Plasma and vitreous selenium concentrations in patients with type 2 diabetes and diabetic retinopathy

Chunmiao Wang, MMEda,b, Ruijin Ran, MDb,*, Xin Jin, MMEd, Xiaohong Zhu, MMEda

Abstract

Background: This work aimed to determine and compare plasma and vitreous selenium (Se) concentrations in patients with type 2 diabetes and diabetic retinopathy (DR).

Methods: A total of 60 type-2-diabetes patients including 20 without DR, 20 with non-proliferative DR (NPDR), and 20 with proliferative diabetic retinopathy (PDR), were involved in this study. Blood plasma samples were collected from above 60 patients and 20 normal controls (without diabetes). Twenty control vitreous samples were obtained from the eyes presenting a macular hole and epimacular membrane. Vitreous samples were also collected from PDR patients receiving one-week intravitreal anti-VEGF therapy or not. Plasma and vitreous Se concentrations were determined by inductively coupled plasma mass spectrometry.

Results: Plasma Se concentrations in PDR patients (163.74 ± 32.68 μg/L) were significantly higher than those in normal control patients (121.59 ± 28.33 μg/L), NPDR patients (130.34 ± 29.11 μg/L), and the patients without DR (81.23 ± 20.59 μg/L) (all P < .001). Similarly, Se concentrations in vitreous samples of PDR patients (56.30 ± 12.03 μg/L) were consistently higher than those in control vitreous samples (26.26 ± 6.53 μg/L). In addition, vitreous Se concentrations in PDR patients decreased to 47.76 ± 9.72 μg/L after intravitreal injection of the anti-VEGF drug ranibizumab for one week, which was significantly lower than those before injection (P = .02). Plasma VEGF levels of diabetic patients were lower than those of the normal controls (P < .001). On the contrary, the vitreous VEGF level in the PDR group (913.61 ± 193.32 pg/mL) was significantly higher than that of the normal control group (101.23 ± 21.33 pg/mL) (P < .001).

Conclusion: The elevation of Se concentrations may be an important risk factor in plasma and vitreous with diabetic retinopathy among type-2-diabetes patients. The elevated VEGF may be also closely related to the intracocular Se concentration in PDR patients.

Abbreviations: DR = diabetic retinopathy, NPDR = non-proliferative DR, PDR = proliferative diabetic retinopathy, Se = selenium, VEGF = vascular endothelial growth factor.

Keywords: diabetic retinopathy, ICP-MS, selenium, VEGF, vitreous

1. Introduction

Selenium (Se) is an essential trace mineral of significant importance in human health and serves as a key component of selenoproteins required for various metabolic and antioxidant functions. Enshi prefecture in the west of Hubei province of China is famous as a Se-rich region globally.[1] Mounting studies have proved that Se plays a crucial role in a range of diseases such as Keshan disease, prostate cancer, hyperthyroidism, and cardiovascular disorders.[2] Given its important role in the human body, colleagues in our lab have done some work on Se in its presence in some diseases.[3] It has been suggested that lower Se concentration may be implicated in the weak immune system and increase the risk of mortality in patients with coronary heart disease or myocardial fibrosis.[4]

Moreover, it has recently been discovered that Se could promote glucose transport and interfere with glucose metabolism, exerting an insulin-mimetic effect.[5–8] As a result, the relationship between Se and diabetes has attracted continuous attention in recent years. Intriguingly, inconsistent findings have been reported on the relationship in several studies. It is previously reported that lower Se concentration was related
to the onset of type 2 diabetes.[9–11] On the contrary, some studies have pointed out that the plasma or serum Se concentrations of diabetic patients are significantly higher than the normal population.[12,13] Meanwhile, some researchers have focused on the relationship between Se and ocular surface disease, cataracts, and thyroid-related eye disease.[14] To date, no previous research has investigated the role of Se in diabetic retinopathy (DR).

Here, we in this research determined and compared Se concentrations in blood plasma and vitreous body of DR patients of varying severity for a primary exploration.

### 2. Materials and Methods

#### 2.1. Study participants

This study was approved by the Ethics Committee of the Minda Hospital Affiliated to Hubei University for Nationalities (Enshi, China) and was conducted according to the principles outlined in the Declaration of Helsinki. Clinical characteristics including age, gender, weight, height, and levels of fasting blood glucose and glycosylated hemoglobin were all obtained from the medical information recorded by the departments of endocrinology and ophthalmology of the Minda Hospital from June 2019 to April 2020. According to the international diagnostic criteria for type 2 diabetes and the results of fundus photography and fluorescein angiography, the diabetic patients were divided into the non-DR group (n = 20), non-proliferative DR (NPDR) group (n = 20) and proliferative DR (PDR) group (n = 20).[15,16] Written informed consent was obtained from all included participants.

