

Assessment of selected oxidative stress parameters in patients with Wilson's disease

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Abstract

Introduction: Wilson's disease is a genetic disorder of copper transport resulting in copper accumulation in different organs and their toxic injury. The involvement of antioxidative agent in Wilson's disease has not been fully explained so far. Aim of the study was assessment of antioxidative status in patients with Wilson's disease.

Material and methods: The study involved 20 patients (12 men, 8 women, mean age 39.9 years) with a confirmed diagnosis of Wilson's disease and 20 healthy volunteers representing the control group. During the study, the patients were in disease remission. Only in the patients with neurological form of Wilson's disease some clinical symptoms persisted, although they were much less prominent. In the examined subjects, total serum antioxidative status was assessed by using colorimetric method, whereas glutathione peroxidase and superoxide dismutase activities were assessed spectrophotometrically. The selenium concentration in the erythrocytes was examined by the atomic absorption spectroscopy. Additionally, in all the patients, biochemical parameters of liver injury and copper metabolism were assessed.

Results: Markedly higher activity of the tested enzymes, total serum antioxidative status and erythrocytes selenium concentration were found in the patients when compared to the control group. While assessing the relationship between the analyzed parameters and copper metabolism indicators, a positive correlation between total serum antioxidative status, glutathione peroxidase activity, and 24-h urinary copper excretion was established.

Conclusions: Higher erythrocyte activities of the antioxidative enzymes and total serum antioxidative status in patients with Wilson's disease confirm activation of the antioxidative system in this group of patients.

Key words: Wilson's disease, antioxidative status, glutathione peroxidase, superoxide dismutase, selenium.

Introduction

Wilson's disease is a recessive autosomal inherited disorder of copper metabolism that is caused by a mutation of the ATP7B gene which encodes an ATP-ase – the enzyme indispensable for biliary excretion of copper through the hepatocyte membrane [1]. The disease afflicts 1 in 40,000 individuals. Impaired transport of copper from the hepatocytes into bile leads to a toxic accumulation of copper in the liver followed by other tissues including the basal ganglia, cornea and erythrocytes. Damage to them is caused via generation of free radicals, lipid peroxidation and inhibition of protein synthesis [2]. Copper plays an important role in initiating the generation of reactive oxygen species. As a result of toxic activity of copper and creation of free oxygen radicals, a change in activity of antioxidative enzymes appears. The involvement of antioxidative factors including antioxidative enzymes from free radical scavenging groups in Wilson's disease is not fully understood. The main enzymes of the antioxidative system are superoxide dismutase (SOD) and glutathione peroxidase (GPx). Both enzymes along with catalase, belong to the first-line of cellular defense mechanism against damage caused by reactive forms of oxygen. Antioxidative enzymes degrade organic superoxides to non-toxic hydroxylipids and in this way inhibit chain reaction of lipid peroxidation. Superoxide dismutase is a specific enzyme which is found in different cell organelles. It causes conversion of superoxide radical into elementary oxide and hydrogen superoxide which in turn undergoes further transformation into oxide and water by means of catalase. Glutathione peroxidase on the other hand, reacts with reduced glutathione and hydrogen superoxide which eventually leads to water and oxidized glutathione formation. Glutathione peroxidase is found both in the intracellular and in the extracellular space [3]. Activity of selenium-dependent GPx containing selenium in its active centers depends, along with other factors, on the bioavailability of this catalyst in the serum which in spite of being a trace element plays an important role in different metabolic processes [4-6]. Studies conducted so far show no selenium concentration disturbances in patients with Wilson's disease [7]. Changes in erythrocytes selenium concentration and activities of antioxidative enzymes in this group of patients still remain unclear. Assessment of their activities could probably indirectly contribute to explanation of the mechanism of copper-dependent toxic liver injury.

The main aim of the study was the assessment of selected oxidative stress parameters: activity of GPx, SOD, serum antioxidative status (TAS), and erythrocyte selenium concentrations (SeE) in patients with Wilson's disease.

