Parkinsonism and Related Disorders 63 (2019) 213–216

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

Increased bilirubin levels in Parkinson's disease

Daniel Macías-García[a], Carlota Méndez-Del Barrio[a], Silvia Jesús[a], Miguel Angel Labrador[a], Astrid Adarmes-Gómez[a], Laura Vargas-González[a], Fátima Carrillo[a], Pilar Gómez-Garre[a,b], Pablo Mir[a,b,*]

[a] Unidad de Trastornos del Movimiento, Servicio de Neurología y Neurofisiología Clínica, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain
[b] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain

ARTICLE INFO

Keywords:
Bilirubin
Parkinson's disease
Pathophysiology
Heme oxygenase 1

ABSTRACT

Introduction: Oxidative stress plays a key role in Parkinson's disease (PD) etiopathology. Heme oxygenase, an important enzyme which regulates oxidative balance, converts heme molecules into carbon monoxide, iron and biliverdin/bilirubin. The role of bilirubin has not been fully studied in PD, showing controversial results over the last few decades. Our aim was to investigate the relationship between bilirubin levels and PD. Secondly, we sought to evaluate the link between bilirubin concentration with PD progression, severity and dopaminergic treatment.

Methods: We included 420 PD patients (56% males, mean age: 64 ± 12 years) and 435 healthy controls (47% males, mean age: 58 ± 17 years). Bilirubin levels in both groups were compared using linear regression and multivariate analysis adjusted according to age and sex. Secondly, a case study with the PD cohort was carried out and bilirubin levels were correlated with current treatment, duration and severity of disease.

Results: Bilirubin levels were significantly higher in PD patients than in controls (PD: 0.56 ± 0.26 mg/dl, controls: 0.45 ± 0.22 mg/dl; $p < 0.001$). In PD patients, we demonstrated a negative correlation between bilirubin levels and disease duration ($p < 0.05$). Higher bilirubin concentrations were identified in PD patients with Hoehn & Yahr stage ≤3. No relationship between bilirubin and treatment was found in PD patients.

Conclusions: Increased bilirubin levels are particularly related to the first years of PD. Overexpression of oxidative enzymes could play an important role in PD etiology, leading to higher bilirubin levels in the early stages of PD.

1. Introduction

Parkinson's Disease (PD) is one of the most common neurodegenerative disorders. Although its pathogenesis is widely studied, the role of many genetic and environmental factors remains unknown [1,2]. Oxidative stress is thought to play a key role in PD etiopathology and is commonly understood as an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant mechanisms [1]. Heme oxygenase (HO) is an important enzyme which regulates oxidative balance, with overexpression in dopaminergic cells exposed to oxidative stress [3]. HO converts heme molecules into carbon monoxide, iron and biliverdin/bilirubin. Bilirubin is considered to be a natural antioxidant and its serum concentration has been considered as a possible marker of HO isoform 1 (HO-1) activity [4,5]. Bilirubin plays a dual intracellular role with a concentration-dependent activity. Bilirubin acts as a potent ROS scavenger in situations with lower intracellular levels. However, increased intracellular concentration may have a toxic effect. There is a dynamic equilibrium between plasmatic bilirubin levels and bilirubin concentration in extravascular tissues [6]. The role of bilirubin has not been fully studied in PD, and has shown controversial results over the last few decades [7–9]. Although it has been suggested that bilirubin plays a protective role in cardiovascular disease and other disorders [10], recent studies have found increased levels of bilirubin in newly-diagnosed PD patients [9].

From the point of view of pathogenic factors in PD, levels of bilirubin and HO-1 expression is a new pathway that should be studied. Our study aimed to investigate the relationship between serum bilirubin concentration and PD. Secondly, we sought to study the relationship between bilirubin levels and severity of disease and dopaminergic replacement therapy in PD.

