

## A modern view on potential biomarkers of Parkinson's disease (review)

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Parkinson's disease (PD) is one of the most widespread neurodegenerative diseases. In spite of the large number of researches, the problem of earlier diagnosis and targeted pathogenetic therapy remains relevant. For more than 20 years, scientists have been continuing to study potential diagnostic and prognostic PD biomarkers. An accurate diagnostic biomarker can help identify PD before motor symptoms occur, or when motor and non-motor symptoms are insufficient to diagnose, and also can be used to differentiate between idiopathic PD and other forms of parkinsonism.

**The aim** of the research is to analyze the last studies of potential PD biomarkers in human biological fluids.

**Conclusions.** Most studies of recent years indicate that the level of total  $\alpha$ -synuclein, its oligomers in blood plasma and its formed elements is elevated in patients at the early stages of PD, and it can be a valuable prognostic biomarker for disease progression, in particular its motor symptoms. Studies of the level of this potential biomarker not only in blood plasma and its formed elements, but also in neuronal exosomes, are promising. The negative impact of oxidative stress in PD is a significant trigger for irreversible pathogenetic processes that affect the development of neurodegenerative changes. Perspectives of further researches may lay not only in identifying the concentrations of nitrotyrosine and oxidative stress components and antioxidants in the blood of PD patients, but also in determining of the effect of antiparkinsonian and neuroprotective drugs on the antioxidant system in order to pathogenetically justify their use for reducing of oxidative stress. It is promising to study the activity of melatonin in the context of its relationship with the components of oxidative stress and antioxidants by determining their concentrations in blood of PD patients.

### Key words:

Parkinson disease, biomarkers, synuclein, glutathione, glutathione peroxidase, melatonin, nitrotyrosine.

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## Сучасний погляд на потенційні біомаркери хвороби Паркінсона (огляд)

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Хвороба Паркінсона (ХП) – одне з найпоширеніших нейродегенеративних захворювань. Незважаючи на велику кількість досліджень, що присвячені ХП, проблема ранньої діагностики та таргетованої патогенетичної терапії залишається актуальною. Науковці вже понад 20 останніх років досліджують потенційні діагностичні та прогностичні біомаркери ХП на різних стадіях захворювання. Точний діагностичний біомаркер може допомогти ідентифікувати ХП до виникнення моторних симптомів або коли моторних і немоторних симптомів недостатньо для встановлення діагнозу, а також його можна використовувати для диференційної діагностики між ХП та іншими неврологічними захворюваннями, зокрема під час диференціювання між ідіопатичною ХП та іншими формами паркінсонізму.

**Мета роботи** – проаналізувати останні дослідження потенційних біомаркерів ХП у біологічних рідинах людини.

**Висновки.** У більшості проаналізованих досліджень останніх років визначено, що рівень загального  $\alpha$ -синуклеїну, його олігомерів у плазмі крові та її формених елементах є підвищеним у пацієнтів на ранніх стадіях ХП, а також є цінним прогностичним маркером щодо прогресування захворювання, зокрема його моторних симптомів. Перспективними є дослідження рівня потенційного біомаркера не тільки у плазмі крові та її формених елементах, але й у нейрональних екзосомах. Негативний вплив оксидативного стресу при ХП – суттєвий тригер незворотних патогенетичних процесів, які впливають на розвиток нейродегенеративних змін. Перспективними можуть бути не тільки дослідження з виявлення концентрацій компонентів нітрозативного та оксидативного стресу, антиоксидантів у крові пацієнтів з ХП, але й з визначення впливу протипаркінсонічних і нейропротективних препаратів на стан антиоксидантної системи для патогенетичного обґрунтування їх використання для нівелювання проявів оксидативного стресу. Перспективним є дослідження активності мелатоніну в контексті його зв'язків із компонентами оксидативного стресу та антиоксидантами шляхом визначення їхніх концентрацій у крові пацієнтів з ХП.

### Ключові слова:

хвороба Паркінсона, біомаркери, синуклеїн, глутатіон, глутатіон-пероксидаза, мелатонін, нітротирозин.

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## Современный взгляд на потенциальные биомаркеры болезни Паркинсона (обзор)

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Болезнь Паркинсона (БП) – одно из наиболее распространённых нейродегенеративных заболеваний. Несмотря на большое количество исследований, посвящённых БП, проблема ранней диагностики и таргетированной патогенетической терапии остаётся актуальной. Ученые более 20 последних лет исследуют потенциальные диагностические и прогностические биомаркеры БП на разных стадиях заболевания. Точный диагностический биомаркер может помочь в идентификации БП до появления моторных симптомов или когда моторных и немоторных симптомов недостаточно для установления диагноза, а также может быть использован для дифференциальной диагностики между БП и другими неврологическими заболеваниями, в частности при дифференцировании между идиопатической БП и другими формами паркинсонизма.

**Ключевые слова:**  
болезнь

Паркинсона,  
биомаркеры,  
синуклеин,  
глутатион,  
глутатион-  
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**Цель работы** – проанализировать последние исследования потенциальных биомаркеров БП в биологических жидкостях человека.

**Выводы.** В большинстве проанализированных исследований последних лет указано, что уровень общего  $\alpha$ -синуклеина, его олигомеров в плазме крови и ее форменных элементах повышен у пациентов на ранних стадиях БП, а также может быть ценным прогностическим маркером прогрессирования заболевания, в частности, его моторных симптомов. Перспективными являются исследования уровня потенциального биомаркера не только в плазме крови и ее форменных элементах, а и в нейрональных экзосомах. Отрицательное влияние оксидативного стресса при БП – существенный триггер необратимых патогенетических процессов, которые влияют на развитие нейродегенеративных изменений. Перспективными могут быть не только исследования по установлению концентраций компонентов нитрозативного и оксидативного стресса и антиоксидантов в крови пациентов с БП, но и по определению влияния противопаркинсонических и нейропротективных препаратов на состояние антиоксидантной системы с целью патогенетического обоснования их использования для нивелирования проявлений оксидативного стресса. Перспективным является исследование активности мелатонина в контексте его взаимоотношений с компонентами оксидативного стресса и антиоксидантами путем определения их концентраций в крови пациентов с БП.

