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Oxidative stress parameters and their relation to motor subtype of Parkinson's disease and levodopa treatment status

Milan Grofik[1], Michal Cibulka[2], Jana Oleksakova[1], Stefan Sivak[1], Daniel Cierny[3], Zuzana Tatarkova[4], Marian Grendar[5], Vladimir Nosal[1], Robert Ruzinak[1], Egon Kurca[1] and Martin Kolisek[2]

[1] *Clinic of Neurology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[2] *Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[3] *Department of Clinical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[4] *Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*
[5] *Department of Bioinformatics, Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia*

Abstract. Parkinson's disease (PD) is an oxidative stress-linked neurodegenerative disorder, with the highest prevalence among seniors. The objective of this study were: (1) to analyse levels of following oxidative stress parameters: total antioxidant capacity (TAC), uric acid (UA), total glutathione (tGSH), bilirubin (Bil) and albumin (Alb), in blood of PD patients and healthy controls; (2) to find possible associations of examined oxidative stress parameters with PD subtypes and levodopa treatment status; and (3) to evaluate power and relevance of the aforementioned oxidative stress parameter for the prediction of onset and progression of PD by utilizing Random Forest machine learning (RFML). Oxidative stress parameters were determined in 125 PD patients and 55 healthy controls. Evaluated with frequentist statistics, our data revealed that UA is the only oxidative stress parameter associated with PD. However, when the PD cohort was divided in gender-dependent manner, tGSH and Bil were also significantly associated with PD in subgroup of female patients. RFML rendered no predictive power of any of the tested oxidative stress parameters in respect to PD, its subtypes, and/or status of levodopa treatment. In conclusion, despite the positive association of UA with PD (in complete cohort of PD patients) and of tGSH and Bil with PD but only in female patients, these oxidative stress parameters are of no use in clinical practice due to the lack of the predictive/diagnostic power.

Key words: Parkinson's disease — Oxidative stress — Biomarkers — Uric acid — Glutathione

Abbreviations: Alb, albumin; Bil, bilirubin; CRE, copper reducing equivalents; GSH, reduced glutathione; GSSG, oxidized glutathione; H&Y scale, Hoehn and Yahr scale; MPD, mixed type of Parkinson's disease; PD, Parkinson's disease; PIGD, postural instability and gait difficulty; RF, Random Forest; RFML, Random Forest machine learning; TAC, total antioxidant capacity; TDPD, tremor-dominant Parkinson's disease; tGSH, total glutathione; UA, uric acid.

Correspondence to: Michal Cibulka, Biomedical Centre Martin, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Mala Hora 4D, 03601 Martin, Slovakia
E-mail: michal.cibulka@uniba.sk

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. It is a complex neurodegenerative disorder whose pathogenesis is not fully understood.

One of the important mechanisms is oxidative stress (Gaki and Papavassiliou 2014). Brain requires high oxygen supply and significant amount of oxygen is converted to reactive oxygen species which overproduction increases oxidative stress in PD patients (Valera-Alberni and Canto 2018). It is suggested that the dopamine metabolism, high levels of iron and calcium in substantia nigra, mitochondrial dysfunction, and neuroinflammation contribute to the increased oxidative stress and dopaminergic neurons' loss in the brains of PD patients (Chang and Chen 2020). The niveau of oxidative stress in PD might be modulated by the multiple factors, such as the subtype of PD, treatment strategies, and/or levodopa treatment. However, the experimental evidence for the latter statement is often controversial in available literature. For example, some published works assumed pro-oxidative and other anti-oxidative effects of levodopa (Colamartino et al. 2018).

Oxidative stress arises primarily because of antioxidant systems not being able to effectively eliminate free radicals (Blesa et al. 2015).

In PD, antioxidant parameters such as levels of uric acid (UA), total glutathione (tGSH), bilirubin (Bil), albumin (Alb), and the total antioxidant capacity (TAC) were the most intensively studied. Currently, these oxidative stress parameters are not considered only as being potential biomarkers of the onset and progression of PD, but also as being putative therapeutic targets in PD treatment, in particular UA, GSH and Bil (Chen et. al. 2012; Dias et al. 2013; Crotty et al. 2017; Yu et al. 2017; Jayanti et al. 2021; Wang et al. 2021; Leathem et al. 2022).