* Corresponding author. Unidad de Trastornos del Movimiento, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío, Av. Manuel Siurot s/n, 41013, Seville, Spain.
E-mail address: pmir@us.es (P. Mir).

https://doi.org/10.1016/j.parkreldis.2019.01.012
Received 15 May 2018; Received in revised form 21 December 2018; Accepted 12 January 2019

2. Methods

We included 420 PD patients (56% males, mean age: 64 ± 12 years) and 435 healthy controls (47% males, mean age: 58 ± 17 years). Patients were recruited from the Movement Disorder Clinic at Hospital Universitario Virgen del Rocio in Seville, Spain. Healthy controls were non-blood relatives of PD patients and volunteers from the primary care centers in the same geographical area as the PD patients. The diagnosis of PD patients was made using the United Kingdom Parkinson's Disease Society Brian Bank criteria. Exclusion criteria were any conditions that could affect serum bilirubin concentration. Firstly, we excluded subjects who had treatment with statins or chemotherapy, alcoholism, liver disease, biliary surgery or history of biliary colic, and recent hemolytic anemia. To avoid the influence of the well-known bilirubin-induced neurotoxicity, we excluded subjects with bilirubin levels high enough to produce it (serum bilirubin concentration > 2 mg/dl). Additionally, in the healthy control group, we considered as exclusion criterion the presence of any relevant neurological disease. Demographic and clinical data as well as current treatment was recorded in both groups. To assess the severity of the disease, we used the modified Hoehn & Yahr scale (HY) and we gathered our PD population into three groups depending on HY stage (group 1 = HY 1–2; group 2 = HY 2.5–3; and group 3 = HY 4–5). In addition, data regarding dopaminergic replacement therapy was recorded. Serum total bilirubin concentration was determined in peripheral blood and analyzed in the laboratory of our Hospital.

Total bilirubin levels, hemoglobin concentration and tracers of liver function were compared in both groups by linear regression with multivariate analysis adjusted according to age and sex. Additionally, we compared bilirubin levels in both groups with multivariate analysis adjusted according to liver enzymes and hemoglobin in order to avoid confounding factors. To determine if the difference in bilirubin levels was linked to specific disease states, we compared bilirubin concentration in different HY groups to controls with multivariate analysis. However, correlation with motor stage of patients (Unified Parkinson's Disease Rating Scale – Part III) was not possible due to these data not being available for many patients. Demographic data was compared between PD and controls by Chi-square. We designed an age- and sex-matched control set in order to compare bilirubin levels between groups avoiding other possible confounding factors associated with sex or age (View Supplemental Material Methods).

In the PD group, we used bivariate linear regression to analyze the relationship between bilirubin levels and disease duration, treatment and demographics variables adjusted according to confounding variables. Results were considered statistically significant for $p < 0.05$.

Statistical analysis was performed using IBM SPSS Statistics software version 24.0. The study was approved by the local ethics committee, and written consent was obtained from all patients and healthy controls participating in the study.

3. Results

Demographic data and serum bilirubin levels are shown in Table 1. PD patients showed statistically significant higher serum bilirubin levels than healthy controls (0.56 ± 0.26 mg/dl vs 0.45 ± 0.22 mg/dl; $p < 0.001$). No difference in hemoglobin levels was found between groups; however, liver enzymes were slightly higher in controls than in PD patients (Table 1). The difference in bilirubin levels remained significant ($p < 0.001$) after adjusting according to age, sex, liver enzymes and hemoglobin (Fig. 1). Furthermore, this difference amongst groups was observed after repeating the analysis in our age- and sex-matched control set (Supplemental material – Table S1). When we compared bilirubin levels among PD patients with a different HY group and controls, we found that those on mild and moderate stages (HY ≤ 3) had higher bilirubin levels than controls. These results remained statistically significant after adjusting according to age, sex, liver enzymes and hemoglobin (Supplemental material - Table S2).

Table 1
Demographic data, liver profile and serum bilirubin levels in controls and PD patients.

	Healthy Controls (N = 435)	PD patients (N = 420)	P value	Laboratory reference values
Age (*years* ± *SD*)	58 ± 17	64 ± 12	< 0.001	–
Sex (% ♂/% ♀)	47%/53%	56%/44%	0.005	–
Serum bilirubin Concentration (*mg/dl*)	0.45 ± 0.22	0.56 ± 0.26	< 0.001*	0.2–1.2
GOT (IU/l)	23.3	17.7	0.07	10–40
GPT (IU/l)	20.6	14.4	< 0.05	10–40
GGT (IU/l)	26.2	22.7	< 0.05	10–50
Hemoglobin (*g/l*)	139,9	139,3	0.63	136–180

* Adjusted according to sex, age, liver enzymes and hemoglobin.