#### 2.2. Collection of plasma and vitreous samples

All subjects fasted for at least 8 h at night before blood collection. A total of 5 mL of peripheral venous blood was taken from each participant at 8 AM, the following day. Collection tubes with ethylenediamine tetraacetic acid were used to prevent clotting of the blood samples. After centrifugation at 3000 r/minute at 4°C for 10 minute, the supernatant was stored in a sealed EP tube in a refrigerator at −80°C for later analyses. Vitreous samples from control eyes with a macular hole and epimacular membrane (n = 20), and from PDR patients without anti-VEGF (vascular endothelial growth factor) treatment (n = 20) were obtained through vitrectomy. Vitreous samples from PDR patients receiving one-week intravitreal anti-VEGF treatment (using ranibizumab) (n = 20) were taken through vitrectomy. About 0.25 to 0.35 mL of vitreous humor was extracted before irrigation. Collected samples mixed with blood were centrifuged to obtain the supernatant for determination. All samples were stored in sealed EP tubes at −80°C for later analyses.

#### 2.3. Determination of plasma and vitreous Se concentrations

Plasma and vitreous Se concentrations were determined by the Thermo Scientific™ TQcon™ RQ IC-MS (inductively coupled plasma mass spectrometry). This real-time trace element analyzer is characterized by fast detection speed, wide linear range, high sensitivity, and strong specificity; its quantification for samples with particularly low content of trace elements can be done even at the nanogram level.[17–19] Briefly, the samples were completely thawed at room temperature and then homogenized on a vortex mixer. Afterward, 200 µL of plasma or vitreous sample was diluted with a prepared diluent containing 65% (v/v) highly purified Triton X-100, 65% nitric acid HNO3, and purified water, to a volume of 4 mL. A prepared sample was vaporized and introduced into the nebulizer gas flow in aerosol form, and then the admitted ions were pushed to the beam axis by a radio-frequency guide field. A single collision cell technology mode with CH4 addition was used for Se determination. As proved, carbon loading could improve the sensitivity of high-ionization potential analytes, and the application of methane gas in plasma could give a sensitivity increase of seven folds for selenium.[20] The results of Se concentration were expressed as pg/L.

#### 2.4. Determination of vitreous and plasma VEGF levels

VEGF levels in vitreous and plasma samples were determined using a commercial enzyme-linked immunoassay kit according to the manufacturer’s instructions. Optical density was read at 450 nm using a microplate reader (SpectraMax Gemini UVmax; Molecular Devices). The VEGF level of each sample was calculated from the standard curve, and the result was expressed as pg/mL.

#### 2.5. Statistical analysis

All data were analyzed using GraphPad Prism 8.0 software (GraphPad Software, San Diego). The measurement data were expressed as mean ± standard deviation (SD). Differences between the two groups were analyzed using the paired or unpaired t test. Comparisons between more than two groups were performed using a one-way analysis of variance with a Bonferroni post hoc test. P values < .05 were considered statistically significant.

### 3. Results

#### 3.1. Clinical data

The baseline clinical and ocular characteristics of the participants are presented in Table 1. There were no significant differences in age, body mass index, and level of fasting blood glucose and glycosylated hemoglobin.

| Clinical features of diabetes and control patients. | Control group | Non-DR group | NPDR group | PDR group | p  |
|---|---|---|---|---|---|
| Age, yr | 59.0 ± 10.12 | 59.80 ± 10.18 | 61.80 ± 10.25 | 64.05 ± 10.60 | .386* |
| Body mass index, kg/m² | - | 24.82 ± 2.84 | 25.03 ± 3.04 | 25.43 ± 2.99 | .219* |
| Duration of diabetes, yr | - | 3.45 ± 2.37 | 7.55 ± 3.79 | 13.75 ± 4.77 | - |
| Fasting blood glucose, mmol/L | - | 7.24 ± 1.96 | 7.46 ± 1.71 | 7.94 ± 2.42 | .613* |
| HbA1c, % | - | 6.94 ± 1.42 | 8.51 ± 2.48 | 9.95 ± 3.05 | - |
| Plasma Se concentration, µg/L | 121.59 ± 28.33 | 81.23 ± 20.59 | 130.34 ± 29.11 | 163.74 ± 32.68 | .000** |
| Vitreous Se concentration, µg/L | 26.26 ± 6.53 | - | - | 56.30 ± 12.03 | - |

Non-DR group = non diabetic retinopathy group, NPDR group = non-proliferative diabetic retinopathy group, PDR group = proliferative diabetic retinopathy group, Se = selenium, HbA1c = glycosylated hemoglobin.

