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ABSTRACT

The Parkinson's (PD) disease is a difficult health problem. Aging is the only probable cause of PD without clearly identified underlying molecular mechanisms. Still, it seems that oxidative stress and mitochondrial damage combined with harmful genetic and environmental factors are the main origins of death in dopaminergic neurons from substantia nigra pars compacta. While influx of new findings on pathogenesis, and development of new diagnostics for PD is increasing, still it diagnosis mostly depends on the physical examination and clinical diagnostic criteria with high misdiagnose rate. This is further complicated with fluctuation of the PD symptoms over the time and hinders objective and unbiased monitoring of the disease progression. PD is often diagnosed in the advanced stage and when majority of dopaminergic neurons are lost, so neuroprotective therapies are not possible. Given the difficulties with clinical diagnosis of the PD there is a pressing need to identify reliant diagnostic biomarkers. Intensively tested biomarker candidates are α-synuclein, DJ-1, 8-hydroxy-2'deoxyguanosine, 8-hydroxy-guanosine, glutathione S-Transferase Pi protein for oxidative damage, and homocysteine with C-reactive protein as inflammatory biomarkers. Currently none of them are not enough specific and selective. Biomarkers with potentially good specificity, selectivity and accessibility are miRNAs, able to provide precise and non-invasive diagnosis. More fundamental research is warranted to provide critical data to determine real reasons behind the PD. Parallel to obtaining data for the origins of the PD, development of the suitable clinical biomarkers should follow.

Parkinson's Disease

Parkinson´s disease (PD) is a chronic neurodegenerative disease that affects motor skills in more than 6 million people worldwide [1]. It is the second most common progressive disease, after dementia [2] caused by Alzheimer disease (AD) [3]. The occurrence of the PD is associated with age (increase with aging) [4], sex (males are more effected than females), and race (whites are more diagnosed than African Americans and Latinos) [1]. The PD symptoms can be categorized in motor and non-motor group. Motoric PD symptoms differ with the stage of the disease, and are usually manifested after the 80% of neurodegeneration [1]. They include bradykinesia (slow initiation of movement), tremor, inability to pass over the obstacles, problems with balance, and forward-leaning gait [1,5]. Non-motor symptoms associated with the PD are dementia, mood swings, hypersexuality, depression, apathy, anxiety, impulsiveness, and others [1]. Recently it was suggested that loss of olfactory ability is associated with onset of the PD and olfactory tests can be potentially used as early sensitive clinical marker [6].

The PD is mostly idiopathic disease, yet 15% of the affected patients have member of their family with the PD. The PD has four stages: (i) premotor PD stage (olfactory impairment, cognitive and mood problems, slower bowl movement); (ii) early PD (rigidity, restlessness, tremor, and bradykinesia); (iii) moderate PD (motor symptoms increase, constipation, and mood disorders); and (iv) advanced PD (motor and non-motor problems worsen, occurrence of gait, and dementia) [1,7].

Epidemiological risk factors for the PD are: age (the most prominent), environmental factors such as exposure to pesticide rotenone and/or herbicide Agent Orange, heroin use (via MPTP that is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), and genetics [1]. Surprisingly enough, smoking (nicotine) and consumption

of coffee (caffeine) showed protective roles against the PD [8], however risk factors for other chronic conditions overweight the benefits of smoking associated with the PD [1]. Much healthier protectants are bioactive compounds (e.g. polyphenol quercetin) from foods [9] that can act as antidote for some of abovementioned risk factors (e.g. for rotenone) in normal/damaged neurons [10]. Epigallocatechin-3-gallate found in green tea prevents conformational changes in α-synuclein associated with formation of Lewy bodies (LB). As explained below, the LB are one of the potential culprits for the neurodegeneration in PD [1]. Berry fruits and their products are good sources of various polyphenolics [11-13] that have protective roles on mitochondrial function greatly involved with etiology of the PD [9].

Etiology of The Parkinson's Disease

It is known that the motoric impairments in PD are associated with lack of dopamine in the brain. Currently no cure exists for the PD, but only disease modifying treatments that help patients improve quality of their lives [14]. Classical approach to PD treatment is dopamine replacement therapy (with L-DOPA), but effectiveness of this approach declines with years of treatment [15]. Novel approaches include deep brain stimulation, cell based therapies and others [1].