Parkinson's disease (PD) is one of the most widespread neurodegenerative diseases [1] with no cure in sight [2]. In more than 200 years since the PD was officially recognized worldwide, the scientific community has learned quite a bit about the peculiarities of the pathogenesis, the course of the disease and developed international guidelines and recommendations for the management of patients at different PD stages [3]. Clinical manifestations of PD are caused by the death of dopaminergic neurons of the cerebral substantia nigra, degeneration of the nigrostriatal, mesolimbic and mesocortical pathways, decreased dopamine concentration, as well as imbalance of brain neurotransmitter systems (cholinergic, noradrenergic) [4]. It is known that this disease is characterized by a combination of motor (rest tremor, rigidity, hypokinesia) [5] and non-motor symptoms in the form of cognitive and psycho-emotional disorders (increased anxiety, apathy, depression of varying severity, etc.) [6,7], which can significantly impair a patient's quality of life. Such important non-motor symptoms of PD as hyposmia and sleep disorders may occur before motor symptoms [8], and their negative impact on patient's life quality is often underestimated [9]. But still the question of earlier diagnosis of PD [4], as well as targeted pathogenetic therapy, remains relevant [10].

To this end, for more than 20 years, scientists have been continuing to study potential diagnostic and prognostic PD biomarkers at different stages of the disease [11]. An accurate diagnostic biomarker can help identify PD before motor symptoms occur, or when motor and non-motor symptoms are insufficient to diagnose, and can be used to differentiate between idiopathic PD and other forms of parkinsonism [2]. Numerous studies of various potential PD biomarkers are ongoing, but only a few are important for clinical practice [12]. Studies of biomarkers in human biological fluids (blood plasma, its formed elements, cerebrospinal fluids, etc.) are quite common [2], as for rational implementation in clinical practice, detection of biomarkers requires maximum convenience and safety for the patient.

## Aim

The aim of the research is to analyze the last studies of potential PD biomarkers in human biological fluids.

It is known, that PD is a type of  $\alpha$ -synucleinopathies [13], as  $\alpha$ -synuclein is a central component of the patho-

genesis of this disease [14]. The level of  $\alpha$ -synuclein is studied in autopsy materials [15], cerebrospinal fluid and blood [16]. The most technically simple and safe lifetime method of detecting the concentration of potential PD biomarkers is their measuring in blood.

Over the past three years the global research database has been replenished with new data that have a common tendency to recognize  $\alpha$ -synuclein and its oligomers in blood plasma and its formed elements as one of the best potential PD biomarkers, even in the early stages of the disease. Thus, in 2017, Vicente Miranda H. et al. decided to determine whether posttranslational modifications of  $\alpha$ -synuclein in blood can be valuable biomarkers of PD. The study involved 58 PD patients and 30 healthy participants (groups were comparable in age). The authors found that the levels of Y125-phosphorylated, Y39-nitrated and glycosylated  $\alpha$ -synuclein were elevated in patients with PD [17]. Jian Ding and co-authors studied the plasma levels of  $\alpha$ -synuclein, T-tau, P-tau181 and A $\beta$ -42-proteins in 73 PD patients, and association of these levels with motor manifestations. Plasma  $\alpha$ -synuclein levels were significantly higher in PD patients compared to control group, and were also higher in the group of patients with postural instability and gait disturbance compared to the tremor-dominant subtype group [18]. Chin-Hsien Lin et al. studied plasma  $\alpha$ -synuclein levels in 80 PD patients and 34 healthy controls. There was a significant increase in  $\alpha$ -synuclein levels in PD patients compared with controls and in  $\alpha$ -synuclein levels in PD patients with a higher Hoehn–Yahr stage without any correlation with motor symptoms severity (according to Unified Parkinson's Disease Rating Scale (UPDRS) part III scores). However, plasma  $\alpha$ -synuclein levels were significantly higher in PD patients with concomitant dementia than in patients with mild cognitive impairment or normal cognition and were negatively correlated with Mini-Mental State Examination (MMSE) scores [19]. But there were other investigations with less optimistic results. For example, Nor A. Samat et al. did not reveal a significant difference between the plasma  $\alpha$ -synuclein levels of PD patients and cognitive impairment in groups distributed according to Montreal Cognitive Assessment test (MoCA) and Parkinson's Disease Cognitive Rating Scale (PDCRS) results [20].

In the same year a team of scientists led by S. Pchelina aimed to investigate glucocerebrosidase 1 (GBA) enzymatic activity in dried blood spots with assessing

plasma oligomeric  $\alpha$ -synuclein levels in sporadic PD (sPD) patients, in PD patients with mutations in the GBA gene (GBA-PD) and in controls. They found that plasma oligomeric  $\alpha$ -synuclein levels were increased in GBA-PD group compared to sPD and controls. Authors suggested that the decrease in enzymatic activity of lysosomal hydrolases in GBA mutation carriers may contribute to PD pathogenesis by increasing the level of neurotoxic oligomeric  $\alpha$ -synuclein species [21]. Matsumoto J. et al. paid attention that erythrocytes contain  $\alpha$ -synuclein concentrations ~1000-fold higher than the cerebrospinal fluid, as a source of potentially pathogenic  $\alpha$ -synuclein. Also erythrocytes produce  $\alpha$ -synuclein-rich extracellular vesicles (EVs), which can cross the blood-brain barrier (BBB), especially under inflammatory conditions provoked by peripheral administration of lipopolysaccharide. According to the authors this transport likely occurs via adsorptive-mediated transcytosis, with EVs that transit the BBB co-localizing with brain microglia. Examination of microglial reactivity upon exposure to  $\alpha$ -synuclein-containing erythrocyte EVs in vitro and in vivo revealed that uptake provoked an increase in microglial inflammatory responses, and EVs derived from the erythrocytes of PD patients elicited stronger responses than did those of control subjects [22].

In 2018 Hua Wang et al. published the results of a study, in which they investigated the levels of plasma  $\alpha$ -synuclein and central nervous system (CNS)  $\alpha$ -synuclein in 256 PD patients in the early stages in baseline and 2-year follow-up. The results demonstrated that baseline and longitudinal increase in total  $\alpha$ -synuclein predicted progression of cognitive decline in hyposmic PD patients with dopamine transporter (DAT) binding reduction. On the other hand, a longitudinal decrease in nervous system derived exosomal (NDE)  $\alpha$ -synuclein predicted worsening cognitive scores in hyposmic PD patients with DAT binding reduction. Finally, in PD patients with faster DAT progression, decreasing NDE/total  $\alpha$ -synuclein ratio was associated with a larger reduction in DAT from baseline to follow-up. Authors suggest that, though underlying mechanisms remain to be defined, alterations in plasma total and NDE  $\alpha$ -synuclein concentrations are likely associated with PD progression, especially in the aspect of cognitive impairment, in early stages of the disease [23].

