Aqueous humor selenium level and open-angle glaucoma

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Abstract

Purpose: Selenium supplementation was seen to be linked to glaucoma disease in a previous study (Lillico A. JE, Reid M et al. (2002) Selenium Supplementation and Risk of Glaucoma in the NPC trial University of Arizona, Tucson, AZ., Arizona Cancer Center). As aqueous humor levels of selenium seemed to be associated with primary open-angle glaucoma (POAG), the aim of this study was to analyze concentrations of selenium in aqueous humor samples of patients with POAG and pseudoexfoliation glaucoma (PEXG) in comparison to normal samples.

Patients and Methods: Thirty-eight aqueous humor samples from patients undergoing cataract surgery were collected: Eleven patients with PEXG (age 65.8 ± 10.69, female 6, male 5), 12 patients with POAG (age 65.3 ± 10.50, female 7, male 5) and 15 patients without glaucoma (age 70.9 ± 12.83, female 10, male 5, controls). Aqueous humor levels of selenium were measured by Flow-Injection-Inductively-Coupled-Plasma-DRC-Mass-Spectrometry (ICP-DRC-MS).

Results: Maximum likelihood estimation of the least squares means (LS-means) and the relative 95% confidence limits of selenium aqueous humor levels were 6.90 ± 1.03 μg/L (control), 6.74 ± 1.14 μg/L (POAG) and 8.25 ± 1.18 μg/L (PEXG). The data were modeled using a generalized linear model (GLM) analysis, where selenium was set as dependent variable. The model was corrected for group differences in age and gender. The data show no differences among all the calculated differences between the least square means (LS means), taking in consideration the simultaneous 95% confidence limit and the multiple comparison tests with Tukey-Cramer adjustment. The evaluation of the model disclosed that POAG and PEXG patients had no significantly different aqueous humor selenium concentrations compared to controls and to each other. However, the quantile regression analysis of selenium aqueous humor levels showed differences in quantiles for open-angle glaucoma patients considering age and gender.

Conclusion: As no significant difference in aqueous humor concentration of selenium was detected between open-angle glaucoma and controls, however, quantile analysis showed differences in quantiles levels for different age ranges in open-angle glaucoma patients, the trace element selenium seemed to be linked to glaucoma disease, yet not in a major role.

Introduction

Glaucoma is one of most common causes of blindness worldwide [1]. The diagnosis of this neurodegenerative disease is based on an altered optic disc, elevated intraocular pressure (IOP) and perimetric field defects. Up to date the origin of glaucoma is unknown. It seems that the pathogenesis is multifactorial, whereas IOP is a major keyplayer. However, although IOP regulation can be achieved, some patients show a disease progression. Thus the pathophysiology is influenced by several factors, of which not all are known up to now. Next to molecular findings (e. g. interrupted retrograde transport of neurotrophins [2]) or ocular ischemia [3], nutrition and dietary supplementation seem to be involved via oxidative stress mediated changes. [4] [5]; An imbalance between oxidants (free radicals) and antioxidants results in oxidative stress. If free radicals dominate, molecular interactions

and cellular integrity is disturbed. [6] [7]; Considering specific dietary supplementation in diseases, which are linked to oxidative stress (e. g. cancer), the Nutritional Prevention of Cancer (NPC) trial investigated the influence of selenium (Se) substitution on cancer incidence [8]. Selenium supplementation (200 μg per day) decreased significantly cancer incidence. However, glaucoma incidence rose as a side effect with an hazard ratio of 1.78 [9]. This observation generated the hyopthesis of a selenium linked pathogenesis of glaucomatous optic nerve atropy, potentially because selenium can be toxic in large amount supplementation to both animals and humans [10–13]. Bruhn et al. followed this idea of a potential increased selenium concentration in glaucoma patients and researched plasma and aqueous humor levels in primary open-angle glaucoma patients (POAG) [14]. Selenium concentration of plasma and aqueous humor were found to be increased in POAG, however level of significance was not reached. Up to date this is the only study on selenium analysis in aqueous humor of POAG. No trial was performed on aqueous humor selenium level in pseudoexfoliation glaucoma (PEXG) until now. Thus it was the aim of this trail to investigate selenium concentrations in aqueous humor of POAG and PEXG with the Flow-Injection- Inductively-Coupled-Plasma-DRC-Mass-Spectrometry (ICP-DRC-MS). All data were compared to a control group.