UA is one of the endogenous antioxidants considered to be a stress biomarker related to PD. It is present in the blood and brain tissue with the potential to scavenge superoxide, peroxynitrite, and hydroxyl radicals (Pacher et al. 2007). It also forms a strong bond with iron (ferrous, ferric) cations preventing oxidative damage in PD (Jellinger 1999). Another antioxidant that plays a significant role in maintaining redox homeostasis and cell membrane stability is glutathione. It is considered the main intracellular redox system. A decrease in reduced glutathione (GSH) is considered one of the earliest biochemical changes in PD (Martin and Teismann 2009). Bil is considered a natural antioxidant and its serum concentration was found to be a possible marker of heme oxygenase isoform 1 activity. Heme oxygenase is an important enzyme which regulates oxidative balance, with overexpression in dopaminergic cells exposed to oxidative stress. The role of bilirubin has not been fully elucidated in PD and has shown controversial results over the last few decades (Macías-García et al. 2019). Alb is currently established as a plasma antioxidant. Endogenous serum Alb is considered an important extracellular molecule responsible for maintaining the plasma redox state. Serum Alb may act as an antioxidant and antiinflammatory protein, which can protect against the disease, including PD (Wang et al. 2017). TAC measures antioxidant capacity of biomolecules from a variety of samples *via* a single electron transfer mechanism (reduction of Cu^{2+} to Cu^{+}) by antioxidants such as UA. Copper is advantageous over iron-based antioxidant assays because all classes of antioxidants, including thiols, are detected with marginal radical interference.

We hypothesized that the oxidative stress parameters levels differ based on the motor subtype of PD and the levodopa treatment status. Therefore, the main goal of our study was to determine whether the levels of the selected five oxidative stress parameters (UA, tGSH, Bil, Alb and TAC) depend on the motor subtype of PD and/or if the markers' concentrations are influenced by the treatment with levodopa in our PD patients' group.

Materials and Methods

The study cohort consisted of 180 subjects – 125 PD patients (71 male and 54 female) with a mean age of 66.27 years (39–86 years) and 55 healthy age- and sex-matched controls (33 male and 22 female) with a mean age of 65.27 years (45–85 years). The PD cohort represented outpatients examined at the Department of Neurology in Martin, Slovakia, in the period from January 2018 to December 2018.

All patients met the current MDS (Movement Disorders Society) clinical diagnostic criteria for PD. For the staging of the functional disability, we used modified Hoehn and Yahr (H&Y) scale 1–5. Patients aged at the onset of the first symptoms of the disease over 20 years of age (exclusion of juvenile PD with the genetic background of the disease) and patients treated exclusively with basic dopaminergic therapy (levodopa/carbidopa only or with entacapon and dopamine agonists) were included. Out of the total number of 215 examined PD patients, 125 patients met the inclusion criteria. The inclusion criteria were not met by 79 patients who were on invasive and combined (supported and dopaminergic therapy) and 11 newly diagnosed, untreated patients.

We defined several groups and subgroups of participants – the group of healthy controls (HCG), the group of all patients together (PDG), three subgroups based on the motor type of PD, and two subgroups based on the treatment. According to the predominant clinical motor symptom (tremor, bradykinesia, rigidity), PD patients were divided into 3 subgroups: a tremor-dominant PD

subgroup (TDPD) with 37 patients (22 male and 15 female), a bradykinetic-rigid subgroup called "postural instability and gait difficulty" (PIGD) with 35 patients (20 male and 15 female), and a mixed type subgroup (MPD) with 52 patients (31 male and 21 female). To elucidate effect of levodopa the PD patients were divided into two subcohorts: a subgroup treated with levodopa/carbidopa (T-wL) with 97 patients (60 male and 37 female), and a subgroup treated with no levodopa/carbidopa (T-nL) with 28 patients (11 male, 17 female). In the T-wL subgroup were patients treated with levodopa/carbidopa or levodopa/carbidopa/entacapon only or with combination with dopamine agonists. In the T-nL subgroup were patients treated with dopamine agonists only. Patients treated with deep brain stimulation, pump therapy (subcutaneously administered apomorphine or levodopa/carbidopa intestinal gel) were excluded from this study. Similarly, patients receiving adjuvant therapy with rasagiline, biperiden and amantadine were excluded from the study to reduce the effect of these drugs on the monitored oxidative stress parameters.