*p values by one-way analysis of variance for comparision among groups;

**p values by Student’s t test for comparison of means between cases and controls.
glucose between the control group, non-DR group, NPDR group, and PDR group. PDR patients present high HbA1c levels and long duration of type 2 diabetes as compared with other included patients, and the differences could not be excluded.

3.2. Se concentration in plasma

The included diabetic patients were randomized into three groups, non-DR group (n = 20), NPDR group (n = 20) and PDR group (n = 20). As shown in Figure 1, the plasma Se concentrations in DR patients were higher than those in non-DR patients and the normal controls. A significant difference was seen between the PDR group and the control group (P < .001), and between the PDR group and the non-DR group (P = .005) in terms of plasma Se concentration. While there was no significant difference between the NPD group (130.34 ± 29.11 μg/L) and the control group (121.59 ± 28.33 μg/L) (P = .19). It could be attributed to the fact that we did not further classify NPDR patients into mild, moderate and severe groups. In addition, it suggested that the change in Se concentration was not obvious in the early stage of DR. Interestingly, there was a significant difference between non-DR patients (81.23 ± 20.59 μg/L) and the controls (121.59 ± 28.33 μg/L) (P < .001) (Fig. 1). There may be a U-shaped relationship between Se concentration and the presence of DR. It indicates that the concentration of high or too low serum selenium may be related to the occurrence of diabetes.

3.3. Vitreous Se concentration before and after anti-VEGF therapy

Since the vitreous humor of the normal controls, non-DR or NPDR patients cannot be obtained, we took the vitreous humor from the patients with a macular hole and epimacular membrane as the control for further exploration of the relationship between intravitreal Se concentration and PDR. As presented in Figure 2, vitreous Se concentration in the PDR group (56.30 ± 12.03 μg/L) was significantly higher than that in the control group (26.26 ± 6.53 μg/L) (P < .001). Moreover, after one-week intravitreal injection treatment with ranibizumab, vitreous Se concentrations of PDR patients (47.76 ± 9.72 μg/L) were significantly lower than before (P = .02). This may be attributable to our small sample volume, but there was still a significant difference between PDR patients receiving anti-VEDF treatment and the controls (P < .001) (Fig. 2).

3.4. VEGF concentrations in vitreous and plasma

At present, many studies have pointed out that appropriate Se concentration could inhibit tumor neovascularization by reducing VEGF levels in tumor vessels and suppressing the growth of new vessels. Therefore this study measured plasma and vitreous VEGF levels in each group. As shown in Figure 3A, plasma VEGF levels of diabetic patients (non-DR group: 42.00 ± 10.47 pg/mL, NPDR group: 59.95 ± 11.59 pg/mL, PDR group: 60.56 ± 13.35 pg/mL) were lower than those of the normal controls (216.88 ± 21.33 pg/mL) (P < .001). On the contrary, the vitreous VEGF level in the PDR group (913.61 ± 193.32 pg/mL) was significantly higher than that of the normal control group (101.23 ± 21.33 pg/mL) (P < .001) (Fig. 3B).

4. Discussion

Diabetes is on the rise throughout the world and accounts for an estimated 4.2 million deaths in 2019. DR is the fifth most common cause of severe vision loss and blindness worldwide; about 3.7 million cases of visual impairment and 0.83 million cases of blindness were reported to directly relate to its presence by 2010 across the globe. The presence of, or the complex interactions between glucose-induced oxidative stress, inflammation, formation of advanced glycation end products, and other factors that could bring damage to the retina and blood vessels, could cause DR or determine its development. Retinal neovascularization is the main cause of blindness in DR patients, and VEGF might play a key role in the retinal microvascular complications of diabetes and represent an exciting target for therapeutic intervention in DR.

