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Levels of serum trace elements in patients with primary open-angle glaucoma

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ABSTRACT

Purpose: Glaucoma disease is known as multifactorial. Trace elements seemed to be linked via oxidative stress mediated changes to the complex glaucoma pathophysiology. Thus, it was the aim of this study to investigate serum levels of trace elements in primary open-angle glaucoma patients (POAG).

Patients and methods: Peripheral venous blood samples were taken from a total of 40 subjects: 22 primary open-angle glaucoma patients (mean age 58.1 ± 13.9, female 8, male 14) and 18 controls (mean age 38.9 ± 11.6, 6 female 6, male 12). Serum samples of cadmium, cobalt, copper, iron, lead, manganese and zinc were analyzed by Inductively-Coupled-Plasma-Optical Emission Spectrometry (Cu, Fe, Zn) and Inductively-Coupled-Plasma-sectorfield-Mass-Spectrometry (Cd, Co, Mn, Pb, Se). Data were analyzed using ANCOVA and presented as log transformed LS-mean.

Results: Patients with POAG had significantly increased serum levels of iron (2.98 ± 0.03 µg/L vs 2.98 ± 0.03 µg/L) when compared to controls, and of cadmium (1.57 ± 0.05 ng/L vs. 1.40 ± 0.06 ng/L) considering the interaction between age and the class variable (control versus POAG). A gender effect was seen for cadmium, cobalt, copper, and iron in controls and POAG patients. Iron concentration was reduced in dependency of age for both genders in normals, however lesser in POAG patients. No difference was seen in serum levels of lead, manganese, and zinc between patients with POAG and controls.

Conclusion: A significant elevation of serum cadmium and iron levels in POAG patients as well as an additional gender effect of cadmium, cobalt, copper, and iron in normals and POAG patients, may argue for a potential role of these trace elements in the pathogenesis of primary open-angle glaucoma.

1. Introduction

Glaucoma is the second cause for blindness in the industrialized nations [1] with about 6.7 million blind people worldwide each year. Up to now, the complex pathophysiology is not understood. It is known that it is a multifactorial disease with intraocular pressure (IOP) as main risk factor. As glaucoma disease proceeds even under optimal therapeutic lowering of IOP, several other factors seemed to be involved in the pathogenesis [2].

Oxidative stress can be seen as a further pathogenic factor in glaucoma disease. It can induce destruction of neurons [3] and cause alterations of the main outflow pathway of aqueous humor, the trabecular meshwork, with consecutive IOP elevation [4]. As result of an imbalance between oxidants (free radicals) and antioxidants, oxidative stress mediated changes can lead to irreversible cell loss as a consequence of DNA alterations [5]. Trace elements are linked to enzymes (e.g. superoxide dismutase enzymes, SOD), being involved in generating or eliminating free radicals. Zinc, copper, iron, and manganese interact via SOD [6], inducing superoxide anions' conversion into hydrogen peroxide. Superoxide belongs to a group of highly reactive oxygen species and is capable of severely damaging cells and genome. The trace element copper is able to generate oxidative stress via Fenton reaction or reduction of glutathione. This results in a generation of hydroxyl radicals, causing DNA strand brakes [7]. Iron is a redox-active agent [7], involved in several neurodegenerative diseases [8,9]. Manganese acts as a cofactor of manganese superoxide dismutase [10],

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Table 1
CRM and achieved recoveries for the trace elements lead, cadmium, iron, manganese, cobalt, copper and zinc.