We investigated oxidative stress parameters in fasting peripheral venous blood samples (sampled at 8 a.m.): two in plasma: TAC and tGSH, and three in serum: UA, Bil and Alb.

The plasma levels of TAC a tGSH: Fasting peripheral venous blood samples were taken from all patients and healthy controls to tubes containing EDTA. The samples were centrifuged at 4000 × *g*, at 4°C for 5 min, aliquoted, and stored at −80°C until use.

TAC assay kit was prepared according to the manufacturer`s instructions (Cell Biolabs, STA-360) with uric acid as a standard and measured at 490 nm on Microplate Reader Synergy H4 (BioTek, USA). Results are expressed as mmol/l of CRE (Copper reducing equivalents) based on the fact that 1 mmol/l of uric acid = 2189 μmol/l CRE).

The total amount (tGSH) of reduced (GSH) and oxidized glutathione (GSSG) was determined by using commercially available Glutathione Assay Kit (Sigma, S0260). We measured changes in absorbance of samples on a Microplate Reader Synergy H4 (BioTek, USA) at 412 nm compared to a control containing 5% sulfosalicylic acid instead of a sample. The resulting values of tGSH concentration are expressed as μmol/l.

The serum levels of UA, Alb and Bil: Fasting peripheral venous blood samples were taken from all patients and healthy controls to tubes containing Gel&Clot activator. The samples were centrifuged at 4000 × *g*, at 4°C for 5 min, aliquoted, and stored at −80°C until use. The serum levels were measured photometrically in an Olympus AU700 analyser (Beckman Coulter, USA) using appropriate kits (Albumin cat. no. OSR6202, Total bilirubin cat. no. OSR6212, Uric Acid cat. no. OSR6298, Beckman-Coulter, USA).

In our study we have analysed possible association between concentrations (and their transformed values – logarithm of concentrations), pharmacotherapy status (T-wL, T-nL), PD subtype (TDPD, PIGD, MPD), age and gender in a cohort of PD patients and HCG.

The study was approved by the ethics committee of the Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin (EK 1935/2016), and all patients signed an informed consent with participating in the study.

Statistics

Data were analysed by means of multivariate linear regression modelling. Parameters of the model were fitted by the ordinary least squares method and the model was subjected to standard diagnostics. Based on the diagnostics a small number (at most four) of outliers, leverage points or influential observations were removed from the data and the model was refit. Outliers were detected using the quantile-quantile plot of studentized residuals with the 95% confidence band constructed by bootstrap; leverage points were identified by using the hat values and influential observations were detected by using Cook's distance. In case of most predictors, concentrations could not be used for modelling due to a non-Gaussian data distribution. Natural logarithm was chosen as a method of data transformation. If age was not a significant predictor in multivariate regression modelling, two-way type III ANOVA was used, followed by *post-hoc* test with Benjamini-Hochberg correction. Type III ANOVA was used because the data were unbalanced. Data were used for iterative regression model building and consequently for making interferences using the developed model. Hence, the obtained *p*-values should be considered cautiously. Besides multivariate regression and two-way ANOVA, imbalanced Random Forest (RF) analysis was used in order to assess the diagnostic value of predictors.

Results of multivariate linear regression modelling and two-way type III ANOVA are presented for individual predictors as:

(a) General comparison – PDG *vs*. HCG, and male *vs*. female comparison.
(b) Comparison between patients depending on levodopa status and comparison of these two groups (T-wL, T-nL) and controls in respect to gender (male *vs*. female).
(c) Comparison between patients depending on clinical phenotype subtypes of PD patients and comparison of these three groups (TDPD, PIGD and MPD) and controls in respect to gender (male *vs*. female).

