# PROMISING BIOCEMICAL MARKERS OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a chronic neurodegenerative disease associated with specific neurological deficits in patients, it mainly affects dopaminergic neurons in the substantia nigra causing accumulation of the neurotoxic amounts of aggregated α-synuclein protein in the neuronal cell bodies. The paper reports the authors' view of certain pathochemical and biochemical aspects of the Parkinson's disease development in terms of interplay between the metabolic pathways of catecholamines and pigments, particularly the possible pathway of neuromelanin synthesis in the neuronal cell bodies and its importance in the life of cells. Assessment of the use of certain neurodegenerative disorder biomarkers, which are of direct pathognomonic value, in the laboratory diagnosis of the disease is provided. It is suggested to use the results in the field of deeper understanding of biochemical patterns underlying neuronal death for early diagnosis of PD in individuals of different age groups, as well as for further study of pathogenesis based on fundamental biochemistry and pathobiochemistry of intracellular processes.

**Keywords:** Parkinson's disease, dopamine, dopamine receptors, key aspects of pathochemistry and pathogenesis, biomarkers

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# ПЕРСПЕКТИВНЫЕ БИОХИМИЧЕСКИЕ МАРКЕРЫ БОЛЕЗНИ ПАРКИНСОНА

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Болезнь Паркинсона (БП) — хроническое нейродегенеративное заболевание с характерными неврологическими расстройствами у пациентов, поражающее преимущественно дофаминергические нейроны черной субстанции с накоплением в телах нейронов нейротоксичных доз агрегатов белка α-синуклеина. В статье представлен взгляд авторов на отдельные патохимические и биохимические аспекты развития болезни Паркинсона во взаимосвязи путей обмена катехоламинов и пигментов, в частности возможного пути синтеза нейромеланина в телах нейронов и его значение в жизни клетки. Дана оценка определенным, имеющим прямое патогномоничное значение, биомаркерам нейродегенеративной патологии в лабораторной диагностике этого заболевания. Предложено использовать полученные результаты в сфере глубокого понимания биохимических процессов, лежащих в основе нейрональной смерти, для ранней диагностики БП в разных возрастных группах и дальнейшего изучения патогенеза на основе фундаментальной биохимии и патобиохимии процессов, протекающих в клетке.

**Ключевые слова:** болезнь Паркинсона, дофамин, дофаминовые рецепторы, ключевые аспекты патохимии и патогенеза, биомаркеры

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Parkinson's disease (PD) is a chronic neurodegenerative and neuroinflammatory disease associated with the development of motor and non-motor impairments. The disease mainly affects dopaminergic neurons in the substantia nigra causing accumulation of α-synuclein protein and Lewy bodies in the cells. Individuals over the age of 65 are more likely to develop PD; the prevalence is about 140 cases per 100,000 population. The incidence of PD among males is 1.5 times higher, which can be due to exposure to toxic substances, traumatic brain injury, X-linked genetic defects. However, the researchers pay maximum attention to the neuroprotective role of estrogens. Taking into account the low pace of studying the mechanisms underlying this disorder, no description of the key aspects of pathobiochemistry and in-depth studies of particular processes, no understanding of the chemical essence of the behavior of certain substances that are of diagnostic value, this disorder can be considered to be an urgent medical and social issue, especially for individuals of older age groups [1–11]. The study was aimed to familiarize the reader with our opinion about certain pathochemical aspects of Parkinson's disease and laboratory value of some biomarkers.

## Interrelationship between the dopamine and melanin biosynthesis pathways

Neuromelanin is synthesized directly from catecholamines. L-DOPA (L-dihydroxyphenylalanine) is subjected to hydroxylation and decarboxylation in natural conditions as a dopamine precursor. An alternative pathway of the mediator and its precursor metabolism is represented by endosomal accumulation of the mediator that moves to mitochondria by means of specific transporter (VMAT2) to be further biotransformed by monoamine

oxidase (MAO). The over-ac cumulated dopamine and DOPA are oxidized by iron-containing enzymes to quinones and semiquinones and are stored in the form of neuromelanin [12, 13]. The neuromelanin exact structure and functions are still poorly understood. We are going to try to model the cyclic structure of possible neuromelanin polymer formation and suggest the reader to discuss this structure. The structure of dioxyindole is provided below (Fig. 1). This fused heterocycle has rather interesting properties allowing us to shed some light on its biochemical behavior in the cell bodies of the CNS neurons and its role of possible neuromelanin monomer.

