Thieme

Serum Selenium Levels in Glaucoma: a Pilot Study

Selenspiegel bei Glaukompatienten: eine Pilotstudie

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Key words

glaucoma, selenium, trace element, age, gender, ICP-sf-MS

Schlüsselwörter

Glaukom, Selen, Spurenelement, Alter, Geschlecht, ICP-sf-MS

received 14. 8. 2020
accepted 13. 11. 2020
published online

Bibliography

Klin Monatsbl Augenheilkd 2021
DOI 10.1055/a-1333-2816
ISSN 0023-2165

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

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ABSTRACT

Background Trace elements are assumed to be involved in glaucoma pathogenesis via changes in oxidative stress. Especially serum selenium (Se) has been linked to this neurodegenerative disease. Serum Se levels differ between countries due to nutrition and ethnicity. It was the aim of the present study to investigate serum Se levels in primary open-angle glaucoma (POAG) patients and controls in Germany and to consider potential age and gender effects.

Material and Methods The Se concentration of 39 serum samples (22 patients with POAG, 17 controls) were analyzed by inductively coupled plasma-sector field mass spectrometry (ICP-sf-MS) in high resolution mode. Covariance and percentile regression were analyzed. Age and gender were defined as confounding factors and their different trends were investigated. Moreover, age was examined across different quantiles of Se levels.

Results Total serum least-squares means (LS-means) Se levels were 132.02 µg/L (controls) and 134.86 µg/L (POAG). Total serum Se levels did not differ between the study groups ($p > 0.05$). Significant age and gender effects of serum Se were observed. Quantile analysis showed that the 1st serum Se quantile decreased with increasing age in POAG patients in contrast to controls. The odds ratios of the 1st serum Se were 1.3 (with 2nd quantile) and 1.3 (with 3rd quantile), respectively.

Conclusion The serum Se level of the German cohort was almost half of those of the published US cohort (glaucoma 209.11 ng/mL; control 194.45 ng/mL). Age and gender effects were observed; the serum Se level increased with age in women (controls and POAG), however, Se levels decreased with age in men (controls and POAG).

ZUSAMMENFASSUNG

Hintergrund Spurenelemente können über oxidativen Stress vermittelte molekulare Interaktionen in die Pathogenese der Glaukomerkrankung eingreifen. Vor allem für das Spurenelement Selen (Se) wird eine Mitbeteiligung postuliert. Der Selengehalt im Serum wird unter anderem über die Ernährung gesteuert und unterliegt damit kulturellen und ethnischen Variabilitäten. Das Ziel der vorliegenden Studie war es, den Serumselenspiegel bei Patienten mit primärem Offenwinkelglaukom (pOWG) im Vergleich zu einer Kontrollgruppe zu analysieren. Diese Ergebnisse der vorliegenden deutschen Kohorte wurden auf mögliche Alters- und Gendereffekte hin untersucht sowie ins Verhältnis zu der bislang einzig beschriebenen Kohorte in den US gesetzt.

Material und Methoden Die Selenkonzentration wurde von 39 Serumproben (22 pOWG, 17 Kontrollen) anhand der Inductively coupled Plasma-sector Field Mass Spectrometry (ICP-sf-MS) analysiert. Die statistische Analyse umfasste eine Kovarianz-, Perzentilenregressions-, Alters- und Genderanalyse.

Ergebnisse Die Serumselenkonzentration (Ls-mean) betrug 134,86 µg/l für Patienten mit pOWG und 132,02 µg/l für die Kontrollgruppe. Der Serumselengehalt zwischen diesen beiden Gruppen zeigte keinen signifikanten Unterschied

(p > 0,05). Jedoch war ein dezenter Alters- und Gendereffekt zu beobachten. Die Quantilanalyse erbrachte eine Reduktion der 1. Serumselenquantile mit ansteigendem Alter bei den Patienten mit pOWG im Gegensatz zu den Kontrollprobanden. Die Odds Ratio der 1. Quantile betrug 1,3 (jeweils in Bezug zur 2. und 3. Quantile).

Zusammenfassung Der Serumselengehalt von Patienten mit pOWG zeigte sich als ca. halb so hoch wie der publizierte Wert der US-Literaturkohorte (Glaukom 209,11 ng/ml; Kontrolle 194,45 ng/ml). Zusätzlich war ein leichter Alters- und Gendereffekt zu beobachten.