Results

The basic characteristics of patient groups and healthy controls and their oxidative stress parameters values are summarized in Table 1.

Table 1. Oxidative stress parameters in HCG and PD subgroups

	HCG	PD					
		PDG	T-nL	T-wL	TDPD	PIGD	MPD
n	55	125	28	97	37	35	53
Age (years)	65.27 ± 10.55 (45–85)	66.27 ± 9.19 (39–86)	63.82 ± 8.83 (43–86)	66.98 ± 9.17 (39–85)	67.59 ± 13.32	64.74 ± 9.31	66.21 ± 9.82
Sex (male/female)	33/22	71/54	11/17	60/37	22/15	20/15	31/21
Duration of PD (years)	0	8.06 ± 4.58	–	–	–	–	–
Hoehn and Yahr scale	0	2.57 ± 0.86	–	–	–	–	–
TAC (μmol/l CRE)	885.70 ± 151.39	912.60 ± 170.97	841.10 ± 174.76	933.17 ± 164.19	898.84 ± 233.98	948.65 ± 154.23	896.92 ± 167.51
Alb (g/l)	41.34 ± 3.40	41.51 ± 4.14	43.25 ± 3.31	41.03 ± 4.23	14.53 ± 13.91	11.49 ± 2.89	13.0 ± 5.52
Bil (μmol/l)	11.33 ± 4.20	13.05 ± 8.57	15.14 ± 15.63	12.44 ± 4.75	42.09 ± 7.37	40.08 ± 6.12	42.03 ± 2.74
UA (μmol/l)	328.10 ± 83.05	301.74 ± 83.23	315.4 ± 85.19	297.8 ± 82.23	321.54 ± 101.73	300.51 ± 70.55	287.65 ± 84.63
tGSH (μmol/l)	18.00 ± 9.61	16.02 ± 8.83	16.99 ± 8.99	15.74 ± 8.76	13.99 ± 7.49	14.88 ± 6.08	18.29 ± 10.77

HCG, healthy controls group; PD, Parkinson's disease; PDG, PD patient group; T-nL, PD patient subgroup treated without levodopa; T-wL, PD patient subgroup treated with levodopa; TDPD, tremor-dominant PD; PIGD, postural instability and gait difficulty; MPD, mixed type PD; *n*, number of patients; TAC, total antioxidant capacity; CRE, copper reducing equivalents; Alb, albumin; Bil, bilirubin; UA, uric acid; tGSH, total glutathione.

Total antioxidant capacity

There was no significant association between TAC and health status or a gender of probands. Age was a significant predictor ($p = 0.009$), positively correlated with ln[TAC] in analysed cohort. There was no significant association between TAC and levodopa status or a gender of probands. Association between TAC and PD subtype or gender of probands was not significant. Age was significantly (positive correlation; $p = 0.004$) associated with ln[TAC] in analysed cohort.

Bilirubin

Because age was not a significant predictor in multivariate regression modelling, two-way type III ANOVA with *post hoc* test was used. ln[Bil] was significantly (p.adj. = 0.002) higher in female PD patients than in female controls in *post-hoc* test. ln[Bil] was significantly (p.adj. = 0.045) higher in female PD patients, treated with levodopa than in healthy female probands. ln[Bil] was significantly higher in female PD patients with MPD subtype (p.adj. = 0.023) as well as in female PD patients with TDPD subtype (p.adj. = 0.008) when compared to healthy female probands.

Albumin

There was no significant association between ln[Alb] and health status or a gender of probands. Age was a significant predictor ($p = 0.045$), negatively correlated with ln[Alb] in analysed cohort. Because age was not a significant predictor, two-way type III ANOVA with *post hoc* test was used. In a cohort of female PD patients without levodopa pharmacotherapy, ln[Alb] was significantly higher than in control cohort (p.adj. = 0.008) as well as in cohort of female PD patients, treated with levodopa (p.adj. = 0.008). There was no significant association between PD subtypes, controls and ln[Alb] values.

