Journal of Trace Elements in Medicine and Biology 62 (2020) 126637

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Difference in iron metabolism may partly explain sex-related variability in the manifestation of Wilson's disease☆

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ARTICLE INFO

Keywords:
Copper
Iron
Phenotype
Sex
Wilson's disease

ABSTRACT

Background/aim: Wilson's disease (WD) is a hereditary disorder characterized by abnormal metabolism of copper. For unknown reasons, the clinical picture of this disease appears to be sex-dependent. Because the metabolism of copper and iron is interrelated, we aimed to evaluate whether the variability in the clinical picture of WD could be explained by the sex difference in iron metabolism.

Methods: A total of 138 WD patients were examined in this study: 39 newly diagnosed, treatment naive patients and 99 individuals already treated with decoppering drugs. The serum concentration of ceruloplasmin (Cp) and copper were measured using an enzymatic colorimetric assay and by atomic absorption spectroscopy, respectively. The parameters of iron metabolism were determined by using standard laboratory methods and enzyme immunoassays.

Results: In the treatment naive group men had a higher median serum concentration of ferritin (290.5 vs. 81.0 ng/mL, $p < 10^{-4}$), and hepcidin (Hepc) (55.4 vs. 22.8 ng/mL, $p < 10^{-3}$) compared to women, and tended to have higher concentration of iron, hemoglobin (HGB) and number of red blood cells (RBC). In the treated group men had higher median ferritin (122.0 vs. 46.0 ng/mL, $p < 10^{-4}$), Hepc (23.5 vs. 10.8 ng/mL, $p < 10^{-4}$), iron (102.5 vs. 68.0 μg/dL, $p < 10^{-4}$), HGB (15.0 vs. 13.2 g/dL, $p < 10^{-4}$), and RBC (5.0 vs. 4.5 M/L, $p < 10^{-4}$) than women.

Conclusion: Iron metabolism differs between men and women with WD, which may partly explain the sex difference noted in the disease manifestation.

1. Introduction

Wilson's disease (WD) (Online Mendelian Inheritance in Men (OMIM) 277900) is a rare disorder characterized by the accumulation of copper in organs such as the liver, cornea, brain, kidney, and heart. Copper accumulation occurs due to functional impairment of the copper-transporting P-type ATPase, ATP7B, caused by pathogenic mutations in both copies of the *ATP7B* gene [1,2]. The clinical phenotype of WD is variable, but the two main forms of this disease: hepatic and neuropsychiatric can be distinguished. The first symptoms may occur in the early years of life, but in few cases the symptoms may also occur in the older age [3–5]. Some observations suggest that the clinical picture of WD depends on sex [6–10].

The variability of WD-related phenotype has been speculated to be associated with the sex hormones because estrogens may exert antioxidant, neurotrophic, and anti-inflammatory effects that have previously been described for some other conditions, including neurodegenerative and liver diseases, in which sex-related differences are noted.

Another theory that explains the sex difference in WD manifestations is related to iron metabolism. Among healthy people, the metabolism of this element differs based on sex [11–13]. The metabolism of copper and iron is interrelated and shares joint metabolic pathways. In the

Abbreviations: ATP7B, adenosine triphosphatase 7B; Cp, ceruloplasmin; Hepc, hepcidin; HGB, hemoglobin; RBC, red blood cells; sTfR, soluble transferrin receptor; TIBC, total iron-binding capacity.

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https://doi.org/10.1016/j.jtemb.2020.126637
Received 7 May 2020; Received in revised form 5 August 2020; Accepted 26 August 2020
Available online 28 August 2020
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experimental WD model, not only copper accumulation but also the presence of iron in the liver was observed [4,14,15]. In this model, a low-iron diet was found to be effective in preventing liver failure and other complications. Furthermore, a few studies on humans have suggested that in WD patients, iron metabolism may be impaired and the resulting accumulation in the liver and/or brain tissues may worsen the clinical course of the disease [16–20].

Thus, it was hypothesized that the phenotypic variability observed between men and women with WD is a result of sex differences in iron metabolism. However, this has not been previously verified. Therefore, in the present study, we aim at verifying the above hypothesis by analyzing the parameters of iron metabolism in men and women, who were either treated or untreated with decoppering drugs.