Introduction

Glaucoma pathophysiology is not completely understood up to now. It is assumed that this neurodegenerative disease is caused by a multifactorial pathogenesis leading to irreversible blindness. Next to its main risk factor, an elevated intraocular pressure, a vascular dysregulation [1], and oxidative stress [2 – 6] are assumed to be involved in this pathophysiology. In 2002, the Nutritional Prevention of Cancer (NPC) trial observed an increased incidence of glaucoma (hazard ratio of 1.78) as a side effect of selenium (Se) supplementation (200 µg per day), which was administered in order to reduce cancer incidence [7, 8]. A subsequent clinical study and, to date, the only one regarding Se in the plasma of patients with primary open-angle glaucoma (POAG) investigated the plasma and aqueous humor (AH) Se levels using high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC ICP-MS) [9]. A trend towards higher plasma and AH Se concentrations was observed, however, not reaching statistical significance. Additional tertile analyses yielded a link to glaucoma disease. A further study on Se in the AH of patients with OAG investigated the Se concentration using the ICP-DRC-MS technique, which provided suitably low detection limits for even very low Se concentrations (LOD 25 ng/L). Se concentrations in that study were found considerably lower compared to Bruhn and a link to glaucoma was seen only in quantile, yet not in the total level of Se. Since Bruhn mentioned some methodical limitations such as “concentrations were very close to the limit of detection” and their “analyses suggests that greater variation in aqueous humor selenium may exist” [9, 10], a further analysis of serum Se levels is of interest using a fully suitable method with quality-controlled measurements. The Se level differs between countries as nutrition and nutritive supply varies between each country. Considering a gender difference of the Se level on cardiovascular disease mortality (country: USA) [11] and a link of an increased Se level to an increased risk of hyperglycemia and dyslipidemia (country: China) [12], a potential influence of Se on microcirculation might be proposed. The aim of the present study was to investigate the serum Se level in patients with POAG and controls in Germany with the inductively coupled plasma-sector field mass spectrometry (ICP-sf-MS) considering potential age and gender effects.

Material and Methods

Patients

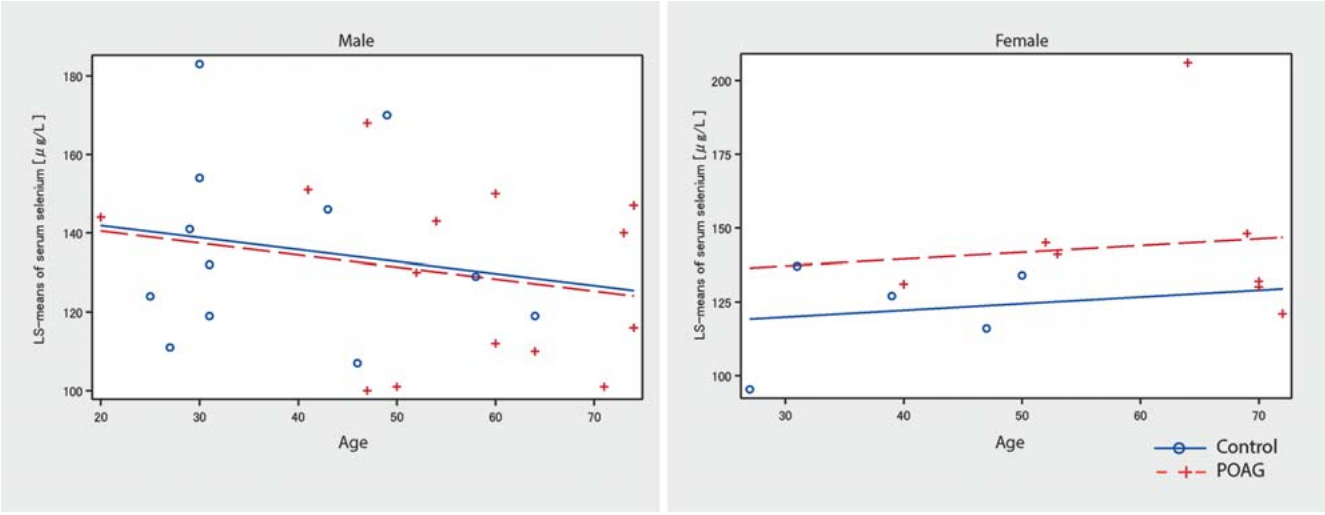
Thirty-nine subjects (22 patients with POAG [female: 8, male: 14], 17 controls [female: 5, male: 12]) were included. Diagnosis of glaucoma was based on a confirmed untreated IOP > 21 mmHg, measured by Goldmann applanation tonometry and optic disc alterations, which were classified after Jonas, and confirmed 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 probands showed normal IOP and healthy optic disc. Supplementary intake was an exclusion criterion. The study was approved by the local ethics committee. Performance in accordance with the tenets of the Declaration of Helsinki was ensured. Written informed consent was obtained from each patient.