The correlation between  $\alpha$ -synuclein and GBA genes and their mutations, associated with a higher risk of PD developing, has also been investigated. Nikolaos Papagiannakis and co-authors took erythrocyte membranes from 36 PD patients with mutation carriers in the  $\alpha$ -synuclein gene (A53T-PD) and GBA gene (GBA-PD), and patients without known mutations ( $n = 56$ ), and age-/sex-matched controls ( $n = 56$ ). A statistically significant increase of  $\alpha$ -synuclein dimer and dimer to monomer ratio was found in GBA-PD and in patients without these mutations. In contrast, dimer levels of A53T-PD were not different from controls. No difference was found in  $\alpha$ -synuclein monomer levels as well [24].

Simona Daniele et al. found the correlation between erythrocyte  $\alpha$ -synuclein levels in PD patients ( $n = 28$ ) compared with control group ( $n = 45$ ) and the severity of the disease and motor deficiency [25]. Amrendra Pratap Singh with co-authors studied the levels of  $\alpha$ -synuclein

in 38 PD patients and 33 healthy controls and found a significant increase in plasma  $\alpha$ -synuclein concentration in PD patients [26].

In contrast to previous studies, Michalina Malec-Litwinowicz and co-authors did not find a significant statistical difference between plasma  $\alpha$ -synuclein levels in 58 PD patients and 38 controls. They found an inverse correlation between plasma  $\alpha$ -synuclein levels in PD patients, who had postural instability and gait disturbances, and the Hoehn–Yahr stage [27]. And Jennifer G. Goldman et al. did not find a significant difference between plasma  $\alpha$ -synuclein levels in PD patients and healthy controls [28].

Instead, A. Emelyanov et al. studied the blood  $\alpha$ -synuclein levels in 458 PD patients and 353 controls. Elevated levels of  $\alpha$ -synuclein single nucleotide polymorphisms and total  $\alpha$ -synuclein were found in the homogenous cell fraction of CD45+ blood cells in PD patients compared to control subjects [29].

Later, in 2019 Xiaoli Si determined, that CNS-produced exosomal  $\alpha$ -synuclein in blood plasma can be regarded as a biomarker for the differential diagnosis between essential tremor and early stages of PD [30]. Using newly developed electrochemiluminescence assays, Chen Tian et al. found that the level of total and aggregated  $\alpha$ -synuclein were higher in the erythrocytes membrane fractions of PD patients than in healthy controls [31]. Adeline S. L. Ng. with co-authors detected plasma  $\alpha$ -synuclein levels in 170 PD patients and 51 controls by single molecule array technology. Plasma  $\alpha$ -synuclein levels in PD patients were significantly higher than in healthy controls. In PD,  $\alpha$ -synuclein levels did not vary by Hoehn–Yahr stages of the disease or UPDRS motor scores, but were significantly higher in PD patients with poorer cognition than controls [32]. According to the large meta-analysis, performed by Anastasia Bougea et al., plasma  $\alpha$ -synuclein levels in patients with PD were also significantly higher compared to control groups [33].

Researchers, whose studies were published in 2020, also observed the relationship between plasma  $\alpha$ -synuclein levels in PD patients and severity of motor symptoms. Chun-Wei Chang et al. revealed that the serum  $\alpha$ -synuclein level showed a significant correlation with patients in Hoehn–Yahr stages 1–3, implying that the serum  $\alpha$ -synuclein level may be a potential marker of motor symptom severity in patients with early PD [34]. Zheng Fan with co-authors also found the correlation between elevated plasma total  $\alpha$ -synuclein level in PD patients and the severity of their motor symptoms [35]. However, there is now a tendency for scientists to study the  $\alpha$ -synuclein level not just in blood plasma, but in its neuronal exosomes. Past studies showed that  $\alpha$ -synuclein and other proteins can be transported to the blood through exosomes [36]. Therefore, M. Niu team of investigators included in their study 36 PD patients in the early stages of the disease, 17 patients with advanced PD, 20 patients with idiopathic rapid eye movement sleep behavior disorder and 21 healthy controls. 18 of 36 patients in the early stages of PD were examined twice: at the beginning of the study and after another 22 months. As a result, a significant increase of the  $\alpha$ -synuclein levels in plasma neuronal exosomes of PD patients in the early stages compared with healthy controls was found. In addition,

Spearman's correlation analysis revealed that neuronal exosomal  $\alpha$ -synuclein concentrations were correlated with Movement Disorders Society UPDRS III/(I + II + III) scores, Non-Motor Symptom Questionnaire scores and Sniffin' Sticks 16-item test scores of patients with PD. After a mean follow-up of 22 months in patients with early-stage PD, a Cox regression analysis adjusted for age and gender showed that longitudinally increased  $\alpha$ -synuclein rather than baseline  $\alpha$ -synuclein levels were associated with higher risk for motor symptom progression in PD [37].

And in April 2020 Jiang C. et al. published the results of their study, according to which increased levels of neuronal exosomal  $\alpha$ -synuclein predicted the development of PD, persisted with the disease progression and helped to differentiate PD from other forms of atypical parkinsonism [38].

The development of oxidative and nitrosative stress also plays a leading role in the pathogenesis of neurodegenerative diseases. It is known that increased levels of free radicals, oxidized proteins and lipids and decreased activity of superoxide dismutase, catalase, glutathione, etc. make dopaminergic neurons more susceptible to oxidative stress [39,40]. Oxidative stress reflects an imbalance between the hyperproduction of free radicals and dynamic ability to detoxify their excess [41]. Oxidative stress is known to be associated with the etiology of many neurodegenerative diseases, including PD [42], and PD is associated with a decrease in nigral glutathione [43]. Although the exact mechanisms of neurodegeneration and persistent pathological changes remain unknown, the critical role of oxidative stress in pathogenesis of neurodegenerative diseases is associated with several proteins, including the central  $\alpha$ -synuclein for PD [41]. Deas et al. (2016) suggested that the interaction between  $\alpha$ -synuclein oligomers and metal ions could induce oxidative stress in rats with modeled PD. Neurons with  $\alpha$ -synuclein oligomers had higher levels of oxidative stress in the form of decreased glutathione levels and increased lipid peroxidation [44]. The state of the antioxidant system and the activity of the oxidative stress components and their detection in blood of PD patients is still being studied. Thus, according to C. Vida et al. (2019), high levels of oxidative stress and damage were observed in all blood cells of PD patients in the form of reduced glutathione peroxidase (GPx) activity and increased content of oxidized glutathione and malondialdehyde contents. Authors suggested that an accelerated immunosenescence in PD stage 2, and that several of the parameters studied could be appropriate peripheral biomarkers in the early stages of PD [45]. The GPx activity in PD patients was significantly higher than in healthy controls in Gökçe Çokal B. et al. study [46]. However, in 2018 Zexu Wei et al. did not find a significant difference between the GPx level in blood of PD patients and control group individuals [47]. Also, the study by Yongsheng Yuan et al., which was published in 2016, found a simultaneous decrease in both glutathione and GPx plasma levels in PD patients [48]. A study by Laurie K. Mischley (2016) found the association between low glutathione concentrations and stronger PD severity according to the MDS UPDRS. The authors suggest that blood glutathione may be used as PD biomarker, but note that future studies should determine whether this