2. Materials and methods

A total of 138 patients with WD, which included 39 previously untreated patients (newly diagnosed with WD) and 99 patients who were treated with anticopper drugs at the Institute of Psychiatry and Neurology (Warsaw, Poland), were included in the study. The detailed criteria for the inclusion and exclusion of patients in the study are presented in Table 1. All the included patients met the diagnostic criteria of WD recommended by the 8th International Conference on Wilson Disease and Menkes Disease. The diagnosis of WD was made based on the assessment of clinical symptoms (strictly as described earlier) [21], abnormal copper metabolism (reduced concentration of Cp and copper in the serum, increased urinary excretion of copper in 24 h), and the presence of the Kayser–Fleischer ring, as well as genetic tests. If there was uncertainty in the diagnosis, the inclusion of Cu64 in Cp was measured after 24 and 48 h. Copper metabolism was also evaluated in the same laboratory using the same methods. According to the initial manifestation of the disease, the patients were grouped as hepatic and neuropsychiatric. The patients classified as hepatic were those who showed signs of chronic or acute liver disease (increase in liver enzymes with an increased level of bilirubin in the blood and abnormal international normalized ratio (INR) and/or changes in the liver echogenicity, signs of portal hypertension, decompensated liver cirrhosis, or acute liver failure) without any neuropsychiatric symptoms. On the other hand, the patients classified as neuropsychiatric showed hepatic injury signs and symptoms accompanied by neurological symptoms, such as tremor, dystonia, ataxia, dysarthria, and stiffness, or had psychiatric symptoms, including behavioral disorders, depression, manic psychosis, or cognitive impairment. Some patients were diagnosed presymptomatically on the basis of family tests. Blood samples were collected prospectively from all the included subjects for analyzing the parameters of iron and copper metabolism in the laboratory. Biological material was collected as separate portions for use in individual measurements so that repeated freeze–thaw cycles can be avoided. Samples were stored at −70 °C and used for testing immediately after thawing.

All the participants gave written informed consent to participate in the study. The study protocol was approved by the local Ethics Committee and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for human experiments.

Table 1
Inclusion and exclusion criteria for the studied group.

	Patients with WD
Inclusion criteria	1 WD diagnosis
	2 Age over 18 years
	3 Written consent to participate in the study
Exclusion criteria	1 Presence of systemic diseases (cancer, current infection) or a carrier of hepatitis virus
	2 Blood transfusion performed up to a month before qualifying for the study
	3 Iron supplementation, used up to a year before qualifying for testing
	4 Alcohol abuse
	5 Lack of consent to participate in the study

The serum concentration of Cp was measured by performing an enzymatic colorimetric test [22]. The concentration of iron, copper ferritin, transferrin, RBC, and HGB, as well as TIBC, were measured using standard methods in a certified medical laboratory (Alab, Warsaw, Poland) accredited by Polish Centre of Acreditaiton. Serum lactoferrin was evaluated using the Human Lactoferrin ELISA Test Kit (catalog number EL20111-1; Test Pro) with a test range of 0.625–40000.0 ng/mL. Serum Hepc concentration was measured using a Hepc-associated immunosorbent assay kit (catalog number SEB979Hu; Cloud Clone Corporation) with a test range of 62.5–4000.0 pg/mL. Serum sTfR level was tested using a sandwich enzyme immunoassay kit (catalog number RD194011100; BioVendor) with a test range of 0.4–2.0 μg/mL.

2.1. Statistical analysis

All data were analyzed using Statistica v.9.0 program (StatSoft, Krakow, Poland). Quantitative variables with normal distribution characterized by mean and standard deviations (SD) were compared between groups using parametric tests (Student's *t*-tests or analysis of variance – ANOVA). Quantitative variables with abnormal distribution characterized by median and interquartile intervals (IQR) were compared between groups using the Mann–Whitney *U* test or nonparametric ANOVA followed by the Mann–Whitney test. Categorical variables characterized by number and percentage were compared between groups using the chi-square test and Fisher's test.

For all aforementioned statistical tests $p < 0.05$ was considered significant.

3. Results

A total of 138 patients were examined in the study. This included 39 newly diagnosed patients and 99 patients who were previously treated with decoppering drugs (D-penicillamine or zinc sulfate).

In the untreated group, women were more often diagnosed with hepatic WD than men, however the groups were small in number of patients. No sex difference was observed in the treated group when the clinical phenotype or duration of the anticopper treatment was compared. The baseline characteristics of the patients are shown in Table 2.