Material and methods

The study involved 20 patients (12 men and 8 women, mean age 39.9 years) with Wilson's disease who were treated at the Departments and Outpatient Clinics of Medical University of Gdańsk and in the Hepatological Outpatient Clinic of State Infectious Diseases Hospital in Gdańsk in Poland. Only patients with a confirmed diagnosis of Wilson's disease were included in the study. The diagnosis had been made in patients with liver injury and/or neurological symptoms using the following criteria: decreased concentration of serum ceruloplasmin, increased 24-h urinary excretion of copper and presence of Kayser-Fleischer ring in the cornea. In some of the patients genetical tests were performed. Assessment of a mutation in ATP7B gene was performed in the Laboratory of the Department of Internal Medicine IV Gastroenterology and Hepatology of University of Vienna.

Twenty equally matched healthy controls also participated in the study (mean age 36.7 years).

Patients with coexisting hepatotoxic factors: hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and those abusing alcohol or drugs were ruled out of the study.

During the study, the patients were in disease remission and did not present symptoms of active hepatic injury (oligosymptomatic group). Mild clinical symptoms still persisted in some patients with neurological form of Wilson's disease although they were much less prominent. The patients had been treated with d-penicillamine and/or zinc sulphate for 2-9 years.

In all the subjects, TAS, erythrocyte GPx and SOD activities were assessed. The evaluation was carried out at the Department of Clinical Nutrition and Laboratory Diagnostics of Medical University of Gdańsk using Randox sets by precisely following the manufacturer's instructions. Serum antioxidative status activity was assessed by means of colorimetric method [8] while SOD and GPx activities were evaluated using Ultraspect III spectrophotometer. In the examined groups, SeE was also determined by means of atomic absorption spectroscopy using hydride generation technique (HG-ASS). The tests were carried out at the Department of Physical Chemistry of Medical University of Gdańsk by the use of Cheman, Merck and Aldrich reagents. Moreover, serum copper and ceruloplasmin concentration, 24-h urinary copper excretion, biochemical markers of liver injury such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ -glutamyltranspeptidase (GGTP) activities were determined. Furthermore parameters such as bilirubin, albumin and γ -globulin concentration and values of normalized international prothrombin

index (INR) were also assessed using standard methods. Serum used for the study was collected only after a consent of a patient to conduct the mentioned above tests.

The data were analyzed using nonparametric methods. The Mann-Whitney U test was used for between-group comparison. Spearman method was applied for correlation assessment. The level of significance was set at $p < 0.05$. All calculations and figures were performed using the Statistica 8 software package (Statsoft Inc., Tulsa, USA).

The Local Ethics Committee consented to carry out the present study.

Results

Comparison of GPx, TAS and SOD activities in the patients and healthy volunteers indicated markedly higher activities of the tested enzymes and TAS in the sera of the patients when compared to the control group (Table I). Assessing erythrocyte selenium concentration, markedly higher values were also found in the group of the patients in comparison to the control group (Table I).

Comparison of the tested parameters in the patients presenting neurological symptoms of the disease and oligosymptomatic ones did not show statistically significant differences. However,

a trend towards lower values of all tested parameters were noticed in patient with total remission of the disease (Table II).

The results obtained in the course of the study were also analyzed according to treatment types as shown in Table III. Enzyme activities remained unchanged in patients undergoing different treatments. However, TAS values were significantly higher in the patients treated with zinc preparations only or combination of zinc sulphate and d-penicillamine as compared to the patients treated with d-penicillamine alone.

Copper metabolism parameters in the group of the patients are shown in Table IV. Analysis of the relationship between the tested parameters and copper metabolism showed only a positive correlation between TAS and GPx activities and 24-h urinary copper excretion ($p = 0.035$, $r = 0.47$; $p < 0.001$, $r = 0.7$) (Figure 1, 2).

Biochemical markers of liver injury were within the normal range, so no correlation between them and the tested parameters were studied.