is a modified risk factor for PD progression and whether glutathione administration can improve the condition of PD patients [49]. Therefore, these results are quite controversial. But there are interesting results of novel studies, in which the effects of certain anti-Parkinson disease medications on the glutathione level were investigated. Thus, levodopa (but not carbidopa) [50] and intranasally administrated selegiline increased the concentration of this antioxidant in blood of PD patients [51]. Also, there is a noteworthy study by Laurie K. Mischley et al. (2015), in which the safety and tolerability of intranasal glutathione in PD patients was determined [52].

Oxidative stress occurs in several stages, the most important of which is the overproduction of reactive oxygen species (ROS) [53]. In addition to ROS, reactive forms of nitrogen also take part in free radical processes. It is known that nitric oxide plays a significant role as a retrograde neurotransmitter in synapses and can perform posttranslational modifications in proteins, providing physiological mechanism for regulation their functions [54]. However, with age and in pathological processes, nitric oxide forms peroxynitrite, while reacting with the superoxide anion. In this case, peroxynitrite reacts mainly with the phenolic ring of tyrosine and forms nitrotyrosine, which greatly impairs the course of physiological processes in proteins. Under nitrosative stress conditions peroxynitrite is able to oxidize protein SH-groups and form 3-nitrotyrosine [55]. With a decrease of the antioxidant system functional activity, there is an increased ROS formation, both in cell and outside. The neuron's sufficiently active thiol antioxidant system, which is able to regulate the NO transport, provides cell resistance to nitrosative stress, as one of the earliest mechanisms of neuronal destruction. Protein nitrotyrosylation is an irreversible process that leads to the accumulation of modified proteins, contributing to the onset and progression of neurodegeneration, particularly in Alzheimer's disease [54]. Nitration due to tyrosine oxidation induces  $\alpha$ -synuclein aggregation, which inhibits its ability to connect with neurotransmitter vesicles [56]. Moreover, PTEN-induced kinase (PINK1), a mitochondrial serine/threonine-protein kinase that activates parkin, is nitrated in pro-oxidant environments [57], which provides harmful effects of parkin S-nitrosylation.

Past decades have been marked by researches about detection of nitrotyrosine concentrations in PD patients [54]. However, there is no information on studies of nitrotyrosine levels in PD patients in open publications in recent years. Instead, studies of the activity of this marker in laboratory animals, as well as determining the effects of certain substances on it, are ongoing. In particular, the protective properties of recombinant adeno-associated-virus (rAAV-Cbs) were revealed, as it reduced the aberrant accumulation of nitric oxide and 3-nitrotyrosine (an indicator of protein nitration) in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced PD in mice models [58]. Daily oral administration of dimethyl fumarate (10 mg/kg, 30 mg/kg, and 100 mg/kg) to PD mice models helped to reduce the expression of nitrotyrosine neuronal nitrite oxide synthase and also reduced the number of  $\alpha$ -synuclein-positive neurons and regulated glutathione levels [59]. There is an interesting study by M. Bandoakwala et al. (2019), which evaluated



the neuroprotective effect of edavaron – a powerful antioxidant – in combination with caffeine – an effective inhibitor of adenosine-A2-receptors, also known as antioxidant, on the course of PD in mice models and on the activity of oxidative stress markers. The authors found a decrease in level of 3-nitrotyrosine in the striatum, as well as the marker of lipid peroxidation – malonaldehyde – in the striatum, cerebrospinal fluid and urine of animals [60].

The development of preventive mechanisms against the progression of neurodegenerative diseases remains one of the main tasks of the scientific community. Melatonin has both chronobiotic and cytoprotective features. As a chronobiotic, melatonin can modify the phases and amplitudes of biological rhythms. As a cytoprotector, melatonin eliminates the effects of inflammation in neurodegenerative diseases in old patients during aging. In experimental models of PD and Alzheimer's disease, melatonin slowed the process of neurodegeneration. Melatonin also eliminated toxic proteins through the glymphatic system of the brain [61]. It is known, in particular, that melatonin reduces  $\alpha$ -synuclein toxicity in PD patients [62]. Therefore, determination of its level in PD patients, as well as the impact of changes in its concentrations on the course of the disease does not lose its relevance. As melatonin has detoxifying properties, it is interesting to study the relationship between its level and the state of the antioxidant system. Thus, Hui Jun Wei et al. (2019) revealed the increase of serum melatonin level and decrease of glutathione level in PD patients, the correlation of both melatonin and glutathione with PD severity and higher melatonin level in PD patients with sleep disorders, while PD patients with cognitive impairment tended to have lower glutathione level [63]. But this result is somewhat contradicted with a previous study by Uysal HA (2018) et al. Measurement of melatonin alone for the diagnosis of sleep disorders in PD patients was insufficient, as no significant difference in its level was found in patients who were diagnosed with such disorders using specific scales, and in those who did not have such disorders [64]. In the context of the relationship between melatonin and the components of oxidative stress, the article by G. G. Ortiz et al. (2017) is interesting. Considering the activity of GPx, as a known oxidative stress marker, and the detoxifying properties of melatonin, the researches tried to reduce the concentration of GPx in PD patients by prescribing 25 mg of melatonin every 12 hours for 12 months. Unfortunately, this method did not significantly affect the level of GPx in PD patients' blood compared to the placebo-group [65].

## Conclusions

Thus, most studies of recent years indicate that the level of total  $\alpha$ -synuclein, its oligomers in blood plasma and its formed elements is elevated in patients at the early stages of PD, and it can be a valuable prognostic biomarker for disease progression, in particular its motor symptoms. However, the conclusions about the relationship between protein levels and cognitive impairment and the development of psycho-emotional disorders are still quite contradictory, and therefore require further researchers. Studies of the level of a potential biomarker not only in blood plasma and its formed elements, but also in

neuronal exosomes, are promising. The negative impact of oxidative stress in PD is a significant trigger for irreversible pathogenetic processes that affect the development of neurodegenerative changes. Perspectives of further researches may lay not only in identifying the concentrations of oxidative stress components and antioxidants in the blood of PD patients, but also in determining of the effect of antiparkinsonian and neuroprotective drugs on the antioxidant system in order to pathogenetically justify their use for reducing of oxidative stress.