Trace element analysis

Blood samples were collected in special metal free tubes for trace element analytics (S-Monovette for metal analysis, Sarstedt) and stored at − 80 °C. The blinded samples were sent to the analytical laboratory at Helmholtz Zentrum München on dry ice for Se determination. Prior to analysis, serum samples remained stored at − 80 °C. After gentle thawing, the serum samples were diluted 1 : 10 with Milli-Q water and ^{103}Rh standard (final concentration 1 µg/L). No HNO_3 was applied since this would have caused protein aggregation with subsequent adulteration of measurements and nebulizer clogging. Subsequently, Se was measured with ICP-sf-MS (ELEMENT II, Thermo Scientific, Bremen, Germany). Instrumental settings were radio frequency power: 1250 W, plasma gas flow: 15 L Ar/min, auxiliary gas flow: 0.85 L Ar/min, nebulizer gas flow: 0.86 L Ar/min, daily optimized, isotopes analyzed in high resolution mode: ^{77}Se, ^{78}Se, and ^{103}Rh as internal standards. Six-point calibration curves from 0–5000 ng/L were linear with r^2 for ^{77}Se = 0.999923 or r^2 (^{78}Se) 0.999842. The limit of quantification (LOQ) was 95 ng/L. Every tenth sample blank-controls and determinations of certified Se control standard (CPI international) were measured. For quality control, control material “human serum” from RECIPE, Munich, Germany was analyzed. The material was reconstituted with the indicated volume of Milli-Q water and further diluted 1 : 10 with Milli-Q water. Se concentration in the reconstituted sample was determined as 134.5 ± 4.3 µg/L (n = 3), being close to the manufacturer’s target value of 132 µg/L.

Statistics

The differences between the serum Se level of patients with POAG and controls were evaluated with an analysis of covariance (ANCOVA), where Se (continuous variable) was set as the dependent variable (least-squares mean; LS-Mean, 95 % CI). The model was corrected for group differences in age and gender. The contribution of the factor was evaluated with the type III sum of squares values. The test significance of differences among the LS-means



▶ **Fig. 1** LS-mean of serum Se level [µg/L] between women and men considering age as a covariate.

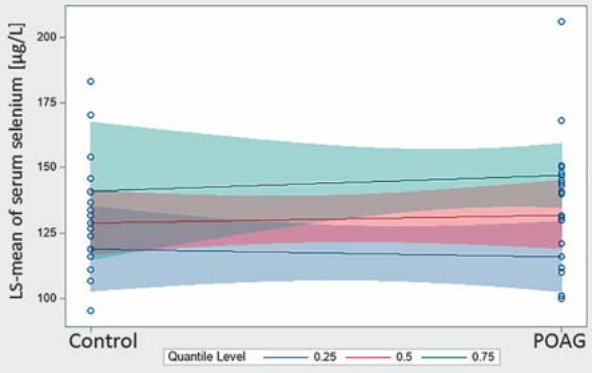
were evaluated after the Dunnett adjustment. The Se level, across the two classes, and the covariate effects on the variables Se were additionally modeled with the quantile regression procedures. Moreover, the covariate effects on the variables Se were additionally modeled with the quantile regression procedure (age: independent variable; Se: dependent variable). Odds ratio (OR) analysis was performed. All statistics were done using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) and EXCEL (Microsoft, 2010).

Results

The LS-mean serum Se level was 132.02 µg/L (95% CI: 120.45–143.58 µg/L) in controls. POAG samples yielded an LS-mean serum Se level of 134.86 µg/L (95% CI: 124.70–145.03 µg/L). ANCOVA analysis after the Dunnett adjustment for the level of serum Se reported a mean difference of − 2.85 between the LS-means of Se (95% CI − 18.24–12.55).