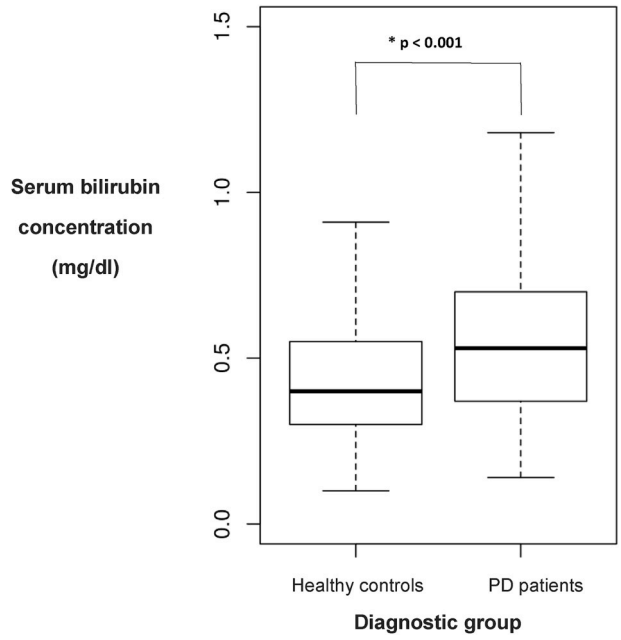

Fig. 1. Difference in bilirubin concentration between healthy controls and PD patients. A boxplot diagram shows the difference in bilirubin concentration between healthy controls and PD determinate by linear regression, *adjusted according to age, sex, liver enzymes and hemoglobin.

In the PD group, we did not find a significant association between bilirubin levels and age, type of dopaminergic treatment (levodopa treatment, dopamine agonist treatment) or levodopa equivalent daily dose (LEDD). However, we found a relationship between bilirubin levels and sex. Higher bilirubin levels were observed in males with PD (Supplemental material - Table S3). We also found a mild but significant negative correlation between bilirubin levels and years of disease duration after adjusting according to sex and LEDD ($p = 0.049$) with a correlation coefficient value of – 0.1 (Supplemental Material - Figure S1).

Although we found differences in bilirubin levels between HY stages compared to healthy controls, within the PD cohort, we did not observe a direct correlation between bilirubin levels and HY stages.

4. Discussion

In this study, we compared serum bilirubin levels in PD patients and healthy controls, and higher bilirubin levels in PD patients were observed. This result remained statistically significant in our sex- and age-matched group, after adjusting according to possible confounding

factors. We also found that bilirubin levels were higher in the first years of the disease and decreased with disease progression, being independent of sex and dopaminergic therapy. To the best of our knowledge, this is the first reported association between bilirubin levels and disease progression. Although we did not observe a direct correlation between bilirubin levels and disease severity, we found that PD patients in early disease stages (HY ≤ 3, functionally independent) have particularly higher bilirubin levels than controls.

These findings concur with previous studies [7,9]. Scigliano et al. found higher bilirubin levels in PD patients, interpreted as a possible effect of dopaminergic replacement therapy [7]. Additionally, Moccia et al. also showed higher bilirubin concentration in drug-naïve PD patients compared to controls [9]. Although they did not observe a relationship with the necessity of initiating dopaminergic treatment after a two-year follow-up, they reported a negative correlation between bilirubin levels and LEDD. In our work, bilirubin levels were not related to dopaminergic therapies or LEDD. Therefore, in our population dopamine replacement therapy does not seem to play a main role in the bilirubin levels of PD patients.

In contrast to our work, Qin et al. described in a Chinese population, lower levels of indirect bilirubin in PD patients compared to healthy subjects, with no difference in total bilirubin concentration [8]. This difference between our results and the previous study might be explained by some factors such as different exclusion criteria or HO polymorphisms, which could vary in different populations.