Conscious movements in human body are executed by muscles and ligaments through two neural projection pathways. Both pathways originate in primary motor cortex (anterior to central sulcus) and are either direct (excitatory) or indirect projections (inhibitory) [1]. Basal ganglia is additional neural structure involved with movement and consisted of 5 parts that are all components of telencephalon: (i) caudate nucleus (CN), (ii) putamen (Put), (iii) nucleus accumbens (Acb), (iv) globus pallidus internal (GPi); and (v) globus pallidus external (GPe) cumulatively named corpus striatum. Subthalamic nucleus (STN) and substantia nigra (SN) are the key components for the PD pathology located in diencephalon. The SN has 2 parts, pars compacta (SNc) and pars reticulata (SNr) that by globus pallidus and thalamus responses influence corresponding part of motor cortex. The SNc contains dopaminergic neurons with black pigment (neuromelanin), hence the name. By direct pathway striatum sends inhibitory projections to the GPi, the GPi inhibits thalamus that causes excitation projections in the motor cortex. Striatum in indirect pathway sends inhibitory projections to the STN via GPe which excites the GPi, and then inhibits the thalamus and the motor cortex. Both indirect and direct pathway are coordinated by the dopamine release from the SNc. In summary, it can be said that the basal ganglia inhibits willful movement in humans. That effect is inhibited by the release of dopamine from the SNc [1]. In the PD, pigmented dopaminergic neurons perish from the SNc over the time [16], as a consequence production of dopamine is impaired, conscious movement tends to be increasingly inhibited, and that causes gradual deterioration of cardinal motor skills in those affected [1].

Molecular Mechanisms of the PD

The underlying mechanisms and causes for PD are still not clear but it is proposed that the PD is multifactorial disease with many unidentified factors [17]. Known factors associated with PD are oxidative stress, mitochondrial damage [1] environmental influences, genetics, and excitotoxicity [4]. Further, it remains a mystery why such small and specific population of dopaminergic neurons from SNc tends to perish in a course of disease while the rest of the brain remains intact [18].

On the molecular level, dopaminergic input in direct pathway is controlled by the expression of the D1 receptors, while indirect pathway is controlled by the D2 receptors. D1 receptors via guanine nucleotide binding protein and adenylate cyclase increase production of cAMP while D2 does the opposite [19]. Increased concentrations of the cAMP increase excitatory activity in striatal neurons by uptake of the Na^{+}/Ca^{2+} [20]. The SNc neurons do not have dedicated proteins to bind excess of Ca^{2+}, so toxic levels of this ion and corresponding apoptosis is avoided by pumping it out of the cell. That process requires large amounts of cellular energy and is heavily dependent on the solid ATP production by mitochondria [1]. On the other hand, mitochondria tends to produce small quantities of reactive oxidative species (ROS) during oxidative phosphorylation that is able to damage its own mDNA and other molecular structures. Additionally, oxidation of dopamine can also increase concentration of the ROS and induce neural death [21].

Calcium imbalance is influenced by the age, environmental factors (e.g. elevated levels of pesticides, toxic metals, neurotoxins, and inflammatory agents), genetics (mutations in mitochondrial proteins), and other [17,22,23]. These are all are various sources of oxidative stress or mitochondrial damage, that cumulatively with Ca^{2+} disbalance may cause neurodegeneration in the PD [22].

Pathophysiological hallmarks for the PD are LBs that contain densely packed α-synuclein and straight filaments [24]. The LBs are found in surviving SNc neurons called Lewy neurites and in other midbrain regions, cerebrospinal fluid (CSF) and plasma. The α-synuclein is a presynaptic neuronal protein associated with PD and numerous theories how it contribute to PD pathogenesis [25]. Still, the most frequent opinion is that it forms damaging oligomeric conformations that are detrimental for cellular balance and induce neuronal death [26]. The connection of α-synuclein with PD is not clear, for instance 10% of elderly over 60 years have incidental LBs in their brains, but only small number of them develops PD symptoms [27]. Nonetheless, it is certain that α-synuclein is important molecule in PD pathogenesis [28]. Structurally speaking, α-synuclein from cytosol does not have any tertiary structure, however when bind with phospholipid membrane it forms α-helix. Overexpression of this protein drives α-helix to β-sheet formation which may form sticky amyloid globules similar to those from Alzheimer's disease [1].