Element	Used RM / CRM	RM type	Reference concentration	Recovery
Cd	RECIPE ClinCal Serum Calibrator	Serum	2.91 µg/L	99.2 %
Cd	ERM-BD-150	Milk	11.4 ± 2.9 µg/kg	100.0 %
Co	RECIPE ClinCal Serum Calibrator	Serum	2.61 µg/L	97.8 %
Cu	RECIPE ClinCal Serum Calibrator	Serum	1.23 mg/L	95.5 %
Cu	ERM-BD-150	Milk	1.08 ± 0.06 mg/kg	94.6 %
Fe	RECIPE ClinCheck Serum Control	Serum	612 µg/L (range: 490–734 µg/L)	99.0 %
Fe	ERM-BD-150	Milk	4.6 ± 0.5 mg/kg	98.9 %
Mn	RECIPE ClinCal Serum Calibrator	Serum	34.6 µg/L	100.2 %
Mn	G-EQUAS	Plasma	4.3 µg/L	98.5 %
Mn	G-EQUAS	Urine	11.8 µg/L	100.8 %
Mn	ERM-BD-150	Milk	0.289 ± 0.018 mg/kg	99.7 %
Pb	ERM-BD-150	Milk	19.3 ± 4 µg/kg	98.8 %
Zn	BCR-637	Serum	1.11 ± 0.22 mg /L	99.6 %
Zn	RECIPE ClinCal Serum Calibrator	Serum	1.812 mg/L	98.2 %
Zn	ERM-BD-150	Milk	44 ± 2 mg/kg	100.7 %

inducing the metabolism of hydrogen peroxide and caspases, closely linked to cell apoptosis. Lead and cadmium can interact with the catalytic centers of enzymes due to their high affinity for sulfhydryl groups. Chronic exposure to lower cadmium levels (e.g. 0.5–10 microM [11]) induced free radicals with carcinogenic effect [12]. Cobalt induced damage of retinal cells (e.g. ganglion cells, bipolar cells, horizontal cells, photoreceptors) and atrophy of the nerve fibers in rabbits' eyes [13].

Previous studies reported about changed oxidative stress markers [14]. Additionally, altered serum and aqueous humor levels of trace elements were observed in glaucoma patients [15]. However, there is no study available up to now investigating serum levels of cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn) and zinc (Zn) parallelly in primary open-angle glaucoma patients (POAG). Thus, it was the aim of the present study to analyze serum levels of these trace elements in serum samples of POAG patients by Inductively-Coupled-Plasma-Optical Emission Spectrometry (ICP-OES; Cu, Fe, Zn) and Inductively-Coupled-Plasma-sectorfield-Mass-Spectrometry (ICP-sf-MS; Cd, Co, Mn, Pb, Se) in comparison to controls.

2. Patients and methods

2.1. Patients

40 patients were recruited from the Department of Ophthalmology and Eye Hospital, Friedrich-Alexander-Universität of Erlangen-Nürnberg (FAU): 22 POAG patients (mean age 58.1 ± 13.9, female 8, male 14) and 18 controls (mean age 38.9 ± 11.6, 6 female 6, male 12). Glaucoma diagnosis was based on an altered optic disc, classified after Jonas [16], a confirmed untreated IOP > 21 mmHg and confirmed visual field loss (MD > 2.8 dB and ≥ 3 adjacent test points on the pattern deviation map with a probability of < 5% or ≥ 2 adjacent test points on the pattern deviation map with a probability of < 1%; Octopus 500, G1 protocol, Interzeag, Schlieren, Switzerland). Control subjects had no glaucomatous alterations or elevated IOP. Exclusion criteria were any ophthalmic (except POAG) and system disease or supplementary intake. Anamnestic data about nutrition were obtained from each patient. All patients underwent slit-lamp biomicroscopy, funduscopy, and Goldmann applanation tonometry. Serum samples were collected in special metal free tubes for trace element analytics (S-Monovette® for metal analysis, Sarstedt) and stored at −80 °C.

The study was designed as a prospective case-control study. It was approved by the local ethics committee (53_14B) and performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was received from each patient.

2.2. Trace element analysis

Serum samples were diluted 1/10 with Milli-Q water and directly measured by ICP-OES (Cu, Fe, Zn) or ICP-sf-MS (Cd, Co, Mn, Pb, Se) since those element concentrations were below the detection limit of ICP-OES.

2.2.1. Sample analysis by ICP-OES

An ICP-OES „Spectro Ciros Vision "system (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) was used for element determination in 1:10 diluted (Milli-Q H_2O) samples. Sample introduction was carried out using a peristaltic pump connected to a Meinhard nebulizer with a cyclon spray chamber. The measured spectral element lines were (nm): Cu: 324.754, Fe: 259.941, Zn: 213.856.

