Clinical and Diagnostical Values of Plasma

α-Synuclein and Melatonin Levels in Early Stages

of Parkinson’s Disease

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Abstract

Parkinson’s disease (PD) is a widespread, progressive neurodegenerative disorder with complex pathogenesis. The combination of its motor and non-motor symptoms significantly impairs a patient’s life quality. The question of targeted patogenetic therapy of Parkinson’s disease is still relevant, which is why biomarkers for early diagnosis and neuroprotective therapy are urgently required.

Objectives: to investigate possible differences between α-synuclein (ASYN) and melatonin levels in plasma of PD patients in I and II Hoehn and Yahr stages and healthy controls and to research possible correlations between (ASYN) and melatonin levels and non-motor symptoms (cognitive impairment and sleep disorders).

Materials and methods. We recruited 67 patients at I-II Hoehn and Yahr (H&Y) PD stages (25 patients at I H&Y PD stage and 42 patients at II H&Y PD stage, respectively) and 20 healthy controls. The Montreal Cognitive Assessment test (MoCA) was used to assess several cognitive domains in PD patients and healthy controls. Patients with MoCA scores of <26 were defined as PD-with mild cognitive impairment (PD-MCI). We used Night Sleep Assessment
Questionnaire (NSAQ) for sleep disorders evaluation and defined patients with NSAQ scores of 23-30 as PD-without sleep disorders, 18-22 as PD-with mild sleep disorders and 6-17 as PD-with severe sleep disorders (Vein A.M., 1998). Plasma for all participants was collected at a fixed time interval between 7:00 AM – 9:00 AM using a 10-ml K2-EDTA tubes (BD Vacutainer). Samples were centrifuged for 15 min at 1000×g at 2-8°C within 30 min of collection. Then 0.5 ml of supernatant plasma was removed from each tube and transferred into a 1.5 ml Eppendorf tubes. All the plasma samples were frozen at –80°C. Enzyme linked immunosorbent assay (ELISA) was performed afterwards.

Results. The level of plasma ASYN in PD patients was significantly higher than in healthy controls (p = 0.001) and it did not depend on PD H&Y stage (Table 1). But the level of melatonin did not differ significantly between PD patients and healthy controls (p = 0.139). The area under the ROC curve (AUC) of plasma ASYN (Figure 3) level to distinguish PD patients from healthy controls was 0.736 (95% CI = 0.630 – 0.825; cut-off value = 70.388 pg/mL). Plasma ASYN level was significantly higher in PD-MCI patients than in PD patients with normal cognition (p = 0.002) (Table 2), and plasma melatonin level was significantly higher in PD patients with severe sleep disturbances than in PD patients with mild sleep disturbances or without sleep disturbances at all (p = 0.002).

Conclusions. Our findings suggest that plasma ASYN level correlates with the disease stage progression and cognitive impairments in PD patients at I-II H&Y stages. Plasma melatonin level was significantly higher in PD patients with severe sleep disturbances only, but not in PD patients with mild sleep disturbances. Plasma ASYN level can differentiate between PD patients and healthy controls.

Keywords: Parkinson’s disease, early stages, α-synuclein, melatonin, cognitive impairment, sleep disorders

Introduction

Parkinson’s disease (PD) is a widespread, progressive neurodegenerative disorder, which has complex pathogenesis [4] The combination of its motor and non-motor symptoms [18] significantly impairs a patient’s life quality [10]. Since dopaminergic therapy has less effect as the disease progresses [14], the question of targeted patogenetic therapy remains relevant [9]. That is why biomarkers for early diagnosis and neuroprotective therapy are urgently required for this chronic disorder [14].

PD is defined as one of the synucleopathies [12] as α-synuclein (ASYN) plays a central role in the pathogenesis of PD [14]. It is known that pathological ASYN has been visualized in multiple peripheral tissues, detected in cerebrospinal fluid, plasma and saliva [1]. But as the diagnostic procedure should be easy in performing, safe for the patient and accurate for the right strategy, investigations of detecting plasma α-synuclein levels in PD patients are still rele-
There are many researches about correlations between plasma ASYN levels and the severity of PD, disease duration, cognitive impairment, etc. [2]. As melatonin reduces ASYN toxicity in PD patients [15], there are researches of its influence on the course of the disease as well [19]. That is why it is interesting to research plasma ASYN and melatonin levels and their relationships between such non-motor PD symptoms, as cognitive impairment and sleep disorders, especially in the early stages of the disease.

The aims of this study were (i) to investigate possible differences between ASYN and melatonin levels in plasma of PD patients in I and II Hoehn and Yahr stages and healthy controls and (ii) to research possible correlations between α-synuclein and melatonin levels and non-motor symptoms (cognitive impairment and sleep disorders).