In particular, we believe that the side chain hydroxyl group being mainly an ortho-director enables aggregation through lateral cross-linking of the benzene rings of the benzpyrrole bicyclic system of indole involving attachment to the linear structure of neuromelanin in ortho position. However, considering the properties of the pyrrole part of the indole molecule, different pattern of interaction should be expected. It is pyrrole rings that enable formation of the product of intermolecular cyclization, similar to unsubstituted porphyrins, yielding tetra-dioxyindole. The +M effect of eight OH groups in the cyclic structure contributes to better stabilization of the delocalized π-conjugated system and enhancement of its chelating properties towards the center (as in heme), however, enhancement of acidic properties by the type of phenol in lateral OH groups of the molecule benzene nucleus should be also taken into account (Fig. 2).

Considering the fact that iron ions ($Fe^{3+}$) accumulate in the cells as part of the ferritin micelles after passing through apoferritin that can be rich with tryptophan amino acid residues, iron ions ($Fe^{2+}$) oxidize and stay in the micelle core in the form of complex complexone with the following biochemical composition: $[(FeO^{*}\mathbf{OH})_{8}(FeO^{*}OPO_{3}H_{2})]$. We can see similar stoichiometric ratio of OH groups (highlighted in bold) that can be involved in retention of the +3 oxydation state iron ions. Furthermore, the chelating part apparently can reduce $Fe^{3+}$ ions to $Fe^{2+}$ is addition to pericyclic adsorption of eight $Fe^{3+}$ ions by the cyclic variant of neuromelanin. This presents a certain danger to the cell's life and underlies neuronal death. The reason is that the iron ions contained in such structure (despite the fact that it is similar to porphyrin structure) cannot have the same function as that contained in the heme transport substances with constant +2 oxydation state or in cytochromes, in which iron is in fact represented by the $Fe^{3+}/Fe^{2+}$ redox couple. This is how the +2 oxidation state iron ions being powerful reducing

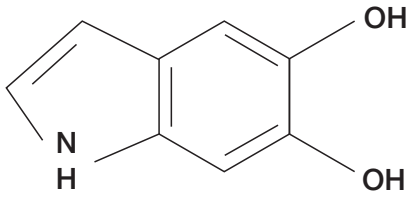


**Fig. 1.** Chemical structure of dioxyindole being the neuromelanin monomer involved in the eumelanin metabolic pathway of tyrosine

agents are accumulated, thereby provoking oxidative stress and eventually cell death.

## Dopamine biochemical properties and functions

Dopamine, a mediator of neuroendocrine and paracrine regulation of the peripheral organs, does not cross the blood-brain barrier. Dopamine receptors belong to the family of the transmembrane metabotropic type G protein-coupled receptors. Five dopamine receptor subtypes were revealed when the gene cloning technique was introduced: $D_1$, $D_2$, $D_3$, $D_4$, $D_5$. Dopaminergic receptors are considered to be "slow" G (from the word "guanine) protein-coupled receptors using secondary intracellular mediators (3',5'-cyclic adenosine monophosphate or cAMP in this case), in contrast to "fast" receptors (such as GABA receptors) that bind directly to the ligand-gated channels. Receptors are divided into two main groups: 1) $D_1$-like receptors ($D_1$, $D_5$) activate adenylate cyclase, activation of such receptors causes muscle relaxation and vasodilation; 2) $D_2$-like receptors ($D_2$, $D_3$, $D_4$), presynaptic in sympathetic nerves, inhibit adenylate cyclase, activation of such receptors enchances the effects of catecholamines. In the central nervous system (CNS) these receptors play an important role in regulation of motion and cognitive function realization. Receptors of the $D_{1A}$ and $D_{2A}$ subgroups regulate the cardiovascular system functions. Disruption of the substantia nigra and abnormalities of the $D_1$-like receptors can be observed in individuals with Parkinson's disease; dopamine production and the effect on the receptors are reduced [8, 9].

## PD laboratory signs

*In blood serum and oral fluid*

Presynaptic α-synuclein protein is involved in the synaptic vesicle transpot and subsequent neurotransmitter release. There are three