The serum level of Se increased with age (covariate of the model) for women of the control and POAG groups. Men of the control and glaucoma groups showed an opposite trend with a decreasing serum Se level with increasing age (▶ **Fig. 1**). The ANCOVA model revealed no difference in total serum Se levels between control subjects and POAG patients ($p > 0.05$).

A quantile analysis of serum Se levels between glaucoma and controls is presented in ▶ **Fig. 2**. The data of quantile analysis are reported in ▶ **Table 1**. The quantile regression (▶ **Fig. 3**) of the serum Se level related to age showed different trends for the different classes (control, POAG). Considering age as a covariate, the 1st serum Se's quantile decreased with increasing age in POAG patients, contrary to control subjects. In addition, the trend in control subjects was the opposite for the extreme part. If the serum Se level was increasing for the young people, the serum Se level was decreasing in older subjects. Interestingly, the level of Se decreased for younger patients with POAG. In summary, we could say that, generally, there is a trend for a decrease of the Se level in all the quantiles in patients with POAG. The ORs were 1.3 for the 1st serum Se quantile with the 2nd quantile, and 1.3 with the 3rd quantile.



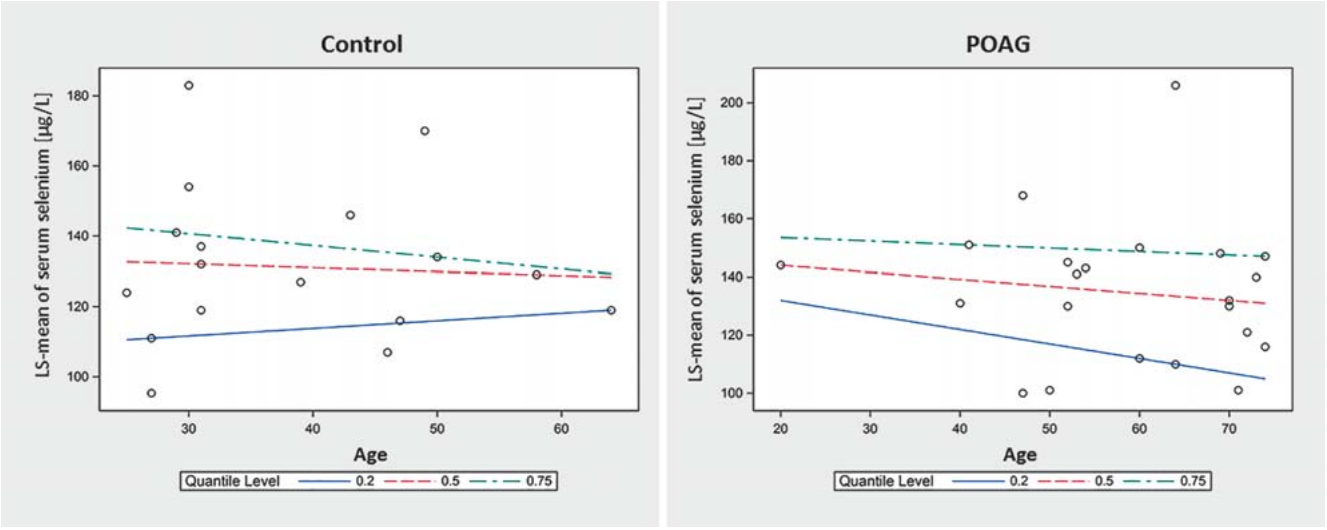
▶ **Fig. 2** Quantile levels of the LS-mean of serum Se [µg/L] of POAG patients and control subjects.

Discussion

Data of recent studies suggested that trace elements might contribute to glaucoma pathophysiology by oxidative stress-mediated changes or even influencing microcirculation [9, 10 – 14]. Se was especially focused on as the NPC trial postulated a potential link of Se to glaucoma conversion. Se supplementation was observed to be linked with an increased risk of glaucoma [7]. In the present German study group, we observed different age trends for female and male controls and patients with POAG. Quantile analysis yielded a decrease of the 1st serum Se's quantile in patients with POAG, contrary to controls. The OR was 1.3 with the 2nd and 3rd Se quantiles, respectively. The total serum Se level and quantile analysis of the Se concentration were not signifi-

▶ **Table 1** Quantile analysis of the serum Se level (95% confidence interval, p value).