In the treated group, no difference was observed between men and women in the serum concentration of copper and Cp. On the other hand, in the untreated group, men tended to have lower serum Cp than women, while no sex difference was observed in the serum copper concentration (Tables 3 and 4).

In the untreated group, the concentration of ferritin and Hepc was significantly higher in men than in women. Similarly, men had higher concentration of iron, RBC, and HGB but lower sTfR and TIBC compared to women (Table 3).

In the treated group, the concentration of iron, ferritin, HGB, Hepc, and RBC were higher in men than in women. However, TIBC was lower in men compared to women (Table 4).

4. Discussion and conclusion

WD is characterized by high phenotypic diversity. Its clinical picture can be modified due to variations in the *ATP7B* gene [10,23–28], methylenetetrahydrofolate reductase (*MTHFR*) gene [29], cytokines [30], prion-related protein *(PRNP)* [31], apolipoprotein E (*APOE*) [32–35], enzymatic antioxidants [36], patatin-like phospholipase domain 3 (PNPLA3) gene [37], or 17-beta hydroxysteroid dehydrogenase 13 (HSD17B13) gene [38]. Several previous studies have shown that the clinical course of WD varies and is dependent on sex [6–10].

Table 2
Clinical characteristics.

Characteristics	Women	Men	*p*-Value
Patients not treated with anticopper drugs n (%)	23 (59)	16 (41)	<0.268
Age at onset of first symptoms (years, median (IQR))	31.3 (10.9)	30.0 (13.5)	<0.742
Age during recruitment to the study (years, median (IQR))	34.7 (12.5)	36.9 (10.1)	<0.563
Clinical form of WD, n (%)*			
- Neuropsychiatric	10 (52.6)	8 (80.0)	<0.148
- Hepatic	9 (47.4)	2 (20.0)	<0.012
Presymptomatic patients, n (%)	0 (0.0)	3 (23.1)	<0.001
Patients on decoppering therapy n (%)	41 (41.4)	58 (58.6)	<0.221
Age at onset of first symptoms (years, median (IQR))	27.1 (11.5)	24.0 (9.6)	<0.140
Age during recruitment to the study (years, median (IQR))	36.2 (10.7)	34.4 (9.6)	<0.881
The duration of treatment (months, median (IQR))	51.0 (41.0)	46.0 (48.0)	<0.163
Type of treatment			
- D-Penicillamine, n (%)	19 (46.3)	30 (51.7)	<0.717
- Zinc sulfate, n (%)	22 (53.6)	27 (46.5)	<0.724
Presymptomatic patients, n (%)	5 (12.2)	4 (6.9)	<0.366
Clinical form of WD, n (%)			
- Neuropsychiatric	24 (66.7)	41 (75.9)	<0.334
- Hepatic	12 (33.3)	13 (24.1)	<0.117

* Lack of data for four women (17.4 %) and three men (18.7 %), ns.

Table 3
Serum copper and iron parameters in WD patients not treated with anticopper drugs.

Parameters	Men n = 16	Women n = 23	*p*-Value
Cp (mg/dL)	14.3 (8.8)	17.2 (9.4)	<0.083
Copper (μg/dL)	62.0 (19.0)	69.5 (23.0)	<0.203
Iron (μg/dL)	136.0 (44.1)	116.0 (60.0)	<0.113
Ferritin (ng/mL)	290.5 (189.4)	81.0 (136.0)	<0.001
Transferrin (g/L)	2.4 (1.28)	2.6 (0.55)	<0.256
TIBC (μg/dL)	281.5 (111.75)	313.0 (106.0)	<0.095
RBC (M/L)	4.6 (1.2)	4.2 (0.6)	<0.182
HGB (g/dL)	13.5 (2.2)	13.05 (1.4)	<0.093
Hepcidin (ng/mL)	55.4 (58.9)	22.8 (26.6)	<0.001
Lactoferrin (ng/mL)	324.0 (244.0)	344.0 (221.0)	<0.917
sTfR (μg/mL)	0.8 (0.3)	0.9 (0.7)	<0.079

Cp, ceruloplasmin; TIBC, total iron-binding capacity; RBC, red blood cells; HGB, hemoglobin; sTfR, soluble transferrin receptor Median and IQR are presented *p*-values for the comparison between untreated men and women with WD.