The RF power was set to 1400 W, the plasma gas was 13 L Ar /min, whereas the nebulizer gas was approximately 0.6 L Ar/min after daily optimization.

2.2.2. Sample analysis by ICP-sfMS

An ELEMENT 2, Thermo-Electron (Bremen, Germany) ICP-sf-MS instrument was employed for the determination of elements which were below the LoD from ICP-OES. ^{103}Rh was administered to each sample at a concentration of 1 µg/L as an internal standard. It was monitored as an internal standard at a final concentration of 1 µg/L in each sample and standard. Sample introduction was carried out using a peristaltic pump connected to a Seaspray nebulizer with a cyclon spray chamber. The RF power was set to 1300 W, the plasma gas was 15 L Ar /min, whereas the nebulizer gas was approximately 0.9 L Ar/min after daily optimization. The threshold levels below which the ICP-sfMS was used, were 1 µg/L for Zn, Fe, Cu, Co and 0.5 µg/L for Cd, Pb, Mn.

Measured element isotopes were: ^{114}Cd, ^{59}Co, ^{55}Mn, ^{208}Pb, ^{77}Se.

No RMs or CRMs are available for glaucoma trace element analysis. Therefore, a control material (with nearly all requested elements) and other CRMs of human (plasma, serum) and animal (milk) origin (only parts of the here requested elements certified, each) were used to cover all investigated elements (Table 1).

2.2.3. Quality control for element determinations

The determination method had been validated previously by regular laboratory intercomparison studies and by regular analysis of adequate certified reference materials.

Routinely every 10 measurements, 3 blank determinations and a control determination of a certified standard for all mentioned elements were performed. Calculation of results was carried out on a computerized lab-data management system, relating the sample measurements to calibration curves, blank determinations, control standards.

2.3. Statistical analysis

Firstly, all numerical variables were transformed logarithmically. The data were analyzed with an analysis of covariance (ANCOVA). In order to control the variance of possible confounding factors, age was set as a covariate. Moreover, the model was adjusted for gender. In the model, each trace element (Cu, Fe, Zn, Cd, Co, Mn, Pb) was set as a dependent variable. In this way, we could investigate if there were any differences between the class variable (control, POAG) in relation to the different trace element levels. Being the experimental design unbalanced (the number of POAG patients differ from the control group) we estimated the least squares means (LS-means), that corresponded to the specified effects for the linear predictor part of the model. For the post hoc multiple comparison tests, we used the Tukey-Kramer adjustment (designed for unequal groups' dimension). Figures and data are presented as logarithmically transformed LS-means. Moreover, before applying the ANCOVA model we investigated the homogeneity of the variance and if the residuals of the model were normally distributed. Therefore, we examined different plots for each species. To test the normality of the residuals, we inspected the normal quantile-quantile (QQ) plot and the plot of the residuals against the percents plots. The plot (predicted variable against residuals) was created to test if there were divergences from the homoscedasticity. The assumptions were satisfied and therefore the ANCOVA model can be performed (Fig. 1). All the statistics were done using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

Raw serum levels and percentiles of cadmium, cobalt, copper, iron, lead, manganese and zinc can be seen in Table 2 for POAG patients and control subjects.

We built the ANCOVA complete model, with both interactions (the class variable with gender and with age) and age as a covariate. A significant interaction between class and age is detected only in serum cadmium level ($p = 0.026$). With increasing age, the slopes of Cd of the control group and POAG group were opposite (Fig. 2). While the fitted models for control's male (1.38 ± 0.06 ng/L) and female (1.41 ± 0.08 ng/L) nearly coincided, POAG patients showed an opposite trend. Female POAG patients (1.68 ± 0.07 ng/L) had higher serum levels of cadmium than male POAG patients (1.46 ± 0.05 ng/L). In summary, serum cadmium levels increased in POAG patients with increasing age, especially in women, yet controls showed a decreasing trend.