**Matherials and methods**

**Subjects**

This study was conducted in Medical Educational and Scientific Center "University Clinic" (Zaporizhzhia State Medical University, Ukraine). We recruited 67 patients at I-II Hoehn and Yahr (H&Y) PD stages (25 patients at I H&Y PD stage and 42 patients at II H&Y PD stage, respectively) and 20 healthy controls. The exclusion criteria were: III-IV H&Y PD stages, dementia, other extrapyramidal disorders, secondary parkinsonism; inflammatory, autoimmune, oncological and mental diseases; decompensated diseases. We divided PD patients into several subgroups: 1) PD patients without cognitive impairment and PD patients with cognitive impairment, 2) PD patients without sleep disturbances, PD patients with mild sleep disturbances and patients with severe sleep disturbances. The study protocol was approved by ethics committee of Zaporizhzhia State Medical University. Written informed consent was provided by all study participants prior to enrollment in the study.

**Cognitive impairment and sleep disorders definition**

The Montreal Cognitive Assessment test (MoCA) was used to assess several cognitive domains in PD patients and healthy controls, as the MoCA explores frontal cognitive domains (i.e., attention, executive functions, and conceptual thinking) [3] and may be more suitable for identifying early cognitive deficits in PD [14]. In the present study we defined patients with MoCA scores of <26 as PD-with mild cognitive impairment (PD-MCI) [20]. We used Night Sleep Assessment Questionnaire (NSAQ) for sleep disorders evaluation and defined patients with NSAQ scores of 23-30 as PD-without sleep disorders, 18-22 as PD-with mild sleep disorders and 6-17 as PD-with severe sleep disorders [6].

**Blood Sampling and Assaying of Plasma Biomarkers: Plasma α-Synuclein, GPx and Melatonin**

Plasma for all participants was collected at a fixed time interval between 7:00 AM – 9:00 AM using a 10-ml K2-EDTA tubes (BD Vacutainer). Samples were centrifuged for 15 min at 1000×g at 2-8°C within 30 min of collection. Then 0.5 ml of supernatant plasma was removed from each tube and
transferred into a 1.5 ml Eppendorf tubes. All the plasma samples were frozen at – 80°C.

**Enzyme linked immunosorbent assay (ELISA)**

**ASYN ELISA.** We prepared all the reagents and added 100µL of standard working solution to each of well. The samples were incubated for 90 min at 37°C. Then we removed the liquid out of each well, immediately added 100µL of biotinylated detection Ab working solution to each well and incubated for 1 hour at 37°C. The solution was decanted from each well after incubation and microplate was washed 3 times. 100µL of Avidin-Horseradish Peroxidase conjugate were added to each well and the microplate was incubated for 30 min at 37°C. The process of the wash process was repeated for 5 times afterwards. Then we added 90µL of substrate reagent to each well and incubated 15 min at 37°C. After incubation 50µL of stop solution was added and we determined the optical density of each well at once, using a microplate reader set to 450 nm. At last the calculations of ASYN concentrations produced by the specific signal were performed.

**Melatonin ELISA.** Follows the same protocol as α-synuclein total ELISA, except at first we added 50µL of standard working solution to each well, then immediately added 50µL of biotinylated detection Ab and incubated for 45 min at 37°C.

**Statistical analyses**

Numerical variables were expressed as the mean ± standard deviation (SD) or median with 95% confidence interval (CI). The Shapiro-Wilk test was used as a test of normality. For variables following a normal distribution, data was compared using the independent samples t-test. For variables not following a normal distribution, data was compared using the non-parametric equivalent of the independent samples t-test – the Mann-Whitney test. A receiver operating characteristic (ROC) curve was used for differentiating between the PD patients and healthy controls via the levels of biomarkers. We performed all analyses using the «STATISTICA® for Windows 13.0» (No. JPZ8041382130ARCN10-J) and MedCalc Version 19.5.3 (free trial). A p-value of < 0.05 was considered significant.

**Results**

**Clinical characteristics**

The mean age of PD patients and healthy controls was 64.35±1.22 and 66.40±0.70, respectively (p = 0.14). The ratio of women in the healthy controls (15/20) was similar to PD patients (52/67) (p = 0.86). There were 18 PD patients with normal cognition (defined as MoCA ≥ 26) and 49 PD patients with mild cognitive impairment (defined as MoCA < 26) (*Table 1*). Also 18 PD patients did not have sleep disorders, 24 patients were defined as PD patients with mild sleep disorders and 25 PD patients experienced severe sleep disorders (*Table 2*).
Clinical and diagnostical values of plasma α-synuclein and melatonin levels … 55

The level of plasma ASYN in PD patients was significantly higher than in healthy controls (p = 0.001) (Table 1, Figure 1) and it did not depend on PD H&Y stage (Table 1). But the level of melatonin did not differ significantly between PD patients and healthy controls (p = 0.139) (Table 1) (Figure 2).