2. Material and methods

2.1. Patients

Consecutive series of thirty-eight patients of the Department of Ophthalmology and Eye Hospital, Friedrich-Alexander-University of Erlangen-Nürnberg (FAU) were involved in the study: Eleven patients with PEXG (age 65.8 ± 10.69, female 6, male 5), 12 patients with POAG (age 65.3 ± 10.50, female 7, male 5) and 15 patients without glaucoma (age 70.9 ± 12.83, female 10, male 5, controls). Informed consent was obtained from all patients. The study was approved by the Local Ethics Committee and performed according to the tenets of the Declaration of Helsinki. Glaucoma was diagnosed as an altered optic disc, which was classified after Jonas [15,16], a confirmed untreated IOP > 21mm Hg and a perimetric field loss (MD > 2.8; ≥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), which was confirmed twice. PEXG diagnosis was based on a glaucomatous optic disc damage, according to the classification after Jonas [15,16], an IOP > 20 mmHg, perimetric field defects, diagnosed as described above, and PEX material deposits on anterior segment structures with an open chamber angle. Controls had no signs of any ophthalmic diseases except cataract. All patients underwent ophthalmic examinations, including slitlamp biomicroscopy, funduscopy and Goldmann applanation tonometry. Detailed anamnestic data were obtained from each patient, including their individual lifestyle and nutrition. Patients, taking nutritional supplements (e. g. selenium supplementation) or other medicals, affecting selenium concentrations, were excluded. Further on, patients with malignant neoplasia, a major systemic disease, hypovitaminoses, psychiatric illness, hypothyroidism, severe psoriasis and gastrointestinal malabsorption in their history were not included. Similarly, cigarette smoking and chronic alcohol use (during the last 6 months) were excluded.

2.2. Methods

Samples of aqueous humor (100 to 150 μl) were collected from all patients undergoing cataract surgery with phacoemulsification. Before performing the corneal tunnel, aqueous humor was obtained through an ab-externo limbal paracentesis via a 27-gauge needle on a tuberculin syringe. No bleeding occurred during performing of the paracentesis. The samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.3. Trace element analysis Selenium concentration was measured by Flow-Injection- Inductively-Coupled Plasma - Dynamic Reaction Cell -Mass- Spectrometry (ICP-DRC-MS) according to [17]. This method is based on the superior detection capability of ICP-DRC-MS using methane as reaction gas [18] and the most abundant 80Se isotope combined with flow-injection (FI) methodology for low sample volumes. To minimize sample consumption we combined the FI mode with a semi-automated injection. This kept the total sample volume below 80 μL for triplicate analysis per sample. The FI sample introduction was conducted by coupling a Knauer 1100 Smartline inert Series HPLC system with a 25 μl injection loop coupled to the ICP-DRC-MS. Flow rate was 1 ml/min of Milli-Q water. Instrumental settings for ICP-DRC-MS [17] and are given in Table 1. Se-working standards were prepared daily form the Se-stock standard solution by appropriate dilution at concentrations between 50–5000 ng/L, providing a 9 point calibration curve. All Se-standard solutions, the aqueous humor samples, and control samples were prepared with a final Rh concentration of 1 μg/L as internal standard. For quality control blanks and control-standards were measured periodically between samples. Methodical figures of merit were given in [17] and are confirmed for this work. In short terms: LOD: 26 ng/L, serial or day-to-day precision both at 2 μg/L: 4.5% or 5.6%, mean recovery (2 μg/L): 101 ± 0.1%. The method was validated by measuring reference materials: Accuracy was determined in reference serum (Recipe, Munich, Germany) at 97% (control value: 62 μg/L) and reference urine (Recipe) at 105% (control value: 23 μg/L).