Considering the other species we have only a significant increase in iron in male POAG patients (3.0 ± 0.03 µg/L) compared to female controls (2.75 ± 0.05 µg/L; $p = 0.004$). All the multiple comparisons

Table 2
Serum levels of lead, cadmium, iron, manganese, cobalt, copper and zinc in the control and the primary open-angle glaucoma patients' group (POAG).

Trace Elements	25th	50th	75th	95th
	Percentile (Control)			
Cadmium [ng/L]	20.2	26.8	37.1	66.2
Cobalt [ng/L]	107.3	134.0	306.5	369.1
Copper [µg/L]	655.0	707.5	851.5	1059.5
Iron [µg/L]	662.8	890.0	1005.3	1346.0
Lead [ng/L]	322.3	619.5	811.0	1527.0
Manganese [ng/L]	538.0	574.0	636.3	768.5
Zinc [µg/L]	590.3	625.0	702.5	787.5
	Percentile (POAG)			
Cadmium [ng/L]	24.7	35.6	44.7	101.8
Cobalt [ng/L]	115.3	136.0	179.5	421.0
Copper [µg/L]	775.3	812.5	966.5	1179.5
Iron [µg/L]	782.3	920.5	1060.0	1254.0
Lead [ng/L]	325.5	482.5	639.5	1207.4
Manganese [ng/L]	547.3	634.5	716.8	1116.7
Zinc [µg/L]	532.5	606.5	637.0	700.8

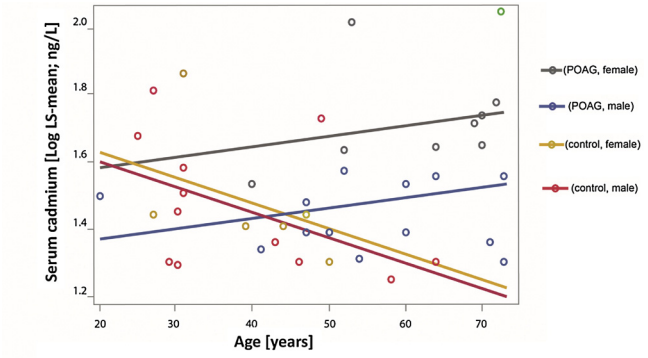

Fig. 2. Log cadmium serum levels (LS-Mean) in controls and POAG patients with respect of age and gender (ANCOVA model).