Table 1. Plasma ASYN and melatonin levels in PD patients at I-II stages and in healthy controls

<table>
<thead>
<tr>
<th>Groups</th>
<th>ASYN, pg/mL</th>
<th>Melatonin, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD (n = 67)</td>
<td>146,90 (98,75 – 261,21)</td>
<td>100,37 (73,52 – 116,84)</td>
</tr>
<tr>
<td>Healthy controls (n = 20)</td>
<td>78,83 (53,34 – 118,99)</td>
<td>106,26 (95,44 – 122,26)</td>
</tr>
<tr>
<td>p</td>
<td>0,001</td>
<td>0,139</td>
</tr>
<tr>
<td>PD, H&amp;Y stages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I (n = 25)</td>
<td>118,00 (88,40 – 247,62)</td>
<td>93,87 (77,25 – 115,84)</td>
</tr>
<tr>
<td>II (n = 42)</td>
<td>177,82 (108,34 – 261,21)</td>
<td>102,47 (66,72 – 116,84)</td>
</tr>
<tr>
<td>p</td>
<td>0,315</td>
<td>0,984</td>
</tr>
</tbody>
</table>

p - Mann-Whitney U-test

The area under the ROC curve (AUC) of plasma ASYN (Figure 3) level to distinguish PD patients from healthy controls was 0.736 (95% CI = 0.630 – 0.825; cut-off value = 70.388 pg/mL). (Figure 3)
Figure 3. ROC curve of ASYN diagnostic value in I-II stages of PD

We found that plasma ASYN level was significantly higher in PD-MCI patients than in PD patients with normal cognition (p = 0.002) (Table 2), and plasma melatonin level was significantly higher in PD patients with severe sleep disturbances than in PD patients with mild sleep disturbances or without sleep disturbances at all (p = 0.002) (Table 3).

Table 2. Plasma ASYN level in PD patients with normal cognition and MCI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ASYN, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-with normal cognition (n = 18)</td>
<td>100.31</td>
</tr>
<tr>
<td></td>
<td>(74.90 – 244.01)</td>
</tr>
<tr>
<td>PD-MCI (n = 49)</td>
<td>184.94</td>
</tr>
<tr>
<td></td>
<td>(116.65 – 261.60)</td>
</tr>
<tr>
<td>p</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Clinical and diagnostical values of plasma α-synuclein and melatonin levels … 57

Table 3. Plasma melatonin level in PD patients with different severity of sleep disorders.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Melatonin, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-without sleep disturbances (n = 18)</td>
<td>124,98 (77,25 – 159,78)</td>
</tr>
<tr>
<td>PD with sleep disturbances (n = 49)</td>
<td>93,87 (66,72 – 109,00)</td>
</tr>
<tr>
<td>PD-with mild sleep disturbances (n = 24)</td>
<td>100,93 (71,59 – 113,13)</td>
</tr>
<tr>
<td>PD with severe sleep disturbances (n = 25)</td>
<td>92,13 (66,72 – 104,28)</td>
</tr>
</tbody>
</table>

p, p^1-2, p^2-3, p^1-3 – Mann-Whitney U-test

Discussion

The search of reliable PD biomarkers has still been continuing. We demonstrated that plasma ASYN levels were higher in PD patients in early stages of the disease than in healthy controls using ELISA. According to AUC data, we can consider acceptable discrimination between plasma ASYN levels in PD patients and healthy controls. We succeed to find strong relationships between levels of plasma ASYN and the severity of cognitive impairment in PD patients. And we also revealed significant difference between the concentration of melatonin level in PD patients with severe sleep disturbances and in PD patients without them.

Many of promising PD biomarkers are being studied nowadays in the context of possible correlations between their levels in human plasma and the severity of non-motor PD symptoms. But in general the results of such researches are quite controversial. For example, according to the meta-analysis, performed by Anastasia Bougea et al. (2019), plasma ASYN levels in patients with PD were significantly higher compared to control groups, but researches did not found any significant association between the ASYN levels and disease duration, disease severity, and quality of studies [2]. On the other hand, Nai-Ching Chen et al. (2020) found out that plasma levels of ASYN were significantly correlated with cognitive impairment, according to Mini Mental State Examination (MMSE) scores [17], in PD patients. In Chin-Hsien Lin et al. research plasma ASYN level correlated with cognitive decline (according to MMSE) but not motor severity in patients with PD [7]. Chun-Wei Chang et al. researched that serum, but not plasma ASYN level showed a significant correlation with patients in H&Y stages...
According to the results of study by Uysal HA (2018) et al., measurement of melatonin alone for the diagnosis of sleep disorders in PD patients was not sufficient [11]. But Linyi L. et al. (2020) found that the plasma melatonin levels in PD patients were significantly higher than those in healthy controls. Moreover, there was a correlation between plasma melatonin levels and sleep quality in patients with PD [13].

**Conclusion**

Our findings suggest that plasma ASYN level correlates with the disease stage progression and cognitive impairments in PD patients at I-II H&Y stages. Plasma melatonin level was significantly higher in PD patients with severe sleep disturbances only, but not in PD patients with mild sleep disturbances. Plasma ASYN level can differentiate between PD patients and healthy controls.

**References**


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