**Table 1**

The instrumental settings for ICP-DRC-MS (inductively coupled plasma mass spectrometry)



2.4. Statistical analysis

All the elaborations were done using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The Anderson-Darling, Cramér–von Mises and the Kolmogorov-Smirnov tests provide that the fitted lognormal distribution is a good model for the distribution of Se. We took in consideration three different distributions: Lognormal, Weibull and Gamma. Based on this assumption we modeled the data using a generalized linear model (GLM) analysis where selenium (continuous variable) was set as dependent variable. 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 (SS) values. The study of possible significant variation between the three classes was done with the LS means (suitable for unbalanced data). All the differences between means were determined and evaluated taking in consideration all the simultaneous 95% confidence limits calculated for all the possible pairsof LS mean differences. We took in examination also the multiple comparison tests with Tukey-Cramer adjustment. Moreover, the covariates effects on the variable selenium were modeled with the quantile regression procedure. The age was divided in three intervals [47–60], [61–69] and [70–85].

3. Results

3.1. Selenium aqueous humor concentration of POAG, PEXG and controls We used the GLM model to study if there are any differentiations among selenium for the three different classes (control, POAG or PEXG). We evaluated the least squares means (LS-means) estimations, which correspond to the specified effects for the linear predictor part of the model, and the relative confidence limits (Table 2). Maximum likelihood estimation of the LS means and the relative 95% confidence limits were 6.90 ± 1.03 μg/L (control), 6.74 ± 1.14 μg/L (POAG) and 8.25 ± 1.18 μg/L (PEXG). From the estimations (Table 2) we can infer an increase of selenium for the PEXG group compared to the other groups. The levels of controls and POAG groups were almost equal. To get more details for the comparison along the three groups we applied the multiple comparison tests with Tukey-Cramer adjustment (Table 3).

**Table 2**

Maximum likelihood estimation of the LS-means and the relative 95% confidence limits for POAG, PEXG and controls.



**Table 3**

Multiple comparison test results with Tukey-Cramer adjustment.

Least Squares Means for effect group Pr>|t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Se



**Table 4**

Differences between the means (for all the pairs) and the simultaneus 95%

confidence limits for the LS means. We see that there are no significant difference.

Least Squares Means for Effect group



The different tests do not reveal any significant differences in the selenium among the classes comparison. Moreover, looking at all pairs of LS means differences and the respective confidence limits we detect again no significant effects at the 0.05 level (Table 4). Fig. 1 shows that the 0.25 quantile remains nearly constant, the 0.5 and 0.75 quantiles show only a slightly increasing trend from controls to POAG to PEXG. Moreover, we considered the age diveded in three main classes [47–60 years] (Fig. 2a), [61–69 years] (Fig. 2b) and [70–85 years] (Fig. 2 c), in order to have a separated analysis on grouped observations. This is demonstrated in Fig. 2. While in the first phase a slight increase is seen for the three quantiles (0.25, 0.5, 0.75) we could observe that in the second phase (age from 60 to 69 years) the 0.25 selenium quantile is decreasing. The 0.5 quantile maintained constant both, in the [61–69 years] and [70–85 years] ranges. The third quantile showed an increase in the [70–85 years] ranges, however, being almost equal in the [61–69 years] ranges. The plots in Fig. 3 are considering the model dived by gender. For men almost equal selenium quantiles were observed in controls, POAG and PEXG (Fig. 3 a). However, in women an increase for the first and second quantile was observed for POAG and PEXG (Fig. 3b).

**Figure 1:** Fit by quantiles of the Selenium variable.



**Figure 2**: Quantile regression for the first age range [47-60 years] (a), second age range [61-69 years] (b) and the third age range [70-85 years] (c).