Discussion

Research concerning antioxidative system activity in patients with alcoholic or post-inflammatory liver injury has become quite popular [9-12]. In both

Table I. Characteristic of the analyzed parameters in the group of patients with Wilson's disease and the control group

Parameters	Patients (n = 20) mean values \pm SD	Controls (n = 20) mean values \pm SD	p
TAS [mmol/l]	2.07 \pm 0.54	1.56 \pm 0.54	0.003
GPx [U/g Hb]	42.3 \pm 2.55	29.0 \pm 10.2	0.003
SOD [U/g Hb]	2452.7 \pm 516.5	1767.1 \pm 449.1	0.0004
SeE [μ g/ml]	0.15 \pm 0.114	0.076 \pm 0.028	0.001

Table II. Characteristic of the analyzed parameters in the group of symptomatic and oligosymptomatic patients

Parameters	Patients with neurological symptoms (n = 7) mean values \pm SD	Patients in remission (n = 13) mean values \pm SD	p
TAS [mmol/l]	2.11 \pm 0.48	1.89 \pm 0.37	> 0.05
GPx [U/g Hb]	43.81 \pm 10.4	39.8 \pm 12.4	> 0.05
SOD [U/g Hb]	2485.1 \pm 425.2	2392.3 \pm 515.8	> 0.05
SeE [μ g/ml]	0.169 \pm 0.13	0.114 \pm 0.03	> 0.05

Table III. Characteristic of the analyzed parameters in the group of patients with different type of treatment

Parameters	Only zinc salts mean values \pm SD	Zinc salts and d-penicillamine mean values \pm SD	Only d-penicillamine mean values \pm SD
TAS [mmol/l]	2.52 \pm 0.33	2.47 \pm 0.71	1.98 \pm 0.36
GPx [U/g Hb]	39.5 \pm 14.55	48.2 \pm 15.32	44.7 \pm 10.55
SOD [U/g Hb]	2360.0 \pm 406.2	2211.7 \pm 391.1	2778.0 \pm 634.6
SeE [μ g/ml]	0.21 \pm 0.22	0.23 \pm 0.09	0.110 \pm 0.01

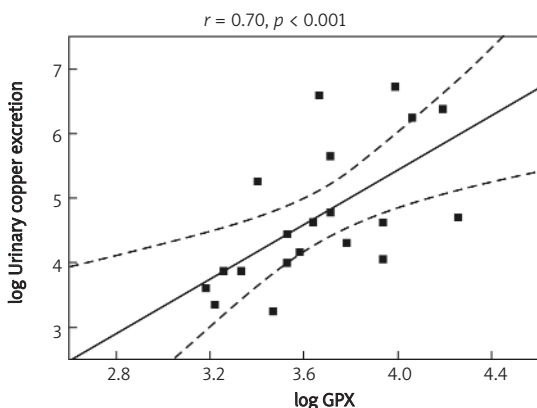


Figure 1. Correlation between GPx activities and 24-h urinary copper excretion

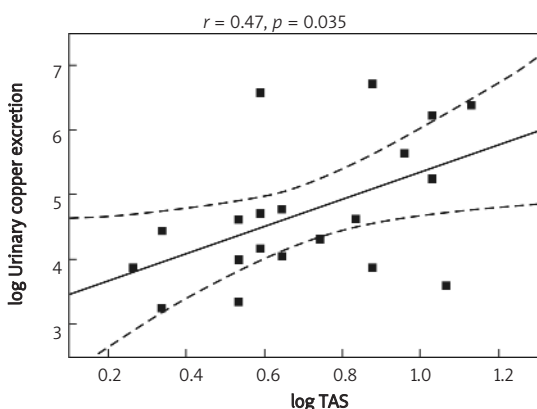


Figure 2. Correlation between TAS activities and 24-h urinary copper excretion

Table IV. Characteristic of the examined laboratory parameters in the group of patients with Wilson’s disease

Parameters	Mean values ± SD
Copper [µg/dl]	71.5 ±42.6
Ceruloplasmin [mg/dl]	21.5 ±13.6
Copper (urine) [µg/24 h]	340.82 ±280.16

groups, a significant reduction of the tested parameters in comparison to the healthy controls has been stated [9-15].

Our studies were concerned with the activity of antioxidative enzymes that are known to play a vital role in the processes involved in cell defense against toxic superoxides and oxidative radicals. Samuele *et al.* proved that the oxidative stress plays a crucial role in degradation of the hepatocytes and neurons of the central nervous system in the course of Wilson’s disease [16]. So far other antioxidative factors have been investigated in Wilson’s disease, however, few published papers address the activity of antioxidative enzymes in this group of patients

[17, 18]. The papers mainly deal with decrease in vitamin E concentration in patients with chronic liver diseases including Wilson’s disease [19, 20]. Antioxidative activity of this vitamin is now quite often used in treatment of neoplastic disease [21, 22]. It has been proven that vitamin E analogues such as α -TOS stimulate apoptosis of proliferating endothelial cells and inhibit angiogenesis and tumor growth.