Genetic research identified that mutation in six human genes is cause of monogenetic PD [29]. These are following cellular proteins (4/6 are mitochondrial): (i) α-synuclein (SCNA) [30,31]; (ii) LRRK2 leucine-rich repeat kinase 2 (PARKS); (iii)

PINK1 PTEN-induced putative kinase 1 (PARK6); (iv) Parkin E3 ubiquitin ligase (PARK 2); (v) protein DJ-1 oxidative stress sensor (PARK7); and (vi) ATP13A - possibly cation-transporting ATPase (ATP13A2) [29]. Genetic mutation in α-synuclein increases expression of this protein with increased formation of synaptic vesicles and extension of dopamine release [32]. That results with overproduction of the LBs and fast progression of the PD. It is interesting to note that whether or not α-synuclein is overexpressed or mutated in transgenic animals it did not cause death of the neurons [33]. Recent study showed that this might be due to animal models that differ between wild type genes and those found with familial PD. Judging by the loss of the dopaminergic neurons, animal models with familial PD and gene A53T more effectively established PD model [34].

Other gene responsible for the development of the PD is LRRK2. Similar to the α-synuclein its function is not clear. It is a large protein with kinase activity and protein-protein interactions [1].

As mentioned before, maintenance of mitochondrial function is crucial for survival of neurons. PINK1 and Parkin are two proteins responsible for such monitoring and protein recycling. PINK1 is a protein maintained at low levels at healthy mitochondria (probably cleaved from the surface of mitochondria by some unidentified protease). Due to the loss of electrical charge from the mitochondrial surface, PINK1 remains attached by some unknown mechanism while Parkin binds to the PINK1, hence targeting such nonfunctional mitochondrion for destruction by the lysosomes [35]. Genetic mutation in PINK1 and Parkin disables cellular damage control, and paves the way for agglomeration of the ROS and other detrimental compounds within the neurons, that can lead to the cell death and the PD.

DJ-1 and ATP13A are yet two other proteins involved with etiology of the PD via maintenance of mitochondrial function by some unidentified mechanisms. DJ-1 is a small molecule that seem to act as cellular sensor for the oxidative damage, and the ATP13A is lysosomal protein [1].

It is important to note that one of the main reasons that mechanisms and causes of PD are still not known, is the location of the PD pathology that is problematic to investigate and due to fluctuations in clinical phenotype [27]. Additional layer that distorts the real picture behind the PD is aging. Although known, that older age is strongest predictor of the PD still it is not clear why such long time is needed to develop PD symptoms [4]. Normal brain aging and development of PD share commonalities e.g. influence on mitophagy and mitochondrial damage, formation of α-synuclein, UCH-L1, and DJ-1. Cells such as astrocytes, stem cells and microglia from the subventricular zone show similar physiological responses with aging and the PD [36].

Based on above mentioned, aim of this review was to give insight in the current state of literature with regards to available biomarkers for biochemical detection of the PD.

Biochemical Biomarkers of Parkinson's Disease

A biomarker is a "*characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention* [37]." Therefore, an ideal PD biomarker must: (i) differentiate amongst all subtypes of the PD in the premotor stages; (ii) follow changes with the all disease stages; (iii) be useful for monitoring the effects of novel therapies; (iv) differentiate PD from other neurodegenerative diseases (e.g. progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD), essential tremor (ER), etc.); (v) be reproducible, and (vi) be inexpensive and non-invasive [38,39]. For instance, early differentiation among PD and MSA symptoms has important therapeutic repercussions (both clinical and prognostic), and assessment may be confounded if it is based only on clinical examination [5,27]. Besides the MSA, diagnosis of PD in early stages may be confounded by other medical conditions with overlapping symptoms (e.g. ER and PSP) [5].

Biomarkers are typical for particular condition and they can be used as indicators of biological processes relevant to some diseases. Further, they should have a positive predictive value which provides risk assessment that patient with a positive result has the disease [40]. Naturally, true positive predictive value of a biomarker should be increased by increasing its sensitivity (probability that patients has biomarker and disease) and/or by increasing specificity (probability that patients does not have biomarker and disease) [41,42].

The PD has poor clinico pathological correlation, meaning it is difficult to predict clinical phenotype just by knowing the pathology, and vice versa [43]. In other words, levels of biomarkers capable of detecting PD pathology may not correlate with relevant clinical data [27]. Example of a good biomarker is C-reactive protein used for prediction of coronary artery disease, as its levels rise (positively correlate) with increased chances for getting this disease, and drop with application of the successful medical treatment [44]. Currently such specific/ideal PD biomarker(s) are not yet known, nor the best method for their identification [27,38].