Uric acid

We found a significant association between ln[UA] and age of probands (positive correlation; $p = 0.004$) and health status ($p = 0.015$) with ln[UA] being significantly lower in PD cohort. There was an association between ln[UA] and treatment status, which was significant for female PD patients treated with levodopa (negative correlation; $p = 0.011$). Age of probands was significantly ($p = 0.013$) positively correlated with ln[UA]. There was an association between ln[UA] and clinical phenotype, which was significant for male PD patient cohort ($p = 0.009$) as well as PIDG patient cohort ($p = 0.047$). For both, the correlation was negative.

Total glutathione

Because age was not a significant predictor in multivariate regression, two-way type III ANOVA was used. ln[tGSH] was significantly (*p*.adj. = 0.002) higher in female controls than in female PD patients. In a cohort of female PD patients with levodopa pharmacotherapy, ln[tGSH] was significantly lower than in control cohort (*p*.adj. = 0.011). There was a significantly higher ln[tGSH] in female control group than in female PD group with mixed (*p*.adj. = 0.046) as well as tremorous (*p*.adj. = 0.046) clinical phenotype. ln[tGSH] was also significantly higher in male PD group with mixed phenotype when compared to male PD group with tremorous phenotype (*p*.adj. = 0.037).

Random Forest analysis with age, gender and level of analytes as predictors

RF analysis was chosen as an analytical tool enabling to determine diagnostic performance of predictors. None of the predictors or combination of predictors exhibited significant diagnostic value in any of the setup modes summarised in Table 2.

Discussion

Oxidative stress contributes to the sequence of pathophysiological processes leading to degeneration of dopaminergic neurons in PD. However, an intimate linkage between oxidative stress and other components of the process of neurodegeneration (such as mitochondrial dysfunction, excitotoxicity, NO toxicity, inflammation) makes it difficult to determine whether oxidative stress leads to, or is a consequence of, these events (Jenner 2003).

The decrease of TAC, decreased levels of serum Alb, UA, and tGSH and increased levels of total and direct/conjugated Bil were associated with PD (Adiga et al. 2006; Wei et al. 2018; Wang et al. 2019; Jin et al. 2020). These parameters are all linked to oxidative stress and are often being discussed as being potential markers of various diseases and/or degenerative pathologies.

Total antioxidant capacity

In this study, we did not show a significant difference between the TAC values of PDG and HCG. We also did not find an effect of levodopa and disease subtype on TAC values. The sex of the probands did not affect the TAC levels either, but we observed the effect of age (positive correlation). To our knowledge, in the available literature, there is only one work showing the lower TAC levels in patients with PD compared to the control group (Adiga et al. 2006). This was explained by the decrease in serum Alb levels as an essential component of TAC in PD patients, which was due to insufficient intake or impaired amino acid absorption during levodopa treatment. Even though, in general we have not observed a significant decrease of Alb in cohort of PD patients, but in the subcohort of levodopa-treated women the levels of Alb were significantly lower compared to levodopa-untreated PD female patients. This observation agrees with the work of Adiga et al. (2006) regarding the Alb, however our data showed that TAC values do not correlate with Alb levels even in this subcohort.

Albumin

Transferrin, caeruloplasmin, haptoglobin, UA, Bil, alpha-tocopherol, glucose, and Alb constitute extracellular antioxidant defences in blood plasma, but albumin is the most potent one (Sitar et al. 2013). During many acute or chronic disease conditions, levels of biomarkers of oxidative protein damage increase and this observation continues with considerable oxidation of human serum Alb (Sitar et al. 2013).

In our study, we did not show a significant difference between Alb values in PDG and HCG. This fact is consistent with a meta-analysis of five studies involving a total of 351 PD patients and 260 controls (Wei et al. 2018). We did not find an effect of gender and disease subtype on Alb values, but the effect of age (positive correlation) and levodopa treatment (negative correlation) was significant. As mentioned above, female patients treated with levodopa had significantly lower Alb levels compared to female patients treated without levodopa.

Bilirubin

Mild hyperbilirubinemia exhibits protective effects against various chronic diseases mediated by increased oxidative stress (Wagner et al. 2015). However, unconjugated bilirubin at high concentrations produces severe neurological damage and death associated with kernicterus due to oxidative stress and other mechanisms (Rawat et al. 2018).