**Figure 3:** Quantile regression for the mean (a) and women (b)



4. Discussion

A potential link of selenium to glaucoma disease was hypothesized by the Nutritional Prevention of Cancer (NPC) trial, presenting an increased incidence of glaucoma under selenium supplementation [8]. As a further study yielded an increased OR of POAG for middle selenium tertile of aqueous humor [14], this study aimed to investigate aqueous humor selenium level in POAG and PEXG compared to control subjects. Data of this study yielded an unchanged selenium aqueous humor level of POAG patients compared to controls. Notably, also the previous paper from Bruhn et al. reported no significant difference (p=0.73 [2]) between POAG and controls in aqueous humor samples (POAG: 46.31 μg/L (range: 44.91 to 47.70); controls: 46.02 Mg/L (range: 45.05 to 46.99)). Further we found that PEXG patients’ selenium aqueous humor concentration was increased according to LS means (c.f. Table 2). Moreover, comparisons along the three groups were done applying multiple comparison tests with Tukey-Cramer adjustment. There contrary, the model did not reveal any significant differences in Se level when we compare all pairs of LS means. Considering age-group dependent trends, a slight increase for all the three selenium quantiles were observed in POAG and PEXG patients in age groups [47–60 years] but a decrease for the first selenium quantile of the [61–69 years] group (c.f. fig.2). and in turn again an increase of the third quantile in the third age range [70–85 years] in these two patients groups. Such varying trends are yet unexplained as well as their possible effects from a medical viewpoint not completely clear. Bruhn et al. described an OR of 0.2 for the middle tertile of selenium aqueous humor with a significant association to glaucoma [1]. Also different trends were observed for women and men, with women showing an increase for Se of the second and third quantiles in POAG and PEXG patients, whereas all quantiles were almost equal in men (c.f. Fig. 3). We therefore conclude that Se may have some effect on PEXG progression, which seem to be age and gender related, which however might be at the end a minor factor due to trends being weak with low significance. Interestingly the selenium concentrations of aqueous humor in POAG patients of our study were about seven fold lower than previously published data of Bruhn et al. (6.74 μg/L vs. 46.31 ng/ml [14]). These differences may be explained by a set of different factors, over all methodical ones, which in minor degree may be superimposed by nutritional aspects Methodical differences could decisively explain the concentration difference to Bruhn et al.: The analytical methods used in the present study, were reliable, more adequate than those used in the study of Bruhn et al., consecutively playing a major role in determining the difference in Se levels of the present and Bruhn’s study: Regarding the used selenium determination method with its methodical figures of merit and limitations Bruhn et al. apparently did not calculate such values in their work but relied on data gained from another laboratory, which were previously published by Nyman et al. [19]. Nyman had developed this method five years earlier, i.e. whether in the study of Bruhn et al [14] analytical accuracy was achieved at methodical LoD was actually not tested. Their linear (suitable) detection range on the lower end starts just at 24.5 μg/l, which is already several-fold the value of all our data. Notably, Bruhn states that their “aqueous humor selenium concentrations were very close to the limit of detection” and their “analyses suggests that greater variation in aqueous humor selenium may exist” [20]. Contrary, our method achieved a LoD of 0.0246 μg/L, being ca. Fig. 2. Quantile regression for the first age range [47–60 years] (a), second age range [61–69 years] (b) and the third age range [70–85 years] (c). Fig. 3. Quantile regression for the men (a) and women (b) 100–300 fold below concentrations in our samples and consequently samples could be measured quite comfortably. The difference in sensitivity of both methods is explained by the use of DRC interference elimination in this work using methane as reaction gas [18], which itself enhances signal intensity according to its carbon input [21] and due to detection at 80Se isotope with 49.7% isotopic abundance [22]. In contrast, Nyman´s and Bruhn´s method was based on an instrument without DRC capability and thus could not profit from signal enhancement by methane. They used “standard mode” which does not permit detection at abundant (sensitive) 80Se isotope, but forced them to choose Se-isotope with possibly low interferences but also low abundance such as 82Se (only 9.2% abundance). Nevertheless, 82Se signal could be superimposed by the Zn-O-interference, at this m/z having 27.83% abundance [22]. The latter is relevant as Zn concentrations in the samples are in the range of 4–50 μg/L, which could erroneously