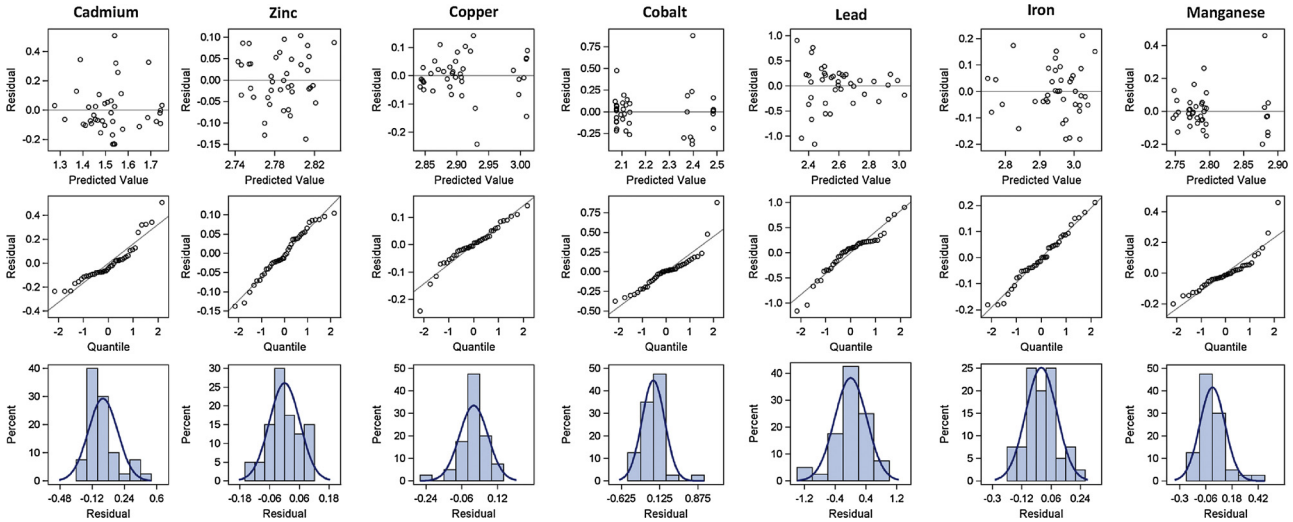

Fig. 1. Fit diagnostics plots for each species (logarithmic transformed): The quantile against the residual and the residual against the percent plots test if the error terms were normally distributed. The plot (predicted variable against residual) tests if there were divergences from the homoscedasticity. Plot inspection did not retrieve violation of the assumptions to excute an ANCOVA model.

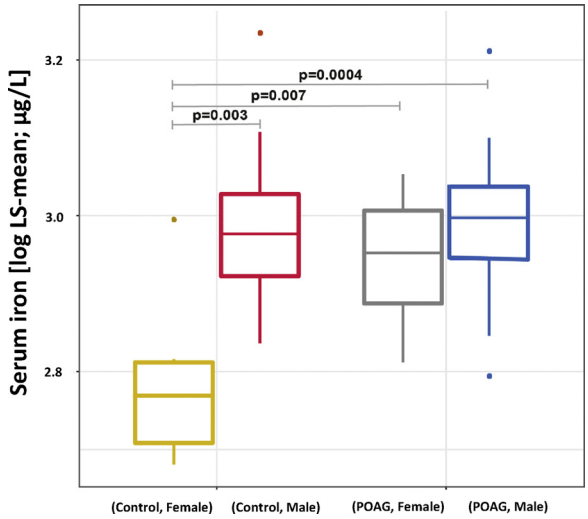

Fig. 3. Box plots of log iron serum levels (LS-Mean) in controls and POAG patients considering gender: iron was significantly different between male and female controls (p = 0.003), between female controls and female POAG patients (p = 0.007) and between female controls and male POAG patients (p = 0.004, multiple comparison and corrected by Tukey test); female controls showed the lowest iron levels, being significantly lower compared to all other groups.

(we applied the Tukey-Kramer adjustment) were presented in Fig. 3. Female POAG patients (2.97 ± 0.04 µg/L) showed an increased serum level of iron in comparison to female controls (p = 0.007). Additionally, a difference was observed between the iron concentration of male and female controls (male control: 2.94 ± 0.04 µg/L; p = 0.003).

For the models where the interactions between age and the class variable were not significant, we examined a reduced ANCOVA model. We considered only one interaction (the class variable with gender) and age as a covariate. We observed that different serum levels were strongly related to gender and glaucoma. Serum Co, Cu, and Fe yielded significant p-values in the different level of the multiple comparisons (adjusted with Tukey-Kramer test). All the p-values were listed in Table 3. Female controls showed increased serum levels of Co compared to male controls (p = 0.008). Additionally, female controls yielded increased Co concentrations than male POAG patients (p = 0.021). Copper concentrations were seen to be increased in female POAG patients compared to male controls (p = 0.014) and male POAG patients (p = 0.020). Iron levels were reduced in female controls compared to male controls (p = 0.002) and male POAG patients (p = 0.0004). Analyzing female serum iron level considering glaucoma, POAG patients showed increased concentrations than the control group (p = 0.007).

No significant differences were seen in serum levels of lead, manganese, and zinc between POAG patients and controls with an additional focus on age and gender.

4. Discussion

Oxidative stress is known to be involved in glaucoma pathogenesis [17–19]. However, the exact molecular pathway is not known up to now. One potential molecular mechanism can be seen in altered levels of trace elements in open-angle glaucoma patients. As previous data showed an association of serum level of Selenium [20] and glaucoma as well as an altered aqueous humor concentration of Zn, Fe [21], and Co [15] in glaucoma patients, subsequent analyses of further trace elements are of interest in glaucoma research. However, there is no study available up to now, investigating simultaneously serum levels of Cd, Co, Cu, Fe, Pb, Mn and Zn in POAG patients with an additional focus on gender and age. The data of the present study showed that significantly higher Cd and Fe levels were observed in serum samples of POAG patients compared to controls considering age and gender interactions. Even, increased serum Fe levels remained significant after adjustment for gender in POAG patients. Additionally, significant interactions between the genders were seen for serum concentration of Cu, Co, and Fe in controls versus POAG patients and other multiple comparisons (see Table 3). In Cd, we observed a significant interaction with age in POAG patients (Fig. 2).

