J Surg Med. 2023;7(1):58-62.
DOI: 10.28982/josam.7673

Research article

Journal of Surgery and Medicine
e-ISSN: 2602-2079
https://jsurgmed.com/

Comparison of selenium levels between diabetic patients with and without retinopathy

Hacer Pınar Öztürk Kurt [1], Düriye Sıla Karagöz Özen [1], İpek Genç [2], Mukadder Erdem [3], Mehmet Derya Demirağ [4]

[1] Clinic of Internal Medicine, Samsun University, Samsun Education and Research Hospital, Samsun, Turkey
[2] Clinic of Ophthalmology, Samsun University, Samsun Education and Research Hospital, Samsun, Turkey
[3] Clinic of Biochemistry, Samsun University, Samsun Education and Research Hospital, Samsun, Turkey
[4] Department of Internal Medicine, Samsun University Faculty of Medicine, Samsun, Turkey

ORCID ID of the author(s)

HPOK: 0000-0002-4893-4190
DSKO: 0000-0001-7852-2114
IG: 0000-0002-3291-4654
ME: 0000-0001-7796-3671
MDD: 0000-0001-5667-1805

Corresponding Author
Düriye Sıla Karagöz Özen
Samsun Eğitim ve Araştırma Hastanesi, Kat 2, İlkadım, Samsun, Turkey
E-mail: silakaragoz@yahoo.com

Ethics Committee Approval
The study was approved by Health Sciences University, Samsun Research and Training Hospital Ethical Committee, date: 21.03.2017 number: 2017-5-37.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
The authors declared that this study has received no financial support.

Previous Presentation
This article was presented as verbal presentation at The International Congress of Future Medical Pioneers (ICOFMEP 2021) on 8th May, 2021.

Published
2023 January 22

Abstract

Background/Aim: Diabetic retinopathy is a common ailment that causes visual impairment among adults, and evidence suggests that oxidative stress plays a significant role in its pathogenesis. The objective of this study was to examine the potential association between selenium deficiency and an increased risk of diabetic retinopathy among individuals with type 2 diabetes mellitus.

Methods: This study was a prospective case-control study. 115 patients with a diagnosis of type 2 diabetes mellitus were included. The patients were divided into groups with and without retinopathy. No subgroups were made according to the level of retinopathy. The aim was to compare the serum selenium level of patients between groups. Therefore, other variables that may contribute to the development of retinopathy were also recorded. The duration of diabetes, medications used, and glycosylated hemoglobin levels were recorded. The retinopathy group included 47 patients, and the non-retinopathy group included 68 patients. Selenium levels were measured in plasma samples.

Results: The mean selenium level of the retinopathy group (70.11 [17.28] µg/l) was significantly lower than that of the non-retinopathy group (80.20 [19.10] µg/l) (P=0.005). The median duration of diabetes mellitus was significantly higher in the retinopathy group than in the non-retinopathy group (10 [1-25] and 6 [1-21], respectively; P=0.002). Logistic regression analyses showed that higher levels of blood selenium were independent preventive factors against the occurrence of retinopathy (OR [95% CI]: 0.965 [0.939-0. 991]). The duration of diabetes mellitus was an independent risk factor for retinopathy occurrence (OR [95% CI]: 1.131 [1.050-1.219]). One unit increase in selenium level was associated with a unit decrease in diabetic retinopathy of 0.965 (0.939-0.991).

Conclusion: Our research revealed a correlation between the duration of diabetes and the incidence of diabetic retinopathy. Furthermore, a notable difference was observed in blood selenium levels between patients with diabetic retinopathy and those without it. Specifically, patients with diabetic retinopathy had lower plasma selenium levels compared to the control group. These findings have potential implications for the treatment or prevention of diabetic retinopathy, but more research is needed to determine the efficacy of selenium supplementation for diabetic patients with or without microvascular complications. Future studies should investigate the effect of selenium deficiency on different subtypes of diabetic retinopathy and the impact of selenium supplementation in this patient population.