Table 2. Performance of predictors in respective setup modes of analysed cohorts

Cohorts	Missclasification rate (%)	AUC
PDG *vs.*HCG	46.84	0.614
T-wL *vs.*T-nL	40.27	0.687
T-wL *vs.*HCG	43.09	0.658
T-nL *vs.*HCG	53.29	0.482
PIGD *vs.*MPD *vs.*TDPD *vs.*HCG	65.92 (mean)	–

AUC, Area under the ROC curve; ROC, receiver operating characteristic. For more abbreviations, see Table 1.

Here, we demonstrated significantly higher Bil values in PD patients, but only in women when compared to female controls. It was not observed an effect of age on Bil levels. We found that higher Bil levels are in patients taking levodopa compared to patients without levodopa. Bil levels were also affected by the phenotype of the disease – patients with TDPD and MPD subtype of PD had higher levels than controls, regardless of gender. The relationship between Bil and PD was investigated by Jin et al. in a meta-analysis that included 8 studies with a total of 1463 PD patients and 1490 controls. Metanalysis showed that PD patients had higher total and conjugated Bil values compared to controls (Jin et al. 2020). Our results confirm this fact, but only in the group of women. Elevated Bil levels have also been found in newly diagnosed PD subjects (Moccia et al. 2015). Macías-García and co-workers did not find an effect of age, gender, and treatment on Bil levels in PD patients, but found that patients with shorter disease duration and milder severity had higher Bil levels (Macías-García et al. 2019). Scigliano and colleagues found, in agreement with our results, higher Bil levels in levodopa-treated patients compared to untreated PD patients and healthy controls (Scigliano et al. 1997). Other authors focused on the association of motor PD subtype and total, conjugated and unconjugated bilirubin levels in PD patients and found that PD patients had lower levels of unconjugated Bil, and also that higher levels of unconjugated Bil were associated with TDPD subtype of PD (Li et al 2019). Interestingly, Bil and its enzymatic machinery and precursors have offered potential benefits by targeting multiple mechanisms in chronic diseases, including PD (Jin et al. 2020; Jayanti et al. 2021).

Uric acid

UA, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension, and cardiovascular disease, conditions associated with oxidative stress and risk factors of neurodegeneration (Sautin and Johnson 2008; Firoz et al. 2015; Mazon et al. 2017).

In this study, we found significantly lower UA levels in PD patients compared to controls. This correlates with the results of a meta-analysis combining 13 studies with a total of 2379 PD patients and 2267 controls (Wen et al. 2017). Serum UA levels in the middle-late-stage PD patients with higher H&Y score were significantly lower in this meta-analysis than those patients in the early-stage PD with lower H&Y score (Wen et al. 2017). In our work we did not find any influence of gender, but the influence of age was significant (positive correlation). We also found the effect of levodopa on UA levels in patients with PD, but only in females. Female patients taking levodopa had significantly lower UA levels than women in the healthy control group. We screened out only one study which analysed the effect of levodopa on UA levels. The authors of this study found similar serum levels of UA in patients taking levodopa (Vieru et al. 2016). Andreadou and colleagues found that age and severity of PD did not significantly affect serum UA concentrations, while gender contributes significantly to UA levels (Andreadou et al. 2009). Strong and significant inverse correlations of UA with disease duration and daily levodopa dosage were observed (Andreadou et al. 2009). These associations were gender-dependent and significant for men but not for women (Andreadou et al. 2009). Furthermore, it was demonstrated that serum UA concentration was lower in PD patients in severe stages than in those in moderate stages of PD, and levodopa equivalent daily dose was associated with lower serum UA concentration in men (Jesus et al. 2013). Concerning the influence of motor subtypes of PD, the levels of UA in our study were significantly lower in the MPD and PIGD subgroup compared to HCG, but in women only. The highest UA levels were detected in the TDPD subgroup, which agrees with the results of recent studies of Lolekha et al. (2015), Huertas et al. (2017), and Huang et al. (2018). However, it should be noted that the level of UA is also significantly affected by diet, which is the major limitation of this and also other studies.