Cadmium is ubiquitous, thus being e.g. a constituent of soil or in food. It is a potent factor in generating cell damage with even more toxic potential than lead [12]. Yet, low chronic exposure of Cd produces free radicals, influences gene expression, and thus can be carcinogenic [23], especially in reproductive organs [24]. Following Cd exposure, decreased levels of glutathione and increased levels of protein-mixed disulfides were observed in neuronal cell culture [25]. Additionally, high Cd levels increased lipid peroxidation, reducing cell's ability to

Table 3
Differences of Least Squares Means and p-values of cadmium, cobalt, copper and iron in controls and POAG patients considering gender.

	Difference between means	Simultaneous 95% Confidence Limits (LSMean(i)-SMean(j))		P-values
	Cobalt			
(Control,M) (Control,F)	−0.407	−0.725	−0.089	0.008
(Control,M) (POAG,M)	−0.023	−0.311	0.265	NS
(Control,M) (POAG,F)	−0.292	−0.635	0.051	NS
(Control,F) (POAG,M)	0.384	0.046	0.722	0.021
(Control,F) (POAG,F)	0.115	−0.270	0.500	NS
(POAG,M) (POAG,F)	−0.269	−0.554	0.016	NS
	Copper			
(Control,M) (Control,F)	−0.066	−0.168	0.036	NS
(Control,M) (POAG,M)	−0.027	−0.119	0.066	NS
(Control,M) (POAG,F)	−0.131	−0.241	−0.021	0.014
(Control,F) (POAG,M)	0.039	−0.069	0.148	NS
(Control,F) (POAG,F)	−0.065	−0.188	0.059	NS
(POAG,M) (POAG,F)	−0.104	−0.195	−0.013	0.020
	Iron			
(Control,M) (Control,F)	0.195	0.059	0.332	0.002
(Control,M) (POAG,M)	−0.046	−0.170	0.077	NS
(Control,M) (POAG,F)	−0.019	−0.166	0.128	NS
(Control, F) (POAG,M)	−0.242	−0.387	−0.097	0.0004
(Control,F) (POAG,F)	−0.214	−0.379	−0.049	0.007
(POAG,M) (POAG,F)	0.028	−0.094	0.150	NS

Least Squares Means for Effect Group*Gender (M = male, F = female), NS – not significant.

protect itself against oxidative stress [12]. Consecutively, the activity of superoxide dismutase is reduced and catalase activity is disturbed [26], generating radicals like superoxide anions, hydrogen peroxide or hydroxyl radicals. A further pathogenetic mechanism of generating free oxygen radicals is the high affinity of Cd to sulfhydryl groups, affecting the function of several antioxidant enzymes [12].