Se is of fundamental importance to human health, as an essential trace mineral. A range of diseases associated with an inadequate intake of Se has been reported around the world, which has attracted abundant attention from health departments and agencies as well as relevant researchers. Increasing studies on the relationship between Se and diabetes indicate that Se plays an important role in the occurrence and progression of diabetes. Se could not only exert positive effects on glucose homeostasis and decrease oxidative stress in diabetic patients owing to its antioxidant and insulin-mimetic properties, but also influence the inflammatory response in a variety of ways. It was first discovered that Se can inhibit the inflammatory response in diabetic rats. Se may induce the production of interleukin and tumor necrosis factor-α by inhibiting the activation of nuclear factor-kB which controls the transcription of various genes that have roles in inflammation. One study found...
related to plasma Se concentration. This may be due to the found that the activity of glutathione peroxidases is directly higher the plasma Se concentration. A number of studies have that the more severe the retinopathy of diabetic patients, the cose on plasma Se concentration was ruled out. We also found between the diabetic groups, and the effect of fasting blood glu- there was no significant difference in fasting blood glucose level of PDR patients. Results showed that vitreous Se concentration decreased after intravitreal injection of the anti-VEGF drug, which indicates that vitreous selenoproteins activity or Se content may be related to the stim-ulation of VEGF. We also found that plasma VEGF level in dia-betic patients, especially in PDR subjects, was lower than that in the controls, which may be related to the lesions of and the adhesion to endothelial cells. A Higher VEGF level in the vitre-ous body indicates a higher VEGF level in the retina. Similarly, lower VEGF level in plasma appears with higher VEGF content in endothelial cells owing to its increased adhesion to injured endothelial cells in diabetes. Recently, the formation in intraocular neovascularization might be closely related to regulatory T cells. Another study pointed out that selenium GPX4 might play an important role in maintaining T cell homeostasis via inhibiting lipid peroxidation. These studies indicated that Se or selenoproteins might have a close connection with VEGF in the relevant potential mechanisms in our research.

To summarize, this study found that DR patients have higher Se concentration in plasma and vitreous humor than normal people. The downtrend in vitreous Se concentration after intravitreal anti-VEGF treatment in the patients may be associated with the stimulation of the activity of selenoproteins by VEGF, which indicates that higher Se concentration may be a risk factor for the development or progression of DR. There may be a U-shaped relationship between Se concentration and the presence of DR. It would be helpful to know how exactly Se concentration interacts with the development or progression of DR among diabetic patients. It suggested that DR might have a potential new mechanism mediated by Se or selenoproteins. The related mechanism of Se underlying the effects on DR requires further research.

Author contributions

Conceptualization: Chunmiao Wang, Ruijin Ran.
Data curation: Xin Jin, Xiaohong Zhu.
Formal analysis: Xiaohong Zhu.
Funding acquisition: Ruijin Ran.
Investigation: Chunmiao Wang, Xin Jin.
Supervision: Chunmiao Wang.
References

[1] Lyu C, Chen J, Li L, et al. Characteristics of Se in water-soil-plant system and threshold of soil Se in seleniferous areas in Enshi, China. Sci Total Environ. 2022;827:154372.

[2] Fairweather-Tait SJ, Bao Y, Broadley MR, et al. Selenium in human health and disease. Antioxid Redox Signal. 2011;14:1337–83.

[3] Yao Y, Chen Z, Zhang H, et al. Author correction: Selenium-GPX4 axis protects follicular helper T cells from ferroptosis. Nat Immunol. 2021;22:1599.

[4] Rayman MP. Selenium and human health. Lancet. 2012;379:1256–68.

[5] Zhang Q, Li W, Wang J, et al. Selenium levels in community dwellers with type 2 diabetes mellitus. Biol Trace Elem Res. 2019;191:354–62.

[6] Vinceti M, Filippini T, Rothman KJ. Selenium exposure and the risk of type 2 diabetes: a systematic review and meta-analysis. Eur J Epidemiol. 2018;33:789–810.

[7] Kohler LN, Foote J, Kelley CP, et al. Selenium and type 2 diabetes: systematic review. Nutrients. 2018;10:1924.

[8] Marcocci C, Kahaly GJ, Krassas GE, et al. Selenium and the course of diabetes. J Natl Cancer Inst. 2016;108: djw152.

[9] Labunskyy VM, Lee BC, Handy DE, et al. Both maximal expression of selenoproteins and selenoprotein deficiency can promote development of type 2 diabetes-like phenotype in mice. Antioxid Redox Signal. 2011;14:2327–36.