Considering the powerful negative effect of nitrotyrosine on the formation of pathological  $\alpha$ -synuclein – the key protein in the pathogenesis of the PD development – it is reasonable to make further studies of its changes in PD patients. There are interesting studies of the effect of various antioxidants and potential neuroprotective drugs on the content of nitrotyrosine in animal models of PD, which may have further prospects for more detailed clinical study.

It is known that melatonin is a powerful phase modifier of human biological rhythms, and a cytoprotector with powerful antitoxic properties. Nevertheless, the recognition of melatonin alone as a marker of sleep disturbance in PD patients is controversial. However, it is promising to study the activity of melatonin in the context of its relationship with the components of oxidative stress and antioxidants by determining their concentrations in the blood of PD patients.

Thus, a thorough investigation of modern scientific researches on the potential PD biomarkers justifies the feasibility of their further detection in patients at the early stages of this disease for the earlier diagnosis of PD and its targeted therapy.

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**References**

- [1] Jagmag, S. A., Tripathi, N., Shukla, S. D., Maiti, S., & Khurana, S. (2016). Evaluation of Models of Parkinson's Disease. *Frontiers in neuroscience*, 9, 503. <https://doi.org/10.3389/fnins.2015.00503>
- [2] Li, T., & Le, W. (2020). Biomarkers for Parkinson's Disease: How Good Are They?. *Neuroscience bulletin*, 36(2), 183-194. <https://doi.org/10.1007/s12264-019-00433-1>
- [3] Li, S., & Le, W. (2017). Milestones of Parkinson's Disease Research: 200 Years of History and Beyond. *Neuroscience bulletin*, 33(5), 598-602. <https://doi.org/10.1007/s12264-017-0178-2>
- [4] Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *Lancet*, 386(9996), 896-912. [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3)
- [5] Holden, S. K., Finseth, T., Sillau, S. H., & Berman, B. D. (2018). Progression of MDS-UPDRS Scores Over Five Years in De Novo Parkinson Disease from the Parkinson's Progression Markers Initiative Cohort. *Movement disorders clinical practice*, 5(1), 47-53. <https://doi.org/10.1002/mdc3.12553>
- [6] Schapira, A., Chaudhuri, K. R., & Jenner, P. (2017). Non-motor features of Parkinson disease. *Nature reviews. Neuroscience*, 18(7), 435-450. <https://doi.org/10.1038/nrn.2017.62>
- [7] Pfeiffer, R. F. (2016). Non-motor symptoms in Parkinson's disease. *Parkinsonism & related disorders*, 22 Suppl 1, S119-S122. <https://doi.org/10.1016/j.parkrel.2015.09.004>
- [8] Sui, X., Zhou, C., Li, J., Chen, L., Yang, X., & Li, F. (2019). Hyposmia as a Predictive Marker of Parkinson's Disease: A Systematic Review and Meta-Analysis. *BioMed research international*, 2019, 3753786. <https://doi.org/10.1155/2019/3753786>
- [9] Albers, J. A., Chand, P., & Anch, A. M. (2017). Multifactorial sleep disturbance in Parkinson's disease. *Sleep medicine*, 35, 41-48. <https://doi.org/10.1016/j.sleep.2017.03.026>
- [10] Savitt, D., & Jankovic, J. (2019). Targeting  $\alpha$ -Synuclein in Parkinson's Disease: Progress Towards the Development of Disease-Modifying Therapeutics. *Drugs*, 79(8), 797-810. <https://doi.org/10.1007/s40265-019-01104-1>
- [11] Shao, Y. P., & Le, W. D. (2019). Recent advances and perspectives of metabolomics-based investigations in Parkinson's disease. *Molecular Neurodegeneration*, 14, Article 3. <https://doi.org/10.1186/s13024-018-0304-2>
- [12] Chen-Plotkin, A. S., Albin, R., Alcalay, R., Babcock, D., Bajaj, V., Bowman, D., Buko, A., Cedarbaum, J., Chelsky, D., Cookson, M. R., Dawson, T. M., Dewey, R., Foroud, T., Frasier, M., German, D., Gwinn, K., Huang, X., Kopil, C., Kremer, T., Lasch, S., ... Zhang, J. (2018). Finding useful biomarkers for Parkinson's disease. *Science translational medicine*, 10(454), eaam6003. <https://doi.org/10.1126/scitranslmed.aam6003>
- [13] Alafuzoff, I., & Hartikainen, P. (2017). Alpha-synucleinopathies. *Handbook of clinical neurology*, 145, 339-353. <https://doi.org/10.1016/B978-0-12-802395-2.00024-9>
- [14] Rocha, E. M., De Miranda, B., & Sanders, L. H. (2018). Alpha-synuclein: Pathology, mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Neurobiology of disease*, 109(Pt B), 249-257. <https://doi.org/10.1016/j.nbd.2017.04.004>
- [15] Liddle, R. A. (2018). Parkinson's disease from the gut. *Brain research*, 1693(Pt B), 201-206. <https://doi.org/10.1016/j.brainres.2018.01.010>
- [16] Parnetti, L., Gaetani, L., Eusebi, P., Paciotti, S., Hansson, O., El-Agnaf, O., Mollenhauer, B., Blennow, K., & Calabresi, P. (2019). CSF and blood biomarkers for Parkinson's disease. *The Lancet. Neurology*, 18(6), 573-586. [https://doi.org/10.1016/S1474-4422\(19\)30024-9](https://doi.org/10.1016/S1474-4422(19)30024-9)
- [17] Vicente Miranda, H., Cássio, R., Correia-Guedes, L., Gomes, M. A., Chegão, A., Miranda, E., Soares, T., Coelho, M., Rosa, M. M., Ferreira, J. J., & Outeiro, T. F. (2017). Posttranslational modifications of blood-derived alpha-synuclein as biochemical markers for Parkinson's disease. *Scientific reports*, 7(1), 13713. <https://doi.org/10.1038/s41598-017-14175-5>
- [18] Ding, J., Zhang, J., Wang, X., Zhang, L., Jiang, S., Yuan, Y., Li, J., Zhu, L., & Zhang, K. (2017). Relationship between the plasma levels of neurodegenerative proteins and motor subtypes of Parkinson's disease. *Journal of neural transmission*, 124(3), 353-360. <https://doi.org/10.1007/s00702-016-1650-2>
