Determination of Serum Ceruloplasmin Concentration in Patients with Primary Open Angle Glaucoma with Cataract and Patients with Cataract Only: A Pilot Study

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this article was to describe the role of ceruloplasmin and to report preliminary results of ceruloplasmin concentrations in patients with primary open-angle glaucoma (POAG) with cataract and in patients with only cataract.

Glaucoma, a neurodegenerative disease, is a heterogeneous group of conditions characterized by loss of retinal ganglion cells (RGC), their axons, progressive optic nerve damage, and visual field deterioration.

Material/Methods: The POAG group included 30 patients and the cataract group included 25 patients.

Results: Ceruloplasmin plays an essential role in iron metabolism and inactivating free radicals. In the presented pilot study, serum ceruloplasmin level was lower in the POAG group in comparison to the group with only cataract.

Conclusions: In treating persistent inflammation in the course of glaucoma, antiglaucoma drugs may increase the permeability of the blood-ocular barrier, which may be connected with the lower concentration of serum ceruloplasmin in the glaucoma patients group.

MeSH Keywords: Cataract • Ceruloplasmin • Glaucoma

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Background

Ceruloplasmin, which has Enzyme Entry EC 1.16.3.1, is also called ferroxidase and is a copper-binding protein. Iron is crucial for many metabolic reactions, but may also cause damage, as it is a strong generator of free radicals. Ceruloplasmin is a protein involved in iron homeostasis and it can inactivate free radicals [1]. The protein utilizes iron oxidase activity to prevent the occurrence of toxic iron products. The biological role for ceruloplasmin in iron metabolism may result from the catalytic activity in conversion of Fe 2+ (ferrous iron) to Fe 3+ (ferric iron), thus promoting the incorporation rate of Fe 3+ to apotransferrin [2].

Ceruloplasmin shows a notable increase during acute-phase response and therefore is referred to as a positive acute-phase reactant. High accumulation of ceruloplasmin is noted in various conditions, such as inflammatory reactions, infections, malignancy, tissue damage and necrosis [3].

Superoxide dismutase (SOD) is an intracellular enzyme that catalyses the dismutation of superoxide to hydrogen peroxide and oxygen [4]. Ceruloplasmin mimics the action of SOD, and, as an acute-phase reactant, scavenges oxygen-derived free radicals.

The expression of the mRNA encoding ceruloplasmin in the retina was found to significantly increase with elevated intraocular pressure (IOP) [5]. Farkas et al. discovered that in glaucoma, mRNA and protein levels of the iron-regulating proteins such as transferrin, ceruloplasmin, and ferritin are increased. This suggests that in the pathogenesis of glaucoma, iron and copper metabolism, in association with the antioxidant system, have a significant role [6].

Primary open-angle glaucoma

Primary open-angle glaucoma (POAG) is a progressive, chronic, optic neuropathy in which IOP and other currently unknown factors contribute to characteristic acquired atrophy of the optic nerve and loss of RGC and their axons [7]. The cell death is induced by apoptosis or necrosis [8].

The pathogenesis of those disorders is multifactorial, and cell death occurs in a characteristic pattern. One theory is that there is a relationship between oxidative stress, glaucoma, and inflammation [9,10].

The only known confirmed risk factor for glaucomatous neuropathy is elevated intraocular pressure (IOP), but in some cases reduction of IOP does not stop the progression of the disease. As glaucomatous neuropathy may develop without increased IOP, hypothetically, it is not the only factor involved in

pathogenesis of glaucomatous neuropathy. When the causative agent is unidentifiable, progression of the disease results in visual field loss and, at the end-stage, in irreversible blindness.

Strong evidence has been reported that glaucomatous neurodegeneration is analogous to other neurodegenerative diseases in the central nervous system, such as Alzheimer disease [11].

The role of ceruloplasmin

Ceruloplasmin is a copper-binding acute-phase protein that increases in inflammatory states. The enzyme is mainly synthesized in the liver but is also expressed in nerve tissues, such as the retina and brain [12]. In normal conditions ceruloplasmin cannot pass the blood-brain and blood-retina barrier, but is also synthesized locally in the brain, retina, hepatocytes, spleen, and lungs. Another function of ceruloplasmin is inhibition of metalloproteinase, which is a neutrophil enzyme that promotes oxidative stress in various inflammatory pathologies.