Even though other antioxidant molecules have been described as decreased in PD, the difference in bilirubin levels between PD and HC may correspond to enzymatic changes in CNS related to PD etiology. HO as well as other enzymatic pathways are suggested to play a key role in oxidative stress and mitochondrial dysfunction, both well-known mechanisms involved in PD etiology. HO-1 is inducible by ROS in CNS. An important role of HO-1 in the oxidative balance is demonstrated in brains with neurodegenerative disorders [3]. Considering these findings, increased levels of bilirubin in PD could be interpreted as a consequence of HO-1 overexpression in CNS during early disease stages. Moreover, it has been suggested that this overexpression could be an adaptive response to oxidative stress in *substantia nigra* of PD patients [3,9]. However, we hypothesize that in PD patients with a longer disease course, lower bilirubin levels might be related to either a failure in that antioxidant system or neurodegenerative changes favored by maintained overexpression of HO-1. The cause of ROS imbalance is still unknown, but an environmental and a genetic component are supposed in most PD forms [2]. The role in PD of genetic variations in the gene that codified HO-1 (*HMOX1)* has not been fully studied. Recently, two functional *HMOX1* variations have been described as potential genetic biomarkers of PD [11], although previously Funke et al. did not find an association between *HMOX1* polymorphisms and PD [12].

The strength of our conclusions is tempered by some limitations. Firstly, we did not have different types of bilirubin (direct and indirect) available in our study. They were not measured by our laboratory in the routine blood test unless a marked hyperbilirubinemia (total bilirubin > 1.5 mg/dl) was detected. Most of the patients with hyperbilirubinemia were excluded from our cohort, as we mentioned above. Secondly, the overall hepatic profile was not completely homogenous in both groups. Higher liver enzymes levels (GGT and GPT) were found in controls. This fact does not justify the differences in bilirubin levels between groups since a hepatic dysfunction would have caused higher bilirubin levels in controls instead of PD. In addition, mean values of liver enzymes were in the normal range without detected hypertransaminasemia in either group. However, we adjusted our analysis of bilirubin between groups according to liver enzymes as previously mentioned. Even though selected exclusion criteria were applied, other possible factors interfering in bilirubin or liver enzymes concentration cannot be fully excluded. Due to the retrospective design of our study, we cannot achieve robust conclusions about a predictive value of serum bilirubin levels in PD progression. Finally, we compared groups (PD and controls) which differ in dopaminergic treatment. This could explain the mild difference in liver enzymes between groups. However, as mentioned above, in our study bilirubin levels were not related to dopaminergic replacement therapy or LEDD so it is difficult to believe in a correlation between bilirubin and dopaminergic replacement therapy.

We conclude that total bilirubin concentrations are higher in PD patients. Our results describe for the first time that this increase in bilirubin levels is related to the initial years of PD. Overexpression of HO-1 could play an important role in PD etiology, leading to higher bilirubin levels in the early stages of PD. Regarding these findings, future genetic analysis of *HMOX1* expression in PD patients might be useful to better understand PD etiology.

Contributorship statement

- Daniel Macías-García: conception and design of the study, analysis and interpretation of data; writing of the first draft and review and critique of the manuscript.
- Carlota Mendez-Del Barrio: acquisition of data; review and critique of the manuscript.
- Silvia Jesús: conception and design of the study; interpretation of data; review and critique of the manuscript.
- Miguel Angel Labrador: analysis and interpretation of data; review and critique of the manuscript.
- Astrid Adarmes-Gomez: acquisition of data; review and critique of the manuscript.
- Laura Vargas-González: acquisition of data; review and critique of the manuscript.
- Fátima Carrillo: acquisition of data; review and critique of the manuscript.
- Pilar Gómez-Garre: analysis and interpretation of data; review and critique of the manuscript.
- Pablo Mir: conception and design of the study; interpretation of data; review and critique of the manuscript.

All the listed authors gave their final approval of the final version of the manuscript.

Conflicts of interest

The authors have nothing to disclose.