In the present work, we investigated a group of patients with Wilson’s disease in the remission stage which was initially confirmed by normal liver tests (no liver biopsy was performed at the time of the study). We found significantly higher activities of the tested enzymes and TAS in comparison to the control group. It may seem therefore that increased activity of the antioxidative enzymes is an adaptive mechanism in patients suffering from Wilson’s disease with intense generation of lipid peroxidation and free radicals. In our study, we did not find significant differences in the analyzed parameters between the patients presenting some neurological symptoms and oligosymptomatic patients. However, a tendency towards lower values of the tested parameters was noticed in the patient with total remission of the disease what may result from lower activity of the antioxidative system in this group of patients. And yet some authors have reported different results. Attri *et al.* stated decreased activities of erythrocyte antioxidative enzymes [23]. It should be underlined, however, that the study included 8 patients with Wilson’s disease in the stage of decompensation with acute haemolysis. Significantly higher values of the parameters mentioned above were observed by the same authors in the follow-up examination after treatment – during the clinical remission of the disease as in the present study. Nagasaka *et al.* stated a decrease in GPx activity but only in patients with fulminant liver failure due to Wilson’s disease [17]. In none of our patients, symptoms of acute or active liver injury appeared during the study. Only the patients with neurological symptoms were not in full remission.

Many authors underline a possible connection between activity of the antioxidative enzymes and use of zinc preparations [24-26]. Santon *et al.* stated an increase in activity of hepatic GPx and concentration of metallothioneins in the liver and intestines of patients treated with zinc preparations in comparison to the ones not receiving this element and a control group [26]. However, we observed significantly higher values of the tested parameters even in the patients who did not get zinc preparations. So activation of the antioxidative system is due not only to the type of treatment but may also result from the disease.

In the present study, we also assessed concentration of erythrocytic selenium which is an indispensable component of GPx. We found significantly higher concentrations of selenium in the group of the patients comparing to the healthy controls. Comparison of our own results with any data from the literature is impossible because of lack of papers regarding this matter. Compounds containing selenium play an important role in the organism removing not only toxic oxygenic substances but also metals. However, reactions between selenium and metals result in generation of poorly soluble metal selenides what in turn may lead to accumulation of metals in the parenchymatous organs. A protein called AE1 takes part in the transport of oxyanions – selenates, phosphatic, sulphatic and magnesic oxyanions – through the erythrocyte membranes, so mutual changes in the concentrations of other elements are responsible for maintenance of selenium concentration in the erythrocytes [27].

In our study, we also analyzed correlation between GPx, SOD and TAS activities, SeE concentration and copper metabolism parameters. A positive correlation between 24-h urinary copper excretion and both TAS and GPx activities was found. It should be stressed that these significant correlations were also observed in the group of patients who did not received d-penicillamine – a drug increasing urinary copper excretion. Unfortunately no paper assessing correlation between TAS and activity of the antioxidative enzymes and copper metabolism in patients with Wilson's disease has been found in the available literature. Dalgıç *et al.* found decreased values of other antioxidative stress parameters (vitamin C and β -carotene) in patients with cirrhosis due to different chronic liver diseases including Wilson's disease [19]. In our paper, we did not assess a correlation between the tested enzymes and selenium and the liver parameters because they were within the normal range of values.

The results of the present study leave many problems unexplained but they deal with the problem of the antioxidative system activity in Wilson's disease. Activities of the antioxidative enzymes and TAS in treated patients with Wilson's disease are high what differs from other liver pathologies and what may result from the stimulation of the antioxidative system in this group of patients.

In conclusion, the antioxidative status expressed by means of activities of the antioxidative enzymes is high in patients with Wilson's disease who are in good clinical condition.

Higher concentration of selenium in the erythrocytes requires further research – it may be due to accumulation of this element in case of oxidative stress threat.

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