symptoms among 6-OHDA-treated rats provide us an opportunity to examine much better and more precisely the association between Parkinsonism and the serum level of testosterone and prolactin.

Most studies indicated that PD is accompanied by reduced serum testosterone level (Alam & Schmidt, 2004; Ready et al., 2004; Kenangil et al., 2009; Bronner, 2011; Okun et al., 2014), i.e., in contrast with our findings. Dopamine agonists were reported to increase the serum level of testosterone by inhibiting prolactin secretion (Okun et al., 2014). Apomorphine, i.e., used in the rotational test might have increased the testosterone level by activating dopamine receptors. However, we cannot confirm this mechanism because the half-life of apomorphine is 30-60 minutes, and its effect lasts for up to 90 minutes (Chaudhuri & Clough, 1998). We collected the rat's blood and extracted its serum several hours and even days after the rotational test. Furthermore, the difference in serum testosterone level was observed between severe and mild subgroups which received the same amount of apomorphine. Moreover, there was a negative correlation between the serum levels of prolactin and testosterone (Nitkowska et al., 2015). In our study, serum prolactin level increased in the third week after the toxin; however, it returned to normal value at the sixth week. Thus, the higher testosterone level could arise from the withdraw of the prolactin's inhibitory effect on the gonads.

Besides, in consistence with our results, human studies highlighted that PD is associated with decreased serum

Razaghi, R., et al. (2021). Association Between Parkinsonism and Testosterone and Prolactin. *BCN, 12*(4), 453-464.

461

prolactin levels (Kostić, Lečić, Doder, Marinković, & Filipović, 1996; Winkler, Landau, & Chaudhuri, 2002; Rustembegovic, Sofic, & Wichart, 2006; Nitkowska et al., 2015). Moreover, administrating L-dopa decreases the prolactin secretion by an increase in the production of dopamine (Gordon et al., 2007). Confirming this, several animal studies have shown that 6-OHDA increases the prolactin levels (Gil-Ad et al., 1976; Lin, Mai, & Pan, 1993). Prolactin secretion is inhibited by the hypothalamic DA neurons; therefore, the death of them in patients with PD is associated with increased serum prolactin concentration. The serum prolactin level was returned to normal value in the sixth week after the toxin. The mechanism probably involves the up-regulation of DA receptors in prolactin-secreting neurons.

5. Conclusion

Our results indicated that in male rats, the serum levels of testosterone and prolactin significantly increased after the toxin. An increase in testosterone level was obvious in rats with severe behavioral symptoms and intensive DA neuronal loss in SNc and was appeared 6 weeks after the toxin. Additionally, increased prolactin level has appeared in the third week after the toxin and unlike testosterone was observed in rats with mild or without behavioral symptoms but with significant SNc DA neuronal loss. The degeneration of SNc DA neurons is common between PD in humans and 6-OHDA- induced animal models. Therefore, our results indicated that an increase in the serum concentration of prolactin predicts the death of DA neurons and can use for early diagnosis of PD in humans.

Ethical Considerations

Compliance with ethical guidelines

All procedures of the present study were carried out according to the guidelines of animal experiments of the Research Council at Qazvin University of Medical Sciences.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflicts of interest.

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463

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