Iron, the second trace element, being increased in serum samples of POAG patients even after adjustment of gender, is essential in the human body. Next to its physiological functions, iron can act harmfully by shifting the Fe(II)/Fe(III) ratio toward Fe(II) with subsequent generation of reactive oxygen species [27,28]. Iron is assumed to be involved in several neurodegenerative disorders (e.g. Alzheimer disease) [29]. Elevated Fe levels can promote lipid peroxidation and thus enhance the formation of hydroxyl radicals, especially in the presence of oxygen [30]. Additionally, increased Fe levels were associated with correspondingly higher levels of urinary 8-hydroxydeoxyguanosine enabling this trace element as a potential risk factor of DNA damage at the cellular level [31]. In ocular tissue Fe transport across the blood-retina barrier is conducted by transferrin, divalent metal transporter-1 (DMT-1) and dexras (i.e. guanylyl imidodiphosphate-binding proteins), which were activated via NMDA receptors [32]. In aqueous humor, Fe was observed to be significantly reduced in pseudoexfoliation glaucoma patients compared to controls and significantly different from POAG patients [21]. Increased intravitreal Fe levels were associated with an increased concentration of superoxide radicals in internal photoreceptor segments in adult C57BL/6 mice [33]. As oral Fe and Calcium substitution were seen to increase the risk of developing glaucoma with an odds ratio of 3.80 (Fe) [34], Fe was postulated to be a potential risk factor for POAG [35]. Ceruloplasmin can affect Fe metabolism by catalyzing the reaction of Fe^{2+} to Fe^{3+}. Thus, lower levels of ceruloplasmin can induce hydroxyl radicals. The concentration of Cu is known to influence the presence of serum ceruloplasmin. Additionally, Cu, important in the human and animal organism, acts via cofactor of several enzymes (e.g.cytochrome C oxidase, superoxide dismutase) [6]. Additionally, it is involved in electron transport [36]. POAG patients showed significantly decreased serum ceruloplasmin levels compared to control subjects [37]. As in the present study, a different serum level of Cu was observed between gender in POAG patients, serum levels of Cu and Fe may interact and promote oxidative stress mediated changes.

Up to date, only a few studies are available investigating trace elements in serum samples of POAG patients [15,20,38,39]. Serum levels of Zn and Cu were not statistically different in Turkish glaucoma patients compared to controls [40]. In a retrospective study by Lin et al., elevated serum levels of mercury and lower serum levels of manganese were linked to a greater odds of glaucoma in the South Korean population [41]. Lee et al. observed a link of log-transformed blood Cd levels with low-teen OAG patients (initial IOP $\leq$ 15 mmHg) only in men (odds ratio 1.65), however not in high-teen OAG patients (initial IOP $>$ 15 mmHg and $\leq$ 21 mmHg) in a South Korean population [38]. These differences in trace elements might be due to the different analytic methods and to a less part due to ethnicity with consequently different lifestyle and nutrition. Lee et al. uses graphite furnace atomic absorption spectrometry (GFAAS), a very reliable method, which however, typically has an instrumental detection limit for Cd around or close below 100 ng/L (Lee et al. report 87 ng/L). With their limit of detection serum cadmium concentrations can be measured: Lee et al. wrote that all Cd-values in blood were above LOD. Their concentrations were between 10–11 µg/L, which is about 500–1000 times higher than the serum concentrations in the present study (20–73 ng/L), measured by ICP-sf-MS. With the method used by Lee et al. it is impossible to analyze that low serum Cd-concentration and thus, they cannot observe tendencies as we have seen them at such low concentrations. The present study presents data of serum trace elements in European POAG patients for the first time. Higher serum Cd levels, increasing with age in both genders, and higher serum Fe levels, especially in female POAG patients were seen. As Fe and Cd were known to be transported via the same cellular transporters, interactions between both trace elements may occur and gene alterations of one pathway may influence the other (e.g. seen in HFE H63D) [42]. As most of the present studies investigated only the effect of one trace element, data of these studies seemed to offer an inverse relationship between Cd and Fe [43,44]. However, Abarikwu et al. presented for the first time that both testicular Cd and Fe levels were increased after simultaneous exposure of these trace elements in rats, yet not after single administration of on trace element [45]. This data may offer that Cd and Fe could have synergistic toxic effects.

The present study is not without limitations. Samples size is small, however, detecting statistically significances in these study groups. This argues for a critical involvement of trace elements in glaucoma disease. Additionally, further studies are required in larger patients' groups. Furthermore, the molecular pathway of trace elements in glaucoma diseases is open for further investigation. Specific interest will be focused on the Fe(II)/Fe(III) balance in aqueous humor samples in future studies.

5. Conclusion

Trace elements are involved in oxidative stress mediated changes, thus potentially being involved in glaucoma pathogenesis. A significant elevation of serum level of cadmium and iron in POAG patients might argue for a role of these trace elements in the pathogenesis of primary open-angle glaucoma.

Conflict of interest

All authors declare no conflict of interest.

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