Existing biomarkers for the PD can be divided in clinical biomarkers (correspond to non-motor symptoms), neuroimaging biomarkers (e.g. SPECT- single-photon emission computed tomography; PET-positron emission tomography; and fMRI-functional magnetic resonance imaging), and biochemical biomarkers (that are in focus of this review). The clinical biomarkers can serve as additional confirmation of the specific and sensitive premotor biomarkers, but sole use in diagnostics is not sensitive and specific enough [45]. Neuroimaging markers are expensive and inaccessible besides, imaging can detect neurodegeneration only with full development of PD symptoms. Biochemical markers (especially blood based and saliva) are the most promising option with minimal invasiveness and costs [46]. With regards to their objectives, there are two main groups of the PD markers, those able to: (i) differentiate predisposed individuals from healthy population prior development of the PD symptoms, and (ii) identify PD with established symptoms [27].

Sensitivity of available clinical tests for the PD (symptomatic cases) is roughly 91%, even in highly specialized centers [47]. Therefore more accurate biomarkers are needed in order to objectively diagnose and follow all stages of the PD and applied medical treatments [48]. According to the recent pathological studies, it is considered that presymptomatic phase of PD lasts five years [44,49]. During that period patients may develop subtle clinical prodromal syndrome lasting 4-6 years [49]. Moreover this timeframe can be used for early diagnosis that will precede onset of characteristic extrapyramidal motor symptoms [27].

Biomarkers of Aberrant Protein Aggregation and Degradation

α-synuclein

The CSF, blood, gastrointestinal tract and salivary glands are potential matrices for measuring levels of skin neuro protein [28]. Intensive efforts to study α-synuclein in CSF as a diagnostic PD biomarker have been underway with some promise [50,51]. However, the assessment yielded conflicting results. One set of results reported that levels of α-synuclein decreased in PD patients vs. control group [52,53] while the other reported no differences among those groups [54].

Levels of oligomeric species of α-synuclein, total α-synuclein, and α-synuclein were measured in plasma with aim to develop blood based biomarkers. Results showed that plasma levels of phosphorylated α-synuclein can potentially be used for the PD diagnostic. In addition, total levels of α-synuclein can be applied as substitute marker for the PD development [14]. This is supported with reported positive correlation between the levels of non-phosphorylated α-synuclein in blood plasma with the PD progression [14]. Regardless of the physiological background, abovementioned findings imply that diagnostic of 'total α-synuclein' or 'non phosphorylated α-synuclein' may be employed as surrogate marker for the progression of PD [14]. Likewise, levels of α-synuclein could be used in potential clinical trials for testing drugs targeting α-synuclein pathology and advancement of the PD. However, hemolysis interferes with accuracy of α-synuclein levels in the CSF or plasma. To that end, Wang et. al. examined α-synuclein oligomer in red blood cells by enzyme linked immunosorbent assay. In their study they showed that the ratio of α-synuclein oligomer/total RBC protein was higher in the PD patients vs. controls, while no significant difference was found for α-synuclein oligomer/total protein ratio between PD and MSA [55].

Uric Acid

Relevance of the Uric Acid (UA) in the PD pathophysiology was first suggested by the putative antioxidant properties of the UA [56]. Recognition of the UA as potential biomarker was first documented by analyses of the UA levels and incidence of PD among 7,968 men enrolled in the Honolulu Heart Program [31]. Theoretic framework for involvement of the UA in reduction of oxidative stress is through several mechanisms. For instance, UA is the antioxidant soluble in water that binds free ROS and main electron-transfer entities able to generate free radicals (e.g. iron) [57]. Lolekha et al. compared the UA serum levels across three groups of patients two were PD groups (tremor dominant (TD) and non-tremor dominant (NTD)) vs. control group [58]. The UA serum was higher in the in the PD vs. controls with lower UA in the NTD vs. TD group. Lastly levels of the UA decreased with severity of the PD.