The aim of the study

The aim of the present study was to describe the role of ceruloplasmin and to assess its relationship with glaucomatous neuropathy. For this purpose the following pilot study was carried out.

Material and Methods

The Ethics Committee of the Medical University of Silesia, Katowice (permission number: KNW/0022/KB1/123/10) approved the study protocol. The study adhered to the tenets of the Declaration of Helsinki for experiments involving human tissue samples.

Participants

The primary open-angle glaucoma (POAG) group was exclusively composed of white individuals who were preparing to undergo antiglaucoma drainage surgery for visual field damage despite IOP control and intolerance of topical antiglaucoma drugs. All patients exhibited bilateral visual field defects and noticeable senile lens opacity (POAG group, n=30).

The control group included white individuals who were scheduled for cataract surgery (cataract group, n=25).

The inclusion criteria for the POAG and control groups were as follows: (1) no previous intrabulbar surgery; (2) between 65 and 75 years old; (3) best corrected visual acuity of 0.5 or better (Snellen chart); (4) no myopia or hyperopia >3 diopters (D); (5) non-smokers; (6) no documented or diagnosed and

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Table 1. Patient characteristics.

	Glaucoma patients		VS	Controls	
Sex (men/female)	♂=14 ♀= 16	n= 30	N/S	♂=10 ♀=15	n=25
Age (years)	68±5.42		p=0.21	69±3.72	
Median duration of known glaucoma (years)	5–12 8.6 ± 3.3		Ø	Ø	
Intraocular pressure (IOP)	21.0 mmHg SD: 2.35 95% CI: 20.12–21.88		p=0.000	16.3 mmHg SD: 1.43 95% CI: 15.69–16.85	
BCVA (best corrected visual acuity) Snellen charts	0.72 SD: 0.18		N/S	0.66 SD: 0.16	
N95 amplitude (pattern electroretinography PERG)	2.05 µV SD: 1.09 95% CI: 1.64–2.45		p=0.000	3.3 µV SD: 1.48 95% CI: 2.70–3.89	
N95 implicit time (pattern electroretinography PERG)	97.93 ms SD: 9.41 95% CI: 94.42–101.45		N/S	97.15 ms SD: 7.63 95% CI: 94.01–100.24	

treated ophthalmic or organic diseases (only treated arterial hypertension was permitted); (7) no additional medications except antihypertensive drugs; (8) no macular pathologies; (9) no abnormalities in routine preoperative laboratory tests, especially C-reactive protein (CRP), complete blood count (CBC), and differential; and (10) body mass index (BMI) <30. The participants reported no addictions.

Ophthalmic examination

Glaucoma was classified based on gonioscopy, ophthalmoscopy, central corneal thickness measurement, tonometry (Goldmann model, Haag-Streit, Bern, Switzerland; 0.5% Alcaine), visual field examination (Octopus 301 HS, Interzeag), and policlinic history analysis. The average IOP for each patient was based on 3 measurements (the day before admission, the day of admission, and the day of surgery). All IOP measurements were obtained in the morning (between 8 AM and 11 AM). The IOP policlinic history (within the last 6 months), together with our measurements, aided in the exclusion of POAG patients in acute IOP phases. To exclude patients with macular pathology, optical coherence tomography (OCT; Cirrus HD-OCT 5000, Carl Zeiss Meditec, Dublin, CA) was used.

The patients were examined on the day of blood sample collection.

Electrophysiological examination

The transient pattern electroretinogram (PERG) was examined using Reti-Port equipment (Roland Consult, Germany). The study

conditions followed the recommendations and standards of the ISCEV (International Society for Clinical Electrophysiology of Vision). Fiber electrodes were used. The implicit time (the time to peak) and amplitude of the negative wave N95 were measured.

Biochemistry

The concentration of serum ceruloplasmin was determined spectrophotometrically by using Richterich reaction with p-phenylenediamine. Ceruloplasmin catalyzes the oxidation of colorless p-phenylenediamine changing it in blue-violet dye. PerkinElmer's VICTORX3 Multilable Plate Reader with absorbance filter for 560 nm was used to read samples.

Determination of total oxidation status (TOS)

Total oxidant status was measured according to Erel (Erel, 2005) in blood serum. The assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium. The change in color of the ferric ion by xylenol orange is measured as a change in absorbance at 560 nm. This process is applied to an automated Perkin Elmer analyzer and calibrated with hydrogen peroxide. Data are shown in µmol/l.