Parameter estimates							
Quantile level	**Parameter**	**Estimate**	**Standard error**	**95% Confidence limits**		**t value**	**Pr > |t|**
0.25	control vs. POAG	–	10.6735	− 24.627	18.6265	− 0.28	0.7802
0.5	control vs. POAG	3	9.0266	− 15.29	21.2896	0.33	0.7415
0.75	control vs. POAG	6	14.5605	− 23.502	35.5024	0.41	0.6827



▶ **Fig. 3** Age dependency of the quantile level for the LS-mean of the serum Se level [µg/L], stratified by control and POAG.

cantly different between patients with POAG and control subjects. Using the different model approaches we could observe different trends related to the two main confounding factors: age and gender. Analysis of gender yielded that men and women showed different age trends for serum Se levels. The serum Se level of female controls and POAG patients slightly increased with increasing age. On the contrary, the Se concentration of male controls and patients with POAG decreased with increasing age. Previous data of AH Se levels offered a similar trend in female German patients with POAG and pseudoexfoliation glaucoma patients, showing increased 1st and 2nd quantiles of the AH Se level compared to control subjects with an additional age effect [15].

The total serum Se concentration was not significantly different between patients with POAG and controls in the present German groups. This data conforms with serum Se levels in American patients with POAG [9, 10]. Interestingly, the Se level itself differed between both study cohorts. The US study yielded serum Se levels almost twice as high (glaucoma 209.11 ng/mL; control 194.45 ng/mL) as the German data in patient and control groups, respectively. This trend was also seen in the AH Se levels of US and German glaucoma patients and controls [9, 10, 15]. The US data (glaucoma 46.31 ng/mL; controls 46.02 ng/mL) were even sevenfold as high as the German data (POAG 6.74 µg/L; controls 6.90 µg/L). Bruhn et al. stated that highest tertile of the plasma Se level showed an OR of 11.3 with a consecutive link to POAG ($p = 0.03$). On the contrary, ORs were even lower in the present study (1.3 for the 1st quantile with the 2nd quantile; 1.3 with the 3rd quantile). The greatest impact on the different total serum Se levels and consecutive OR analysis between both studies might be seen in methodical differences, next to nutritional aspects. The linear (suitable) detection range in that study starts on the lower end at 24.5 µg/L, exceeding values to be expected from appropriately prepared (diluted 1/5–1/10) plasma samples for measurement. The [Zn-O] interference has 27.83% relative abundance at $m/z = 82$. Thus, it could superimpose the ^{82}Se signal, resulting in overestimation of the selenium amount [16]. The latter is relevant as Zn concentrations in plasma samples are typically in the range of 800–1200 µg/L, which could erroneously increase Se concentration measurements in substantial amounts. On the contrary, in our measurement with ICP-sf-MS, the ^{77}Se isotope, which was not interfered with in the high-resolution mode, was used. Nutritional, i.e., supplementation, reasons might also contribute to higher Se plasma concentrations in the Bruhn et al. study. A supply with trace elements, especially Se, is common in the US [17], but unfortunately was not controlled in the US study. Yet, one exclusion criterion of the present study was dietary supplementation; thus, this data can be seen as unbiased by dietary supplements. In the 1990s, the serum Se level was assumed to range between 61–99 µg/L in healthy subjects [18], although nowadays, higher optimum levels are reported to be in the range of 100–

130 µg/L [19]. Yet, widely geographical and cultural variability dominate the actual range. Mean Se levels can vary between, e. g., 67.4 ± 38.6 µg/L (Poland) [20] or 73.2 ± 9.9 µg/L (Brasil) [21], which can even reach levels up to 216.2 ± 7.4 µg/L (Taiwan) [22].

The present study has some limitations. The study sample is small; thus, the study should be seen as pilot study. As the study was designed as a prospective one, only probands without uptake of any supplements were included. Nowadays, it is hard to find subjects and glaucoma patients without any supplementary intake, yet the study samples are small. On the contrary, the only study to date in the literature did not control supplementary intake, thus, this data might be biased. Further studies are necessary to confirm the present data in larger study cohorts of different countries.

Conclusion

The serum Se level of controls and patients with POAG were almost half as high as the published US data. Slight gender and age affections on the Se level were noticed in the German cohort; the serum Se concentration increased with age in female subjects (controls and POAG). Yet, the Se concentration showed a decrease with increasing age in men (controls and POAG). Considering the analysis of trace elements and their potential impact on diseases, a potential effect of gender, age, and nutrition, differing between countries, should be kept in mind.

Conflict of Interest

The authors declare that they have no conflict of interest.

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