Coenzyme Q10

Coenzyme Q10 (CoQ10) is another marker associated with oxidative stress. Recently, blood CoQ10 was associated with decreased redox ratio in PD vs. controls [59,60]. Also, in the CSF the percentage of oxidized -to-total CoQ10 increased in subjects with PD [60]. Deficiency of CoQ10 in PD should be further explored as a potential blood biomarker of antioxidant status in the PD [61]. However, the other conditions affecting the status of CoQ10 indicated that the reduction is not specific to the PD [61].

For instance dietary supplementation and individual needs confounds clinical PD status and progression of the disease with regards to CoQ10. To that end, researchers conducted a functional test, (Functional Intracellular Assay (FIA), Spectra Cell Lab, Houston, TX) on 22 PD patients, from 2004-2008 [62]. It was concluded that CoQ10 should be pursued as candidate for the peripheral biomarker of antioxidant status in PD. The FIA test demonstrated that PD cells showed better function in a presence of supplementation with CoQ10, so these test may prove useful to identify groups of the PD patients that may benefit from supplementation [62].

Biomarkers of Mitochondrial Dysfunction and Oxidative Stress

As explained in the section "MOLECULAR MECHANISMS OF THE PD" there is a strong connection between oxidative stress, mitochondrial dysfunction and etiology of the PD [63]. The PD has many known sources of ROS and mechanisms for their production including dopamine metabolism, mitochondrial dysfunction, iron, neuroinflammatory cells, calcium, and aging. It is believed that alterations in oxidative stress contribute to development of the PD [63]. Also, brain of patients affected with the PD showed increased levels of DNA, lipids and protein oxidation markers [64-66].

DJ-1

The DJ-1 emerged as potential biomarker for the PD after identifying mutations in the PARK7 gene [67]. Previous research showed that overall DJ-1 levels decreased in the CSF, but stayed constant in human plasma for the PD patients vs. controls [68]. Despite identified post-translational modifications, it is possible to develop specific and sensitive assay for the 4-hydroxy-2-nonenal (4-HNE) modification of DJ-1 [69]. All this data strongly suggests that oxidative metabolites can be excellent candidates for biomarkers for the PD.

Glutathione S-Transferase Pi Protein

One of the major components of the anti-oxidant system is glutathione that acts through association with a glutathione

S-transferase to bind and reduce ROS [61]. Study on a small population of PD patients found that expression of Glutathione S-Transferase Pi Protein (GSTP) increased number of leukocytes as a reaction to oxidative stress [61]. This was not observed in the erythrocytes or plasma. This suggests that baseline blood levels of GSTP are not altered with PD; rather they are regulated either at the mRNA or translational level. This study examined small group of PD patients while larger studies are needed to confirm suitability of this protein as a biomarker in general and PD population [61]. Such studies should include newly diagnosed, non-medicated patients and unaffected controls with genetic relatedness to individuals with the PD.

Other study showed connection between GSTP and progression of the PD by changes in expression of GSTP1, SH3GL2 and CNPase. Cultured cortical neurons responded to the stress with overproduction of the GSTP1 in order to reduce oxidative and endoplasmic reticulum stress. It was also reported that concentrations of this protein tend to decrease with progression of the PD [70]. Additional study reported postmortem comparison of the CSF in PD group vs. controls and confirmed reduction of the GSTP in PD group [71].

8-hydroxy-2'deoxyguanosine and 8-hydroxyguanosine

Diagnostic of 8-hydroxy-2'deoxyguanosine (8-OHdG) and 8-hydroxy-guanosine in plasma or urine may have controversial significance [72]. For instance, concentrations of 8-OHdG depends on both on oxidative rate and efficacy of the DNA repairing systems [73]. Data showed that urine samples differed in PD vs. controls for both, sole 8-OHdG and for the 8-OHdG/2-dG ratio. In plasma samples, only this ratio was significantly higher among studied groups, implying that it might be reliable diagnostic tool that captures DNA oxidative damage with the PD [73,74]. Other literature data showed no difference in urine and/or plasma samples for the 8-OHdG among PD vs. controls. Authors of this study suggested that further research is needed in order to strengthen the power of the proposed observation [73]. Given the significance of this parameter in other clinical conditions (various cancers and other degenerative diseases) in which oxidative stress plays a key role in pathogenesis of the PD, we believe that the role of this molecule as PD biomarker is still insufficiently investigated.