- [19] Lin, C. H., Yang, S. Y., Horng, H. E., Yang, C. C., Chieh, J. J., Chen, H. H., Liu, B. H., & Chiu, M. J. (2017). Plasma  $\alpha$ -synuclein predicts cognitive decline in Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*, 88(10), 818-824. <https://doi.org/10.1136/jnnp-2016-314857>
- [20] Samat, N. A., Abdul Murad, N. A., Mohamad, K., Abdul Razak, M. R., & Mohamed Ibrahim, N. (2017). Apolipoprotein E $\epsilon$ 4: A Biomarker for Executive Dysfunction among Parkinson's Disease Patients with Mild Cognitive Impairment. *Frontiers in neuroscience*, 11, 712. <https://doi.org/10.3389/fnins.2017.00712>
- [21] Pchelina, S., Emelyanov, A., Baydakova, G., Andoskin, P., Senkevich, K., Nikolaev, M., Miliukhina, I., Yakimovskii, A., Timofeeva, A., Fedotova, E., Abramychyeva, N., Usenko, T., Kulabukhova, D., Lavrinova, A., Kopytova, A., Garaeva, L., Nuzhnyi, E., Illarionshkin, S., & Zakharova, E. (2017). Oligomeric  $\alpha$ -synuclein and glucocerebrosidase activity levels in GBA-associated Parkinson's disease. *Neuroscience letters*, 636, 70-76. <https://doi.org/10.1016/j.neulet.2016.10.039>
- [22] Matsumoto, J., Stewart, T., Sheng, L., Li, N., Bullock, K., Song, N., Shi, M., Banks, W. A., & Zhang, J. (2017). Transmission of  $\alpha$ -synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease?. *Acta neuropathologica communications*, 5(1), 71. <https://doi.org/10.1186/s40478-017-0470-4>
- [23] Wang, H., Atik, A., Stewart, T., Ginghina, C., Aro, P., Kerr, K. F., Seibyl, J., Jennings, D., PARS Investigators, Jensen, P. H., Marek, K., Shi, M., & Zhang, J. (2018). Plasma  $\alpha$ -synuclein and cognitive impairment in the Parkinson's Associated Risk Syndrome: A pilot study. *Neurobiology of disease*, 116, 53-59. <https://doi.org/10.1016/j.nbd.2018.04.015>
- [24] Papagiannakis, N., Koros, C., Stamelou, M., Simitsi, A. M., Maniati, M., Antonelou, R., Papadimitriou, D., Dermentzaki, G., Moraitou, M., Michelakakis, H., & Stefanis, L. (2018). Alpha-synuclein dimerization in erythrocytes of patients with genetic and non-genetic forms of Parkinson's Disease. *Neuroscience letters*, 672, 145-149. <https://doi.org/10.1016/j.neulet.2017.11.012>
- [25] Daniele, S., Frosini, D., Pietrobbono, D., Petrozzi, L., Lo Gerfo, A., Baldacci, F., Fusi, J., Giacomelli, C., Siciliano, G., Trincavelli, M. L., Franzoni, F., Ceravolo, R., Martini, C., & Bonuccelli, U. (2018).  $\alpha$ -Synuclein Heterocomplexes with  $\beta$ -Amyloid Are Increased in Red Blood Cells of Parkinson's Disease Patients and Correlate with Disease Severity. *Frontiers in molecular neuroscience*, 11, 53. <https://doi.org/10.3389/fnmol.2018.00053>
- [26] Singh, A. P., Bajaj, T., Gupta, D., Singh, S. B., Chakrawarty, A., Goyal, V., Dey, A. B., & Dey, S. (2018). Serum Mortalin Correlated with  $\alpha$ -Synuclein as Serum Markers in Parkinson's Disease: A Pilot Study. *Neuromolecular medicine*, 20(1), 83-89. <https://doi.org/10.1007/s12017-017-8475-5>
- [27] Malec-Litwinowicz, M., Plewka, A., Plewka, D., Bogunia, E., Morek, M., Szczudlik, A., Szubiga, M., & Rudzińska-Bar, M. (2018). The relation between plasma  $\alpha$ -synuclein level and clinical symptoms or signs of Parkinson's disease. *Neurologia i neurochirurgia polska*, 52(2), 243-251. <https://doi.org/10.1016/j.pjnns.2017.11.009>
- [28] Goldman, J. G., Andrews, H., Amara, A., Naito, A., Alcalay, R. N., Shaw, L. M., Taylor, P., Xie, T., Tuite, P., Henchcliffe, C., Hogarth, P., Frank, S., Saint-Hilaire, M. H., Frasier, M., Arnedo, V., Reimer, A. N., Sutherland, M., Swanson-Fischer, C., Gwinn, K., Fox Investigation of New Biomarker Discovery, ... Kang, U. J. (2018). Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson's disease Features. *Movement disorders*, 33(2), 282-288. <https://doi.org/10.1002/mds.27232>
- [29] Emelyanov, A., Kulabukhova, D., Garaeva, L., Senkevich, K., Verbitskaya, E., Nikolaev, M., Andoskin, P., Kopytova, A., Milyukhina, I., Yakimovskii, A., Timofeeva, A., Prakhova, L., Ilves, A., Vlasova, I., & Pchelina, S. (2018). SNCA variants and alpha-synuclein level in CD45+ blood cells in Parkinson's disease. *Journal of the neurological sciences*, 395, 135-140. <https://doi.org/10.1016/j.jns.2018.10.002>
- [30] Si, X., Tian, J., Chen, Y., Yan, Y., Pu, J., & Zhang, B. (2019). Central Nervous System-Derived Exosomal Alpha-Synuclein in Serum May Be a Biomarker in Parkinson's Disease. *Neuroscience*, 413, 308-316. <https://doi.org/10.1016/j.neuroscience.2019.05.015>
- [31] Tian, C., Liu, G., Gao, L., Soltys, D., Pan, C., Stewart, T., Shi, M., Xie, Z., Liu, N., Feng, T., & Zhang, J. (2019). Erythrocytic  $\alpha$ -Synuclein as a potential biomarker for Parkinson's disease. *Translational neurodegeneration*, 8, 15. <https://doi.org/10.1186/s40035-019-0155-y>
- [32] Ng, A., Tan, Y. J., Lu, Z., Ng, E., Ng, S., Chia, N., Setiawan, F., Xu, Z., Tay, K. Y., Prakash, K. M., Au, W. L., Tan, E. K., & Tan, L. (2019). Plasma alpha-synuclein detected by single molecule array is increased in PD. *Annals of clinical and translational neurology*, 6(3), 615-619. <https://doi.org/10.1002/acn3.729>
- [33] Bougea, A., Stefanis, L., Paraskevas, G. P., Emmanouilidou, E., Vekrelis, K., & Kapaki, E. (2019). Plasma alpha-synuclein levels in patients with Parkinson's disease: a systematic review and meta-analysis. *Neurological sciences*, 40(5), 929-938. <https://doi.org/10.1007/s10072-019-03738-1>