Keywords: diabetes mellitus, diabetic retinopathy, free radicals, microvascular complication, oxidative stress, selenium

How to cite: Öztürk Kurt HP, Karagöz Özen DS, Genç İ, Erdem M, Demirağ MD. The comparison of selenium levels between diabetic patients with and without retinopathy. J Surg Med. 2023;7(1):58-62.

Introduction

Diabetes mellitus (DM) is a significant public health issue affecting over 460 million people globally [1]. The worldwide prevalence of both the disease and its complications is steadily increasing. Diabetic retinopathy, a common microvascular complication of DM, is an important cause of visual loss among adults [2]. The level of hyperglycemia and the duration of DM are major determining factors for the development of this microvascular complication [3]. The Diabetes Control and Complications Trial showed that normalization of hyperglycemia in patients with type 1 DM is related to a 76% risk reduction for the occurrence of diabetic retinopathy and a 54% risk reduction for its progression [3]. Other factors that increase the risk of diabetic retinopathy are being studied to prevent morbidity.

Research has shown that increased levels of reactive oxygen species in the vitreous fluid are correlated with the progression of diabetic retinopathy [4]. Hyperglycemia leads to the accumulation of advanced glycation end products in various tissues, which plays a crucial role in the development of microvascular complications in DM [5]. The mechanism behind diabetic retinopathy is well understood, and it is recognized that multiple factors contribute to its development. Some of these factors are related to hyperglycemia, while others are not directly connected to it [6]. The accumulation of free radicals in the retina along with oxidative stress results in thickening of the basal membrane, endothelial pericyte loss, leukocyte adhesion, DNA damage, retinal inflammation, and an increase in vascular permeability [7-9].

Selenium is a component of the enzyme glutathione peroxidase (GPx), which catalyzes the reduction of hydrogen and organic peroxides to alcohol and water. Selenomethionine is the depot form of selenium and cannot be synthesized in vivo, so it must be taken either in food or as a supplement. Selenium has an important role in cleaning reactive oxygen species by decreasing lipid peroxidation. For this reason, selenium supplementation is thought to be a preventive factor for some complications of DM [10].

Although this mechanism is important for the progression of diabetic retinopathy, there is still doubt about whether antioxidant nutrients improve diabetic retinopathy. On the other hand, the relationship between trace element deficiency and diabetic retinopathy is among the topics that have been frequently studied recently. Therefore, we measured selenium levels in vivo and interpreted the results. The aim of this study was to investigate whether selenium deficiency is related to an elevated risk of diabetic retinopathy among patients with type 2 DM.

Materials and methods

This study was a prospective case-control study. The patients were selected from patients who were treated at Samsun Research and Training Hospital Internal Medicine outpatient clinics between November 2019 and November 2020. Patients who are older than 18 years old with a diagnosis of type 2 DM were included. Although the patients were already being treated with antidiabetic medications, the diagnosis was retrospectively confirmed according to the recent algorithms of the American Diabetes Association for each patient [11]. Power analysis was done according to 80% power and a 95% confidence interval, and the minimum patient number that must be included in each group was calculated as 46 for detecting a true difference in means [12].

Patients were excluded from the study if they were younger than 18 years of age, pregnant or lactating, being treated with glucocorticoids or selenium supplements, or immunocompromised. Furthermore, patients were also excluded if they were taking drugs that could interfere with selenium levels. Age, sex, and the duration of DM were noted. The duration of diabetes was calculated based on the records in the insurance system and by asking the patients. All patients were examined by the same ophthalmologist to determine whether diabetic retinopathy was found or not.

Fundus photographs were taken for each patient to confirm the diagnosis. Best-corrected visual acuities were obtained, and dilated fundus examinations were performed for each patient after applying cyclopentolate drops. Diabetic retinopathy was diagnosed according to the Early Treatment Diabetic Retinopathy Study [13].

The study group included a total of 115 patients comprising 47 patients with type 2 DM and diabetic retinopathy (retinopathy group), and the control group included 68 patients with type 2 DM without retinopathy (non-retinopathy group). Plasma samples were collected from the patients, and serum selenium levels, fasting plasma glucose levels, and glycosylated hemoglobin (HbA1c) levels were measured. HbA1c levels were measured using the high-performance lipid chromatography technique with a Trinity Biotech Premier Hb9210 device. Fasting plasma glucose levels were measured by the hexokinase method, which was adapted to automatized devices. Reduced nicotinamide adenine dinucleotide phosphate is proportional to the glucose levels and measured by absorbance differences at 340 nm.