Protein carbonyls

The protein carbonyls (PC) were largely studied in connection with the traumatic brain injuries (TBI) and they tend to increase in animas with the presence of TBI [75]. It was proposed that carbonyl assay can be used to assess oxidative damage in the human brain as it was observed that the postmortem brain tissues PD vs. controls have higher levels of the PC [76]. Similarly, elevated PC were also detected in brain regions initially not predicted as PD targets (e.g. caudate nucleus and putamen). This might suggest that biosynthesis of the PC is somehow connected with induction of oxidative activity in neurons. Also there might be association between L-DOPA treatment and the PC, as subjects with incidental LBs disease (putative presymptomatic PD) did not have increased PC in any parts of their brains. These findings imply that brain tissue affected by the PD shows extensive signs of oxidative damage in later stages of disease, and/or that L-DOPA therapy promotes oxidation of proteins [76].

MicroRNAs

MicroRNAs (miRNAs) are short (20-25 nucleotides) non-coding RNA molecules, that regulate genetic expression [77]. It is predicted that they control approximately 60% of all the protein-coding genes [78,79]. Database with more than 25K of various miRNAs sequences (human and other) can be found in “miRbase” [79]. Further, miRNAs are able to impede expression of proteins by translational control [80,81], thus influence survival and function of the neurons [82]. They also regulate the expression of various genes at the posttranscriptional level with important roles in various cellular processes, including regulation of epigenetic machinery, cell proliferation, differentiation, and apoptosis [83]. Such mechanisms target proteins involved in DNA methylation and histone modifications by controlling whether or not chromatin is in its accessible form [84].

Given the importance of miRNAs in various biological processes, it is reasonable to expect that deregulated miRNA expression is associated with a number of medical conditions [84], and that many diseases can be diagnosed with miRNAs as biomarkers [85]. Alterations of miRNA expression were observed in a neuropsychiatric conditions such as schizophrenia, bipolar disorder, major depression, PD and Alzheimer's disease [78]. It was reported that blood miR-30c was down regulated in PD vs. controls [85]. Recent data showed that some miRNAs expressed in human brain may control genetic expression related to AD and PD [86,87]. PD related dementia showed association with reduced concentrations of miR-205 in the frontal cortex and striatum for the PD vs. controls [88,89]. Similarly miR-34b and mir34c production was inhibited in several other brain regions for the PD patients [88-90].

Vallelunga et al. (2014) reported the first study with data analyzing global miRNAs expression in serum of PD and MSA patients vs. controls [86]. Authors employed TaqMan Low Density Array technology and analyzed 754 miRNAs. They validated set of 4 differentially expressed circulating miRNAs (cmiRNAs) for the above groups, and found that 9 cmiRNAs differed for PD/MSA vs. controls. More precisely, miR-339-5p was down regulated, while miR-223, miR-324-3p, and miR-24 were upregulated for both PD and MSA. Further, cmiRNAs were deregulated for PD (down regulation of both miR-30c/miR-148b) and in MSA (upregulated miR-148b). The MSA vs. PD serum had 3 upregulated cmiRNAs (miR-24, miR-34b, and miR-148b). Aforementioned data proposed that cmiRNA signatures are able to segregate among PD, MSA, and healthy individuals. Hence they can be considered specific, noninvasive biomarkers for differential diagnosis [86]. It was suggested that this promising implications should be more toughly tested on a larger population of the subjects [86].

Inflammatory Markers as PD Biomarkers

Homocysteine (Hyc), C-reactive protein (CRP) are the two of the most important PD inflammatory biomolecules. They have been

extensively studied over the last decade and it was concluded that elevated plasma Hcy levels presents a risk factor for dementia and cognitive impairments. Furthermore their association with mild cognitive impairment (MCI), Alzheimer's disease, and PD was well documented. Similar to the Hyc, increased plasma CRP is correlated with number of diseases, including the PD. There are also studies that have examined the combination of Hcy and CRP in the PD that concluded that contribution of these two biomolecules in PD pathogenesis might be equal [91].

Genomics, Proteomics, And Metabolomics

Genomics is an important area of research for development of PD biomarkers [92]. Genetic analysis resulted in the identification of numerous mutations, connected with familial or sporadic PD. The main targets for genetic profiling are above mentioned genes associated with the PD pathophysiology [27,29-31,33-35].

Mass spectrometry is one of the approaches used in proteomics for detection of pathologic proteins resulted from the PD genetic mutations. Such methods were able to identify bioactive neuropeptides that activate G-coupled receptor in murine brains. Additional data is warranted for this methods to be applied for diagnostic purposes [93].