- [34] Chang, C. W., Yang, S. Y., Yang, C. C., Chang, C. W., & Wu, Y. R. (2020). Plasma and Serum Alpha-Synuclein as a Biomarker of Diagnosis in Patients With Parkinson's Disease. *Frontiers in neurology*, 10, 1388. <https://doi.org/10.3389/fneur.2019.01388>
- [35] Fan, Z., Pan, Y. T., Zhang, Z. Y., Yang, H., Yu, S. Y., Zheng, Y., Ma, J. H., & Wang, X. M. (2020). Systemic activation of NLRP3 inflammasome and plasma  $\alpha$ -synuclein levels are correlated with motor severity and progression in Parkinson's disease. *Journal of neuroinflammation*, 17(1), 11. <https://doi.org/10.1186/s12974-019-1670-6>
- [36] Shi, M., Sheng, L., Stewart, T., Zabetian, C. P., & Zhang, J. (2019). New windows into the brain: Central nervous system-derived extracellular vesicles in blood. *Progress in neurobiology*, 175, 96-106. <https://doi.org/10.1016/j.pneurobio.2019.01.005>
- [37] Niu, M., Li, Y., Li, G., Zhou, L., Luo, N., Yao, M., Kang, W., & Liu, J. (2020). A longitudinal study on  $\alpha$ -synuclein in plasma neuronal exosomes as a biomarker for Parkinson's disease development and progression. *European journal of neurology*, 27(6), 967-974. <https://doi.org/10.1111/ene.14208>
- [38] Jiang, C., Hopfner, F., Katsikoudi, A., Hein, R., Catli, C., Evetts, S., Huang, Y., Wang, H., Ryder, J. W., Kuhlenbaeumer, G., Deuschl, G., Padovani, A., Berg, D., Borroni, B., Hu, M. T., Davis, J. J., & Tofaris, G. K. (2020). Serum neuronal exosomes predict and differentiate Parkinson's disease from atypical parkinsonism. *Journal of neurology, neurosurgery, and psychiatry*, 91(7), 720-729. <https://doi.org/10.1136/jnnp-2019-322588>
- [39] Blesa, J., Trigo-Damas, I., Quiroga-Varela, A., & Jackson-Lewis, V. R. (2015). Oxidative stress and Parkinson's disease. *Frontiers in neuroanatomy*, 9, 91. <https://doi.org/10.3389/fnana.2015.00091>
- [40] Khan, Z., & Ali, S. A. (2018). Oxidative stress-related biomarkers in Parkinson's disease: A systematic review and meta-analysis. *Iranian journal of neurology*, 17(3), 137-144.
- [41] Jiang, T., Sun, Q., & Chen, S. (2016). Oxidative stress: A major pathogenesis and potential therapeutic target of antioxidative agents in Parkinson's disease and Alzheimer's disease. *Progress in neurobiology*, 147, 1-19. <https://doi.org/10.1016/j.pneurobio.2016.07.005>
- [42] Islam, M. T. (2017). Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. *Neurological research*, 39(1), 73-82. <https://doi.org/10.1080/01616412.2016.1251711>
- [43] Coles, L. D., Tuite, P. J., Öz, G., Mishra, U. R., Kartha, R. V., Sullivan, K. M., Cloyd, J. C., & Terpstra, M. (2018). Repeated-Dose Oral N-Acetylcysteine in Parkinson's Disease: Pharmacokinetics and Effect on Brain Glutathione and Oxidative Stress. *Journal of clinical pharmacology*, 58(2), 158-167. <https://doi.org/10.1002/jcph.1008>
- [44] Deas, E., Cremades, N., Angelova, P. R., Ludtmann, M. H., Yao, Z., Chen, S., Horrocks, M. H., Banushi, B., Little, D., Devine, M. J., Gissen, P., Klenerman, D., Dobson, C. M., Wood, N. W., Gandhi, S., & Abramov, A. Y. (2016). Alpha-Synuclein Oligomers Interact with Metal Ions to Induce Oxidative Stress and Neuronal Death in Parkinson's Disease. *Antioxidants & redox signaling*, 24(7), 376-391. <https://doi.org/10.1089/ars.2015.6343>
- [45] Vida, C., Kobayashi, H., Garrido, A., Martínez de Toda, I., Carro, E., Molina, J. A., & De la Fuente, M. (2019). Lymphoproliferation Impairment and Oxidative Stress in Blood Cells from Early Parkinson's Disease Patients. *International journal of molecular sciences*, 20(3), 771. <https://doi.org/10.3390/ijms20030771>
- [46] Gökçe Çokal, B., Yurtdaş, M., Keskin Güler, S., Güneş, H. N., Ataç Uçar, C., Aytaç, B., Durak, Z. E., Yoldaş, T. K., Durak, İ., & Çubukçu, H. C. (2017). Serum glutathione peroxidase, xanthine oxidase, and superoxide dismutase activities and malondialdehyde levels in patients with Parkinson's disease. *Neurological sciences*, 38(3), 425-431. <https://doi.org/10.1007/s10072-016-2782-8>
- [47] Wei, Z., Li, X., Li, X., Liu, Q., & Cheng, Y. (2018). Oxidative Stress in Parkinson's Disease: A Systematic Review and Meta-Analysis. *Frontiers in molecular neuroscience*, 11, 236. <https://doi.org/10.3389/fnmol.2018.00236>
- [48] Yuan, Y., Tong, Q., Zhang, L., Jiang, S., Zhou, H., Zhang, R., Zhang, S., Xu, Q., Li, D., Zhou, X., Ding, J., & Zhang, K. (2016). Plasma antioxidant status and motor features in de novo Chinese Parkinson's disease patients. *The International journal of neuroscience*, 126(7), 641-646. <https://doi.org/10.3109/00207454.2015.1054031>
- [49] Mischley, L. K., Standish, L. J., Weiss, N. S., Padowski, J. M., Kavanagh, T. J., White, C. C., & Rosenfeld, M. E. (2016). Glutathione as a Biomarker in Parkinson's Disease: Associations with Aging and Disease Severity. *Oxidative medicine and cellular longevity*, 2016, 9409363. <https://doi.org/10.1155/2016/9409363>