increase Se concentration measurements in substantial amounts. To a smaller extent differences in Se-supply from nutrition in Germany (this study) vs. USA (Bruhn´s study) may contribute to the lower concentrations in our study, too. To a smaller extent this nutritional effect may superimpose the methodical difference being supposedly the major cause. The serum concentration of selenium depends on the selenium level, which has been absorbed by daily nutrition [23]. It is known, that selenium levels in beverage and nutrition vary between countries due to the different diet of the animals [24] and selenium content in the soil of the growing plants [25]. This might be a factor attributing to the different selenium levels between our (Germany) and Bruhn’s (USA) data since Germany is considered as slightly suboptimal supplied, whereas in USA selenium levels are typically rather high [26]. Additionally, the different selenium concentrations might be due to the fact, that our exclusion criteria were absolutely strict to any dietary supplementation. Bruhn et al. stated that their data interpretation is limited because they were unable to get information about each proband’s dietary supplementation which is rather common in the US [14]. Consequently, in addition to the higher Se-supply from nutrition further Se-intake might have been likely (but not controlled in the study) which might have biased selenium levels in aqueous humor of POAG in the previous study of Bruhn et al. Selenium is an essential trace element, which was described first by Jöns Jacob Berzelius in 1817. It shows a similarity to sulfur, as it belongs to chalcogens. The metabolism of selenium in human body is widely discussed in literature (e.g [27,28].) but still not all aspects are well understood. It is known that a total amount of about 10–20 mg selenium can be found in whole human body (for review [23]). Especially in liver, testes and kidney high levels of selenium were found. Additionally, cells of the immune system, platelets and erythrocytes show high selenium levels [23]. Selenium uptake is per gut [29] and its metabolism into selenide (HSe) might be the potentially most important regulation point in selenium metabolism [30,31]. The elimination pathway is mainly via kidney and bladder [30], only little by faeces [31]. Selenium seems to have “two faces”: one the one hand selenium can be neurotoxic (e.g. selenit) [32], on the other hand it can be neuroprotective (e.g. selenoprotein P). Being part of the active site of antioxidant GPX enzymes (glutathione peroxidase) selenium might protect against oxidative stress and participate in the regulation of inflammation [29,33]. The antioxidant selenoprotein P is linked to vascular endothelium and might potentially protect endothelial cells against oxidative stress induced changes [34]. The function of several other selenoproteins (e. g. selenoprotein W, prostate epithelial selenoprotein) is still under investigation [31,35,36]. Our study is not without limitations. Our subjects’ collective is not very large, thus very small differences in selenium concentration might not be able to detect and statistical imprecision might be increased. Thus a study of a larger population should investigate a potential effect of selenium in open-angle glaucoma. Taking together the data of selenium in aqueous humor concentration of open-angle glaucoma, it seemed that they do not have clear and highly significant alteration in selenium aqueous humor level in comparison to controls, however trends in selenium quantiles analysis were observed for different age ranges and gender. This points to some effect of selenium in open-angle glaucoma progression, which might have been not clearly monitored in our study according to above mentioned limitations. As selenium concentration differs between the nutrition and beverage of different countries and is dependent on dietary supplementation, further molecular and long-term studies are necessary to investigate a potential selenium related effect in the pathogenesis of open-angle glaucoma. As a refinement, selenium speciation would be helpful for investigation probable differences of selenoenzymes and their relationship to POAG or PEXG, though the general extremely low sample volume available may prevent such analytical approach (as holds true for this study).

5. Conclusion

Selenium concentration was found to be linked to glaucoma in a previous study [14]. As data of our study showed that selenium aqueous humor levels did not differ between open-angle glaucoma and controls (c.f. [2], too), however trends towards different levels of selenium quantiles were observed considering age and open-angle glaucoma, a linkage of selenium to glaucoma disease can be derived from our data. Further studies are necessary to re-investigate the part of selenium in a larger glaucoma patients’ group.

Conflict of interest

All authors declare no conflict of interest.

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