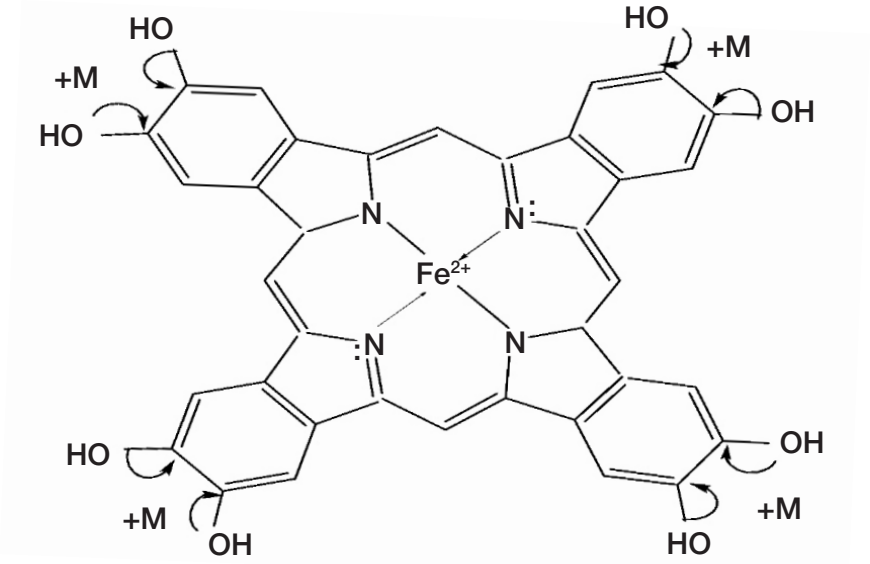


**Fig. 2.** Possible product of intermolecular cyclization: dioxyindole tetramer as a basis of the neuromelain cyclic tructure

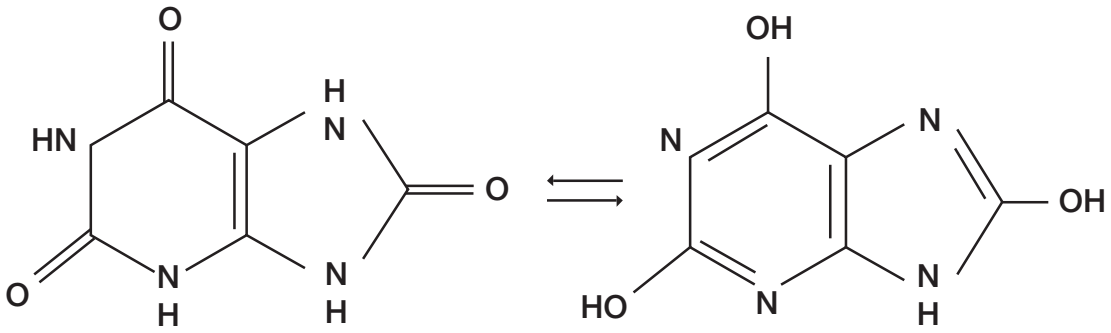


**Fig. 3.** Chemical structure of uric acid in the form of two possible native tautomers

α-synuclein isoforms produced by alternative splicing: 140 amino acids (base form), 126 amino acids and 112 amino acids. The base form of α-synuclein consists of the hydrophobic central region (non-amyloid component), amino-terminal region containing recurring amino acid sequences, and the negatively charged C-terminal region containing several phosphorylation sites and the domain responsible for α-synuclein chaperone activity. The N- terminal region is similar to the lipid-binding domain of apolipoproteins, which indicates the α-synuclein ability to interact with the membrane lipids. There is an assumption that two forms of this protein exist in the cells: native and membrane-binding. The α-synuclein native form is an unfolded protein with the disordered spiral structure. It is the increase in neurotoxic aggregates of this small protein that is important for the disease pathochemistry [3].

The Parkinson disease protein 7 (DJ-1 protein) is involved in cell functions, including transcription regulation and the response to oxidative stress, the processes that are directly related to neurodegeneration. Mutations of the gene encoding this protein are a rare cause of autosomal recessive parkinsonism; such mutations are associated with impaired DJ-1 capability of dimerization, its stability and folding. The DJ-1 protein functions include antioxidant, transcription co-activator and molecular chaperone activity. This protein has different catalytic and non-catalytic functions in different compartments of the cell: the DJ-1 protein acts as a co-activator of various signaling pathway in the cell nucleus, thereby preventing cell death; in mitochondria, it is contained in syntasomes, in which it interacts with the ATP synthase β-subunit. The decrease in the levels of normal protein results in the decrease in its function in oxidative stress [6, 14].

Uric acid: the risk of PD is inversely related to plasma levels of uric acid. Thanks to its double bonds, uric acid can possess antioxidant activity as an unsaturated, electron-deficient heterocyclic structure [7, 8, 10–12]. This property of the compound can underlie total antioxidant capacity of blood plasma and cerebrospinal fluid, thereby protecting the human body against reactive oxygen species and nitrogen (singlet oxygen, hydroxyl radicals, hydrogen peroxide and peroxynitrite).

*In blood plasma*

The emergence of autoantibodies in blood plasma of individuals with PD is associated with chronic neuronal damage and degeneration. Neuropeptides, nucleofactor 200, S100, etc. are products of neuronal degradation (this triggers autoimmune component of the disease pathogenesis resulting in synthesis of specific antibodies (cell adhesion molecule 4, myotilin, fibronectin, elongation factor 1-alpha 1).