Selenium levels were measured in plasma using the inductively coupled plasma – mass spectrometer method. This method has the following stages: the sample is heated to 10000K by electromagnetic induction and ionized by argon plasma. Ionized elements are separated by a mass spectrometer, and finally, levels of elements are measured using a detector. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. This study was approved by Health Sciences University, Samsun Research and Training Hospital ethics committee with protocol number 2017-5-37.

Statistical analysis

A statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) Version 20.0. Continuous variables that were normally distributed were represented as the mean (standard deviation [SD]), while non-normally distributed continuous variables were represented as the median (min-max). Categorical variables were expressed as numbers and percentages (%). The student's t-test and Mann-Whitney U test were used for comparisons of continuous variables, while the chi-squared test was used for comparisons of categorical variables. Logistic regression analysis was utilized to

evaluate independent risk factors. A *P*-value of less than 0.05 was considered statistically significant.

Results

The study included a total of 115 patients comprising 62 (53.9%) females and 53 (46.1%) males. There were 47 patients in the study group (those with retinopathy) and 68 patients in the control group (those without retinopathy). The group with retinopathy had 26 female and 21 male patients, while the other group had 36 female and 32 male patients. The mean age in the retinopathy group was higher (Table 1). However, this difference was not statistically significant. Similarly, no statistically significant difference was found between the groups in terms of gender distribution.

Table 1: Demographic properties and selenium levels of two groups

	Non-retinopathy (n=68)	Retinopathy (n=47)	*P*-value
Age, mean (SD), years	53.2 (9)	56.5 (10)	0.070
Female sex, n(%)	36 (52.9)	26 (55.3)	0.801
Fasting plasma glucose, mean (SD), mg/dl	192 (90)	214 (97)	0.218
HbA1c, mean (SD)	8.1 (2.3)	8.9 (2.3)	0.087
Duration of DM, median (min-max), years	6 (1-21)	10 (1-25)	0.002
Selenium, mean (SD), µg/l	80.2 (19.1)	70.1 (17.3)	0.005

HbA1c: glycosylated hemoglobin, SD: standard deviation

The mean levels of fasting plasma glucose of the study group and the control group were 214 (97) mg/dl and 192 (90) mg/dl respectively. The mean levels of HbA1c were 8.9% (2.3) for the retinopathy group and 8.1% (2.3) for the non-retinopathy group. The mean fasting plasma glucose level and mean HbA1c level were higher in the retinopathy group compared to the non-retinopathy group. However, these differences were not significant statistically (*P*=0.218 and *P*=0.087, respectively).

The median duration of DM was significantly higher in the retinopathy group compared to the non-retinopathy group (Table 1). The mean selenium level was significantly lower in the retinopathy group (70.1 [17.3] µg/l) compared to the non-retinopathy group (80.2 [19.1] µg/l) (*P*=0.005). When age, gender, HbA1c levels, selenium levels, and the duration of DM were used as independent variables in the logistic regression analysis, a higher blood selenium level was an independent preventive factor against the occurrence of retinopathy (OR [95% CI]: 0.965 [0.939-0. 991]) while the duration of DM was an independent risk factor for retinopathy occurrence (OR [95% CI]: 1.131 [1.050-1.219]).

Discussion

Diabetic retinopathy is one of the major causes of visual disturbance among adults worldwide and the leading cause of visual loss in developed countries [14]. Screening diabetic patients for this complication has resulted in improvement in the quality of vision in this population, but unfortunately, the prevalence of diabetic retinopathy is still higher than 40%. Globally, 93 million people have diabetic retinopathy [15].