Determination of total antioxidant capacity (TAC)

Total antioxidant capacity was measured according to Erel (Erel, 2004) in blood serum. In this colorimetric method, radicals are generated and the antioxidant activity of blood serum reduces radical formation. The change in color of ABTS+
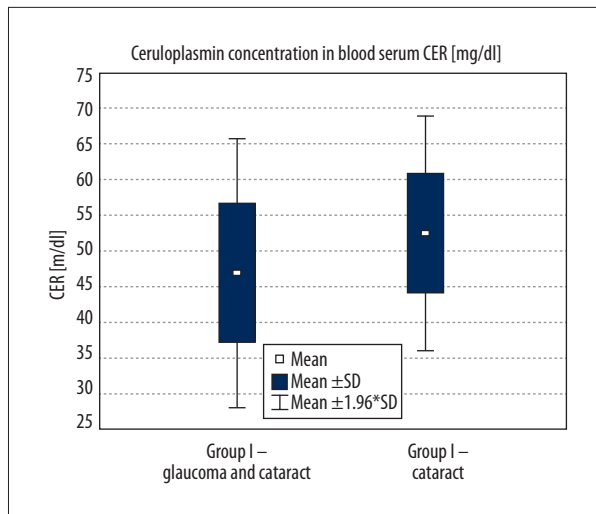
Figure 1. Ceruloplasmin concentration in blood serum.

ions (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) is measured as the change in absorbance at 660 nm. This was conducted in an automated Perkin Elmer analyzer calibrated with Trolox. Data are shown in mmol/l.

Results

Characteristics of the studied groups are presented in Table 1.

There was no statistically significant difference in best corrected visual acuity between groups. Serum ceruloplasmin level was lower in the POAG group (46.95 mg/dl) in comparison to the group with only cataract (52.49 mg/dl), (p=0.013), (Figure 1).

Substantial increases in total oxidative stress (TOS) were noted in POAG patients (22.81 µmol/l) compared with those with cataract (8.08 µmol/l), (p=0.016). The antioxidative reserves (TAC) were similar in both group (1.03 µmol/l and 1.02 µmol/l). N95 implicit time was comparable among groups.

Discussion

We have shown decreased activity of ceruloplasmin in the group with glaucoma and cataract in comparison to the group with only cataract. Despite a relatively low number of patients we are convinced that clinical assessment, inclusion and exclusion criteria, and the use of PERG enabled us to select homogenous groups.

All patients had visual field examinations, bur results are not presented in the study. In the first group there were characteristic changes for glaucoma in visual field, while in the second group the examination did not reveal typical glaucomatous defects.

The reduction of PERG N95 amplitude is a consequence of loss of RGC or their dysfunction, while increase in latency is due to damage to RGC axons [13].

The goal for glaucoma research is to indentify contributing factors in the initiation of this disease. IOP measurement, as an isolated element, does not fully reflect the progression of the disease.

Alzheimer and Parkinson disease are neurodegenerative conditions similar to glaucoma [14,15]. In Parkinson disease, serum concentration of ceruloplasmin is decreased, while in Alzheimer disease the concentration of this enzyme is normal but its activity is reduced [16]. Systemic ceruloplasmin seems to play a protective role in patients with Parkinson disease [17].

To the best of our knowledge, there is no evidence in the literature about correlation between serum ceruloplasmin levels in the blood and glaucomatous neuropathy. Studies have shown that increased expression of the enzyme is present locally in the retina [18]. The upregulation of ceruloplasmin was noted in murine and human glaucoma eyes in the retina, especially in the Muller cells and in the area of the inner limiting membrane. Increased expression is a response to noxious factor or RGC death. This may suggest a protective role of ceruloplasmin in neurodegenerative disease, such as primary open-angle glaucoma [19]. Moreover, ceruloplasmin upregulation seems to be a part of the injury response and was observed in experimental glaucoma in rats [20].

Conclusions

Persistent inflammation in the course of glaucoma and anti-glaucoma drugs may increase the permeability of the blood-ocular barrier [21,22] and may be connected with lower concentration of serum ceruloplasmin in glaucoma patients.

Disclosure statement

The authors of this paper declare they have no conflicts of interest.

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