Total glutathione

The possible role of GSH in pathophysiology of PD is being substantiated by the study of Pradhan and colleagues, who showed that scopoletin, a common potent neuroprotective derivative in most of the nootropic herbs, increased cellular resistance to oxidative stress through efficient recycling of GSH and thus preventing oxidative damage (Pradhan et al. 2020). Scopoletin-treated cells showed increased levels of reduced glutathione making them resistant to perturbation of antioxidant machinery or neurotoxin MPP^+ (Pradhan et al. 2020).

In our study, we found lower levels of tGSH in PDG compared to HCG, but only in women. We did not find an effect of age on tGSH levels. PD patients receiving levodopa had significantly lower tGSH levels than patients without levodopa treatment. The disease phenotype also affected the level of tGSH. Patients – women with TDPD subtype had significantly lower tGSH than control women. Similarly, female patients with the MPD subtype had lower tGSH levels compared to control women. Patients with TDPD subtype had significantly lower tGSH values than patients with MPD. In bibliography, it is problematic to adequately evaluate glutathione levels in PD patients, as different works follow different parameters – total, oxidized, reduced glutathione and their mutual ratios. Regarding total glutathione, the paper of Younes-Mhenni et al. (2007) documented that tGSH levels are not significantly different between PD patients

and healthy controls. But the meta-analysis of Wei and colleagues which studied multiple oxidative stress parameters and covered seven original studies with 397 patients and 481 controls concluded that tGSH levels were significantly lower in PD patients compared to healthy controls (Wei et al. 2018). This conclusion is consistent with our observation, but only in the group of women.

Furthermore, we also showed the effect of treatment and disease phenotype on tGSH levels, which was not yet reported in the literature. Mischley and colleagues found in their study an inverse correlation between tGSH levels and PD severity (Mischley et al. 2016).

In summary, for most of the studied biochemical parameters, there is a statistically significant difference between PD-patients and controls, but a number of other factors come into play, fundamentally affecting the levels of these parameters, such as age, sex, variable PD phenotype, duration of the disease, disease severity or treatment of PD. Based on the above, it is therefore difficult to assign a significant role to a particular biochemical parameter as a diagnostic or prognostic marker of PD. This notion also supports the statistical view, when with conventional frequentist statistics we proved a significant difference between patients and controls, but with the RF machine learning approach, we found that the oxidative stress predictors have negligible or literally no power in predicting the disease or its progression. According to Wang and colleagues, a multivariable logistic regression showed that the decreased levels of serum UA and Alb were independent risk factors in PD. The receiver operating characteristic (ROC) curve analyses showed that the area under the ROC curve (AUC) for serum UA and Alb was 0.669 and 0.883, respectively (Wang et al. 2017). The combination of serum Alb and UA improved the AUC to 0.898 (Wang et al. 2017). However, our RF machine learning analysis did not confirm these findings; in addition, the Wang's team did not include the levodopa treatment factor in their analysis.

Age is being considered among the strongest factors influencing oxidative stress markers, and it should be noted that age is also the most important risk factor for onset of neurodegenerative diseases, such as PD. It is therefore difficult to assess, whether it is the age eventually with age-associated neurodegeneration, which may affect the biochemical parameters of PD (including oxidative stress parameters). In terms of gender, we know that PD affects men slightly more frequently. Most biochemical parameters are gender-dependent. The biological mechanisms underlying such sex specificity remain not unequivocal (Yu et al. 2017). Reasons for this discrepancy may include: 1. sex-specific hormones (in particular estrogen may play a key role in dopamine modulation and neuroprotection of dopamine neurons; Behl et al. 1997); 2. female PD patients weigh less than male PD patients, resulting in a relative increase in the concentrations of levodopa in the plasma of female PD patients (Müller et al. 2000); 3. several cardiovascular risk factors, such as hypercholesterolemia and hypertension, have been reported to be associated with an increased risk of PD in women but not in men (Simon et al. 2007).

Conclusions

Our data lead us to conclude that studied oxidative stress parameters have negligible power to be used in clinical practice as diagnostic or progression-monitoring tools for PD and likely also any other neurodegenerative disease.

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