Metabolomics is recent discipline that observes influence of the proteins on the production of metabolites in cellular systems. Metabolites can be excellent source of potential biomarkers that are able to monitor entire course of the disease (including the onset and influence of therapy). Another advantage is that metabolic biomarkers are readily accessible from the CSF, saliva, skin, serum and urine [15].

Conclusions

In conclusion, PD is serious public health problem that will continue to burden human lives and medical healthcare systems [15]. Currently, aging is the only probable cause of the PD without clearly identified underlying molecular mechanisms [4]. Nevertheless, it is believed that oxidative stress and mitochondrial damage favored by the detrimental genetic and environmental factors are the main origins of death in dopaminergic neurons from SNc [17,94]. Hence, fundamental research (epidemiologic, genetic, animal, etc.) is need to provide this critical data in order to determine real reasons behind the PD. Parallel to obtaining data for the origins of the PD, development of the suitable clinical biomarkers should follow. Although there are plenty of new data on pathogenesis, pathoanatomy, and development of new diagnostic for the PD (SPECT, PET, fMRI), still diagnosis of the PD heavily depends on degeneration of the SN cells [36] and the physical examination and clinical diagnostic criteria. Unfortunately misdiagnose rate is fairly high (10-50%) even by movement disorder specialist [47,95]. This is further complicated as PD symptoms tend to fluctuate with time and hinder objective and unbiased monitoring of disease progression [27]. This disease is often diagnosed when degenerative process is in the advanced stage and when more than 80% of dopaminergic neurons of the SN are lost [1]. In that stage a potential neuroprotective therapies are not possible, only symptomatic ones. Given the difficulties with clinical diagnosis of the PD (particularly in earlier stages of the disease when neuropretection is possible), there is a pressing need to identify reliant diagnostic biomarkers [96].

The development of biomarkers that will predict, diagnose, evaluate, and prognosticate PD is essential for patient's health care and research [27]. In addition, unbiased discovery is underway using techniques including metabolomics, proteomics, and transcriptomics (gene profiling) [97]. Recently, it was also suggested that post-transcriptional regulation has important role in molecular mechanisms for PD [86]. Several potential biomarkers identified in other diseases or in other types of biological fluids are investigated as blood-based biomarkers for the PD.

Among multiple adducts of nucleoside oxidation, 8-OHdG and 8-hydroxy-guanosine are two of the most common modifications of nucleic acids under oxidative stress [72]. Therefore 8-OHdG is recognized as a biomarker of oxidative DNA damage caused by the ROS, and logically it was believed that it might also serve as good biomarker for the PD [73].

Based form the current data on PD pathogenesis, α-synuclein is the first candidate for the potential biomarker [98]. Next to α-synuclein, DJ-1 is the second major candidate [67] for the possible PD biomarker in both CSF and plasma/serum [48].

It is believed that UA has a protective effect on the central nervous system, against oxidative damage [58,99] with levels of the UA responding to the severity and type of the PD. This suggests that the UA may be useful biomarkers able to specify risk, intensity and PD subtype.

Data implies that GSTP can be useful biomarker for the PD, however it should be considered as gauge of the general neurodegeneration rather than disease specific biomarker. Also increased levels of the GSTP with the PD suggests that neurodegeneration is prevented by some "*redox compensatory mechanism"* that may explain reduction of this protein in the CSF.

The PC occurs as a product of the oxidative stress where ROS (carbonate radical) binds with proteins (primary with proline, arginine, histidine, and glutamic acid) and forms PC [75]. As a consequence, newly formed PC have different structure and function and may serve as oxidative stress biomarker.

miRNAs are accessible for diagnosis as they are present in the circulating blood, plasma, serum, CSF, saliva, and elsewhere [78,100-104]. Potentially good specificity and accessibility makes miRNAs an excellent candidates for the PD biomarker able to provide precise and non-invasive diagnosis [78].

Inflammation is important pathophysiological process in the PD and various inflammatory markers were studied as a potential predictive biomarker for this disease. Two of the best biomarkers for PD associated inflammation are Hyc and CRP [91].

Due to documented involvement of several genes in the pathology of the PD it is likely that polygenetic mutations are risk factors for the development of PD. These mutations result in formation of tissues, proteins and peptide that with acceptable specificity and selectivity may became useful biomarkers for the PD [105].

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