Table 4
Serum copper and iron parameters in WD patients treated with anticopper drugs.

Parameters	Men n = 58	Women n = 41	*p*-Value
Cp (mg/dL)	4.96 (5.5)	5.68 (12.5)	<0.381
Copper (μg/dL)	23.0 (27.0)	21.0 (48.0)	<0.683
Iron (μg/dL)	102.5 (52.0)	68.0 (30.0)	<0.000
Ferritin (ng/mL)	122.0 (126.0)	46.0 (52.0)	<0.000
Transferrin (g/L)	2.7 (0.6)	2.9 (0.9)	<0.088
TIBC (μg/dL)	331.0 (72.0)	346.0 (102.0)	<0.047
RBC (M/L)	5.0 (0.5)	4.5 (0.4)	<0.000
HGB (g/dL)	15.0 (1.1)	13.2 (1.5)	<0.000
Hepcidin (ng/mL)	23.5 (26.1)	10.8 (12.7)	<0.000
Lactoferrin (ng/mL)	293.0 (170.0)	286.5 (158.5)	<0.794
sTfR (μg/mL)	0.71 (0.54)	0.82 (0.65)	<0.322

Cp, ceruloplasmin; TIBC, total iron-binding capacity; RBC, red blood cells; HGB, hemoglobin; sTfR, soluble transferrin receptor median and IQR are presented *p*-values for the comparison between men and women with WD treated with anticopper drugs.

Some authors documented that the neuropsychiatric form of WD, was often observed in men, while the hepatic form was more frequent in women [7,8,10]. In the study by Ferenci et al., this finding was significant in the case of children and young adults [8]. Litwin et al. observed that men with the neuropsychiatric form of WD developed symptoms about 2 years earlier compared to women [7]. In the study by Li et al., ventricular dilatation, which is a sign of brain atrophy, was found to be more common in men [9].

Iron metabolism may be an explanation of association between sex and WD phenotype. A high level of iron has been found to significantly predict fibrosis in chronic hepatitis [39], and is also considered to promote neurodegenerative diseases [40,41]. In the examined population, treatment naive women tended to have lower concentration of iron, HGB, and RBC than men, but the difference was not statistically significant. In the group of WD patients treated with anticopper drugs, men were found to have higher levels of iron, RBC, and HGB than women. These results are consistent with the observations reported for the general population that women generally showed lower values of these parameters due to iron excretion with the menstrual blood [42–44]. The higher levels of iron noticed in men with WD corresponded with the higher levels of Hepc and ferritin like as in healthy population [45]. Despite observed gender related differences in iron metabolism we have noted clear differences neither in the time to onset of symptoms of the disease, nor clinical phenotype between women and men. Women more often than men manifested WD with the hepatic only form in the treatment naive group, but the group was relatively small. WD men tended to have lower serum ceruloplasmin concentration than female patients, similarly to population of healthy adults [46]. Cp is required for the cellular outflow of iron, and therefore, defects in its activity can lead to iron accumulation [47,48]. Jin et al. documented that the iron levels were significantly increased in the black matter of patients with Parkinson's disease, but only when the serum Cp was low [49]. Gender related cerulopasmin activity may therefore contribute to WD clinical phenotype vairiability described by other authors [7,8,10].

In summary, we have proved that iron metabolism differs between men and women with WD. These differences may be associated with the sex-related variability of the WD phenotype. We are aware that although we analyzed a relatively large number of patients (considering that WD is a rare disease), the number of untreated patients was small, and as a result, some of the observed differences did not reach statistical significance. Therefore, more research should be performed to verify the observed relationships.

Funding source

This study was supported by grant N N402 471640 from the Polish National Ministry of Education and Science.

Authors' contribution

- Grażyna Gromadzka: conceptualization; data curation; formal analysis and funding acquisition; investigation, methodology, project administration, and supervision; review and editing of the manuscript.
- Diana Wierzbicka: investigation and methodology; preparation of the original draft.
- Adam Przybyłkowski: formal analysis; review and editing of the manuscript.
- Tomasz Litwin: data curation; investigation and methodology; review of the manuscript.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) the work.

Acknowledgments

We thank all those who helped us in carrying out the research and express our gratitude to all the patients for their participation in this study. We would also like to thank the hospital staff who contributed to the collection of blood samples as well as all the clinicians who were involved in the diagnostics/phenotyping of patients.

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