Financial disclosure/conflict of interest concerning the research related to the manuscript

This work was supported by grants from the Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional (ISCIII-FEDER) [PI14/01823, PI16/01575], the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía [CVI-02526, CTS-7685], the Consejería de Salud y Bienestar Social de la Junta de Andalucía [PI-0437-2012, PI-0471/2013], the Sociedad Andaluza de Neurología, the Fundación Alicia Koplowitz and the Fundación Mutua Madrileña. Pilar Gómez-Garre was supported by the "Miguel Servet" (from the ISCIII-FEDER) and “Nicolás Monardes” (from of Andalusian Ministry of Health) programmes. Silvia Jesús Maestre was supported by "Juan-Rodes" programme (from the ISCIII-FEDER). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We would like to thank the patients and healthy individuals who participated in this study. We also thank the *HUVR-IBiS Biobank (Andalusian Public Health System Biobank and ISCIII-Red de Biobancos PT13/0010/0056)* for the human specimens used in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.01.012.

References

[1] J.A. Obeso, M.C. Rodriguez-Oroz, C.G. Goetz, C. Marin, J.H. Kordower, M. Rodriguez, E.C. Hirsch, M. Farrer, A.H. Schapira, G. Halliday, Missing pieces in the Parkinson's disease puzzle, Nat. Med. 16 (2010) 653–661.

[2] A.B. Singleton, M.J. Farrer, V. Bonifati, The genetics of Parkinson's disease: progress and therapeutic implications, Mov. Disord. 28 (2013) 14–23.

[3] H. Schipper, W. Song, H. Zukor, J.R. Hascalovici, D. Zeligman, Heme oxigenase-1 and neurodegeneration: expanding frontiers of engagement, J. Neurochm. 110 (2009) 469–485.

[4] L. Ziberna, M. Martelanc, M. Franko, S. Passamonti, Bilirubin is an endogenous antioxidant in human vascular endothelial cells, Sci. Rep. 6 (2016) 29240, https://doi.org/10.1038/srep29240.

[5] M.F. McCarty, Serum bilirubin may serve as a marker for increased heme oxygenase activity and inducibility in tissues – a rationale for the versatile health protection associated with elevated plasma bilirubin, Med. Hypotheses 81 (2013) 607–610.

[6] J.F. Watchko, C. Tiribelli, Bilirubin-induced neurologic damage - mechanisms and management approaches, N. Engl. J. Med. 369 (2013) 2021–2030.

[7] G. Scigliano, F. Girotti, P. Soliveri, M. Musicco, D. Radice, T. Caraceni, Increased plasma bilirubin in Parkinson patients on L-dopa: evidence against the free radical hypothesis? Ital. J. Neurol. Sci. 18 (1997) 69–72.

[8] X.L. Qin, Q.S. Zhang, L. Sun, M.W. Hao, Z.T. Hu, Lower serum bilirubin and uric acid concentrations in patients with Parkinson's disease in China, Cell Biochem. Biophys. 72 (2014) 49–56.

[9] M. Moccia, M. Picillo, R. Erro, K. Longo, M. Amboni, G. Santangelo, R. Allocca, O. Caporale, M. Triassi, M.T. Pellecchia, P. Barone, C. Vitale, Increased bilirubin levels in de novo Parkinson's disease, Eur. J. Neurol. 22 (2015) 954–959.

[10] F. Peng, Y. Yang, J. Liu, Y. Jiang, C. Zhu, X. Deng, X. Hu, X. Chen, X. Zhong, Low antioxidant status of serum uric acid, bilirubin and albumin in patients with neuromyelitis optica, Eur. J. Neurol. 19 (2012) 277–283.

[11] P. Ayuso, C. Martinez, P. Pastor, O. Lorenzo-Betancor, A. Luengo, F.J. Jimenez-Jimenez, H. Alonso-Navarro, J.A. Agúndez, E. García-Martín, An association study between Heme oxygenase-1 genetic variants and Parkinson's disease, Front. Cell. Neurosci. 8 (2014) 1–8.

[12] C. Funke, J. Tomiuk, O. Riess, D. Berg, A.S. Soehn, Genetic analysis of heme oxygenase-1 (HO-1) in German Parkinson's disease patients, J. Neural. Transm. 116 (2009) 853–859.