[10] Thompson PA, Ashbeck EL, Roe DJ, et al. Selenium supplementation for prevention of colorectal adenomas and risk of associated type 2 diabetes. J Natl Cancer Inst. 2016;108: djw152.

[11] Labunskyy VM, Lee BC, Handy DE, et al. Both maximal expression of selenoproteins and selenoprotein deficiency can promote development of type 2 diabetes-like phenotype in mice. Antioxid Redox Signal. 2011;14:2327–36.

[12] Kohler LN, Florea A, Kelley CP, et al. Higher plasma selenium concentrations are associated with increased odds of prevalent type 2 diabetes. J Nutr. 2018;148:1333–40.

[13] Jie W, Chao Z, Qian-yi G, et al. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. Nutr J. 2015;14:18.

[14] Hwan KT, JaeSang K, Ram KB, et al. Serum selenium levels in patients with Graves’ disease: associations with clinical activity and severity in a retrospective case-control study. Korean J Ophthalmol. 2022;36:36–43.

[15] American Diabetes A. Z. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. Diabetes Care. 2019;42(Suppl 1):S13–28.

[16] Wilkinson CR, Ferris FL, 3rd, Klein RE, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110:1677–82.

[17] Fu L, Nie X-d, Xie H-L, et al. Rapid multi-element analysis of Chinese vinegar by sector field inductively coupled plasma mass spectrometry. Eur Food Res Technol. 2013;237:795–800.

[18] Li XT, Yu PF, Gao Y, et al. Association between plasma metal levels and diabetes risk: a case-control study in China. Biomed Environ Sci. 2017;30:482–91.

[19] Aberami S, Nikhalashree S, Bharathselvi M, et al. Elemental concentrations in Choroid-RPE and retina of human eyes with age-related macular degeneration. Exp Eye Res. 2019;186:107718.

[20] Tindemans T, Dobney A, Wambeke D. Development and application of an analyte/matrix separation procedure for multi-element trace analysis of steel alloys by means of sector-field ICP-mass spectrometry. J Anal At Spectrom. 2014;29:1073–81.

[21] Zhou J, Huang K, Lei XG. Selenium and diabetes—evidence from animal studies. Free Radic Biol Med. 2013;65:1548–56.

[22] Bajpai S, Mishra M, Kumar H, et al. Effect of selenium on connexin expression, angiogenesis, and antioxidant status in diabetic wound healing. Biol Trace Elem Res. 2011;144:327–38.

[23] Jampol LM, Glassman AR, Sun J. Evaluation and care of patients with diabetic retinopathy. N Engl J Med. 2020;382:1629–37.

[24] Ran R, Du L, Zhang X, et al. Elevated hydrogen sulfide levels in vitreous body and plasma in patients with proliferative diabetic retinopathy. Retina. 2014;34:2003–9.

[25] Walder K, Kantham L, McMillan JS, et al. Tanis: a link between type 2 diabetes and inflammation? Diabetes. 2002;51:1859–66.

[26] Cai Z, Dong L, Song C, et al. Methylseleninic acid provided at nutritional levels inhibits angiogenesis by down-regulating Integrin β3 signaling [published correction appears in Sci Rep. 2020 Mar 24;10(1):5673]. Sci Rep. 2017;7:9445.

[27] Kong FJ, Ma LL, Chen SP, et al. Serum selenium level and gestational diabetes mellitus: a systematic review and meta-analysis. Nutr J. 2016;15:94.

[28] Ali MA, Aly EM, Elawady AD. Effectiveness of selenium on acrylamide toxicity to retina. Int J Ophthalmol. 2014;7:614–20.

[29] Van Cauwenbergh R, Robberecht H, et al. Comparison of the serum selenium content of healthy adults living in the Antwerp region (Belgium) with recent literature data. J Trace Elem Med Biol. 2004;18:99–112.

[30] Juno K, Soo CH, Kyu CM, et al. Association between serum selenium level and the presence of diabetes mellitus: a meta-analysis of observational studies. Diabetes Metab J. 2019;43:447–60.

[31] Bleys J, Navas-Acien A, Guillaud E. Serum selenium and diabetes in U.S. adults. Diabetes Care. 2007;30:829–34.

[32] Tian Y, Zhang F, Qiu Y, et al. Reduction of choroidal neovascularization via cleavable VEGF antibodies conjugated to exosomes derived from regulatory T cells. Nat Biomed Eng. 2021;5:968–82.