- [50] Colamartino, M., Duranti, G., Ceci, R., Sabatini, S., Testa, A., & Cozzi, R. (2018). A multi-biomarker analysis of the antioxidant efficacy of Parkinson's disease therapy. *Toxicology in vitro*, 47, 1-7. <https://doi.org/10.1016/j.tiv.2017.10.020>
- [51] Kumar, S., Dang, S., Nigam, K., Ali, J., & Baboota, S. (2018). Selegiline Nanoformulation in Attenuation of Oxidative Stress and Upregulation of Dopamine in the Brain for the Treatment of Parkinson's Disease. *Rejuvenation research*, 21(5), 464-476. <https://doi.org/10.1089/rej.2017.2035>
- [52] Mischley, L. K., Leverenz, J. B., Lau, R. C., Polissar, N. L., Neradilek, M. B., Samii, A., & Standish, L. J. (2015). A randomized, double-blind phase I/IIa study of intranasal glutathione in Parkinson's disease. *Movement disorders*, 30(12), 1696-1701. <https://doi.org/10.1002/mds.26351>
- [53] Jakubczyk, K., Dec, K., Kaldurńska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020). Reactive oxygen species – sources, functions, oxidative damage. *Polski merkuriusz lekarski*, 48(284), 124-127.
- [54] Picón-Pagès, P., Garcia-Buendia, J., & Muñoz, F. J. (2019). Functions and dysfunctions of nitric oxide in brain. *Biochimica et biophysica acta*, 1865(8), 1949-1967. <https://doi.org/10.1016/j.bbdis.2018.11.007>
- [55] Tsikas, D. (2017). What we authors, reviewers and editors of scientific work-can learn from the analytical history of biological 3-nitrotyrosine. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences*, 1058, 68-72. <https://doi.org/10.1016/j.jchromb.2017.05.012>
- [56] Bural, R., Ait-Bouziad, N., Chiki, A., & Lashuel, H. A. (2015). Elucidating the Role of Site-Specific Nitration of  $\alpha$ -Synuclein in the Pathogenesis of Parkinson's Disease via Protein Semisynthesis and Mutagenesis. *Journal of the American Chemical Society*, 137(15), 5041-5052. <https://doi.org/10.1021/ja5131726>
- [57] Feng, J., Chen, X., Guan, B., Li, C., Qiu, J., & Shen, J. (2018). Inhibition of Peroxynitrite-Induced Mitophagy Activation Attenuates Cerebral Ischemia-Reperfusion Injury. *Molecular neurobiology*, 55(8), 6369-6386. <https://doi.org/10.1007/s12035-017-0859-x>
- [58] Yuan, Y. Q., Wang, Y. L., Yuan, B. S., Yuan, X., Hou, X. O., Bian, J. S., Liu, C. F., & Hu, L. F. (2018). Impaired CBS-H<sub>2</sub>S signaling axis contributes to MPTP-induced neurodegeneration in a mouse model of Parkinson's disease. *Brain, behavior, and immunity*, 67, 77-90. <https://doi.org/10.1016/j.bbi.2017.07.159>
- [59] Campolo, M., Casili, G., Biundo, F., Crupi, R., Cordaro, M., Cuzzocrea, S., & Esposito, E. (2017). The Neuroprotective Effect of Dimethyl Fumarate in an MPTP-Mouse Model of Parkinson's Disease: Involvement of Reactive Oxygen Species/Nuclear Factor- $\kappa$ B/Nuclear Transcription Factor Related to NF-E2. *Antioxidants & redox signaling*, 27(8), 453-471. <https://doi.org/10.1089/ars.2016.6800>
- [60] Bandoorkwala, M., Sahu, A. K., Thakkar, D., Sharma, M., Khairam, A., & Sengupta, P. (2019). Edaravone-caffeine combination for the effective management of rotenone induced Parkinson's disease in rats: An evidence based affirmative from a comparative analysis of behavior and biomarker expression. *Neuroscience letters*, 711, 134438. <https://doi.org/10.1016/j.neulet.2019.134438>
- [61] Cardinali, D. P. (2019). Melatonin: Clinical Perspectives in Neurodegeneration. *Frontiers in endocrinology*, 10, 480. <https://doi.org/10.3389/fendo.2019.00480>
- [62] Gallardo-Fernández, M., Hornedo-Ortega, R., Cerezo, A. B., Troncoso, A. M., & García-Parrilla, M. C. (2019). Melatonin, protocatechuic acid and hydroxytyrosol effects on vitagenes system against alpha-synuclein toxicity. *Food and Chemical Toxicology*, 134, 110817. <https://doi.org/10.1016/j.fct.2019.110817>
- [63] Wei, H. J., DU, M., & Bai, H. Y. (2019). Correlations of Melatonin and Glutathione Levels with Oxidative Stress Mechanism in Parkinson's Disease. *Acta Academiae Medicinae Sinicae*, 41(2), 183-187. <https://doi.org/10.3881/j.issn.1000-503X.10775>
- [64] Uysal, H. A., Tiftikcioğlu, B. I., Öcek, L., & Zorlu, Y. (2018). Serum Levels of Melatonin and Sleep Evaluation Scales in the Diagnosis of Sleep Disorders in Patients with Idiopathic Parkinson's Disease. *Noro psikiyatri arsivi*, 56(4), 264-268. <https://doi.org/10.5152/npa.2017.19367>
- [65] Ortiz, G. G., Moráles-Sánchez, E. W., Pacheco-Moisés, F. P., Jiménez-Gil, F. J., Macías-Islas, M. A., Mireles-Ramírez, M. A., & González-Usigli, H. (2017). Efecto de la administración de melatonina sobre la actividad de la ciclooxigenasa-2, la concentración sérica de metabolitos del óxido nítrico, los lipoperóxidos y la actividad de la glutatión peroxidasa en pacientes con enfermedad de Parkinson [Effect of melatonin administration on cyclooxygenase-2 activity, serum levels of nitric oxide metabolites, lipoperoxides and glutathione peroxidase activity in patients with Parkinson's disease]. *Gaceta medica de Mexico*, 153(Supl. 2), S72-S81. <https://doi.org/10.24875/GMM.M17000008>