*In cerebrospinal fluid*

High interleukin 6 (Il6) levels are associated with the 3.5-fold increase in the risk of PD, which is a neuroinflammation biomarker. Interleukin 6 belongs to the family of Il6 cytokines, the other members of which include Il11, Il27, Il31, etc. The Il6 biological activity is related to its ability to activate the target genes involved in cell differentiation, survival, apoptosis, and proliferation. Il6 is involved in intracellular signal transmission, thereby causing activation of tyrosine kinase triggering phosphorylation of transcription factors involved in regulation of the Il6 synthesis. Stimulation of the acute phase inflammatory response, associated with the expression of the Il-encoding gene in the liver and manifesting itself in the increase in the concentration of the acute phase inflammatory proteins (primarily C-reactive protein and haptoglobin), represents the Il6 pro-inflammatory effect. We believe that one of its pathochemical mechanisms can underlie its pro-inflammatory effect on glial cells in case of high levels in cerebrospinal fluid, as well as the decrease in biosynthesis of neurotrophic peptides (belonging to the group of peptide NTF biomolecules, which, like IL6, implement signaling pathways through gp130 in the neuronal membrane apparatus) that contribute to survival of the cerebral neurons in oxidative stress in healthy body. In inflammation, this group of cytokines is likely to play a role of intracellular anti-inflammatory factors similar to anti-inflammatory myokines of the muscle tissue belonging to the same family of biomolecules based on biochemistry [15].

*Uric acid*

In our opinion, biochemical potential of uric acid in neurodegenerative disorders is of interest. As is known, there are two tautomeric forms of this organic acid: lactim and lactam. In the body it obviously can exist in both forms playing a certain role in pathochemistry of some disorders, including PD (Fig. 3). Thus, a well-known fact in the PD pathogenesis is the loss of catecholamine neurons by the patient's body that eventually results in progression of clinical manifestations. This means that the effects of substances capable of increasing the effects of biogenic amines have a certain positive influence on the disease course and the patients' condition improvement. It has been found that the patients' health improvement correlates with the increase in the levels of this metabolite in cerebrospinal fluid. The logical question is: what is the possible mechanism of action of the acid itself? Apparently, there are at least two such mechanisms. According to pharmacological biochemistry, tri- and dimethylxanthines are inhibitors of certain phosphodiesterases. The enzymes of this class are divided into two types based on their localization in the cell: membrane-bound and cytosole. It is possible that the lactim form of this acid that has a certain dipole moment and a polarized OH group (besides, not only one) has strong acidic properties and is unable to cross the cell membrane. However, by analogy with the "mobile" OH group and aromatic nature of the chroman core of tocopherols, it can be retained in the membrane apparatus of

certain cells, including neurons. Furthermore, on the one hand it functions as a local antioxidant, and on the other hand as a phosphodiesterase inhibitor (as a xanthine derivative), thereby prolonging the effects of catecholamines (while biogenic amines have a membrane-mediated mechanism of action). Meanwhile, it is the membrane-bound form of mostly type I phosphodiesterase that is inhibited: it targets cAMP activated by $Ca^{2+}$ cations, $4Ca^{2+}$-calmodulin complex + cGMP in healthy body.

## CONCLUSION

Thus, we have systematized and characterized in terms of biochemistry the following laboratory signs of Parkinson's disease: 1) α-synuclein accumulation; 2) mutations of the gene encoding the DJ-1 protein; 3) changes in a number of blood plasma antibodies; 4) presence of potential biomarkers: Il6, uric acid. A number of processes play an important role in pathochemistry of Parkinson's disease: the decrease in the levels of dopamine and the number of neurons in the substantia nigra; accumulation of neuromelanin with certain chemical structure in neurons; the decrease in tyrosine hydroxylase activity; the final link of the PD pathochemistry is represented by α-synuclein degradation in the CNS and peripheral ANS neurons; it is possible that the decrease in the levels of the uric acid lactim form in the membrane apparatus of the cell bodies of neurons is of some importance. The reported details of the neuromelanin production pathochemistry and biochemistry of the uric acid behavior in native conditions can be biomarkers or biosensors of oxidative stress experienced by the neuronal cell bodies of individuals with this disorder. These details can be of some diagnostic value even in the pre-clinical phase of the disease (before manifestation). We believe that further research is required for better understanding of biochemistry of various intracellular processes and the behavior of substances that are of some pathogenetic and diagnostic value to enable early diagnosis and timely detection of the new disease cases in the population not only in the groups at risk, but also in other age groups.

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