The pathogenesis of diabetic retinopathy associated with hyperglycemia includes oxidative stress, polyol activity, and the hexosamine pathway [6]. It has been demonstrated that diabetic patients are more susceptible to oxidative stress due to impaired defense mechanisms [16]. Recent research has indicated that oxidative stress is linked to retinopathy in both diabetic patients and non-diabetic individuals [17]. There have been numerous studies exploring the use of dietary antioxidant supplements for the prevention or treatment of diabetic retinopathy. One study found that oxidative stress in DM results in retinal activation of caspase-3 and apoptosis of endothelial cells and pericytes. An antioxidant supplement mixture was used and showed potential for improving patient management [18].

Kähler et al. [19] investigated antioxidant treatments in diabetic microvascular complications and reported that selenium and D-alpha-tocopherol supplementation improves neuropathic symptoms. In another trial, Gonzalez de Vega et al. [20] showed that selenium supplementation improves GPx activity even in hyperglycemic conditions. This study was an in vitro analysis of oxidative pathways and antioxidative supplements [20]. In one of the oldest studies on the subject, selenium levels were measured in pediatric diabetic children and were significantly higher in the diabetic group. However, the relationship of this condition with microvascular complications was not mentioned because none of the children in that study had diabetic complications [21].

In our study, the fasting plasma glucose levels of the study group were higher than that of the control group, but this result did not reach statistical significance. This condition may be related to the small sample size. But there was a significant difference between the two groups according to the duration of DM. Longer duration of diabetes was related to an increased risk of diabetic retinopathy (OR [95% CI]: 1.131 [1.050-1.219]). This is compatible with the results of similar studies on risk factors of diabetic retinopathy [3].

The comparison of two groups according to selenium levels showed that plasma selenium levels were lower in patients who had diabetic retinopathy. Regression analysis showed that elevated plasma selenium levels were an independent preventive factor against the occurrence of retinopathy (OR [95% CI]: 0.965 [0.939-0.991]), while a longer duration of DM was an independent risk factor for the occurrence of retinopathy. Selenium levels of aging populations may decrease because of low intake [22]. This is compatible with our results, which show that the retinopathy group had a higher mean age and lower selenium level.

One study from China that included 135 patients investigated the role of serum trace elements and heavy metal levels in diabetic retinopathy [23]. The study found that the serum concentrations of manganese (Mn) and zinc (Zn) were significantly lower in the diabetic retinopathy group, while cadmium (Cd) and cesium (Cs) levels were higher [23]. However, only selenium levels were measured in the study and not other trace elements.

Some other studies have explored the effect of other factors such as protein and lipoprotein glycosylation and their impact on diabetic retinopathy severity. Research has shown that both glycation and oxidative processes play a role in the development of diabetic retinopathy, and changes in the concentrations of Cd, Se, chromium (Cr), Zn, and copper (Cu) can affect the progression of the disease [24]. An animal study conducted in 2015 investigated the effect of chromium supplementation on diabetic retinopathy and found that chromium histidinate (CrHis) supplementation had beneficial effects on the retinas of diabetic rats [25]. Another study found that chromium levels had a predictive value for the occurrence of

diabetic retinopathy at 15.2 μg/L (sensitivity: 70%; specificity: 60.5%) [26].

All these studies measured the levels of trace elements or metals in a cross-sectional timeline, so the results may be affected by the nutrition status of the participants. For example, in our study, all of the participants were living in the same region in the central Black Sea region of Turkey and had similar dietary habits. All participants were offered a Mediterranean-type diet containing balanced macronutrients for weight and age. Fish, grains, eggs, and beans are foods that include high selenium levels and are easily accessible foods in this region. In a study from Black Sea region, the average of selenium level in honeybee pollen was 0.422-0.722 mg kg^{-1} dry pollen. Measurement of trace element levels in regional honeybee pollen is one way to show food sources specific to the regions in which they are collected [27]. In another recent study, selenium levels in fish were enough for daily intake in the Black Sea region (Samsun, Trabzon, and Sinop) and selenium/mercury levels were within permissible limits according to the WHO [28].

Some researchers have studied the effect of trace elements in diabetes control but did not study their effect on microvascular complications. For example, Sonkar et al. [29] showed that the mean levels of zinc, copper, selenium, and magnesium were significantly lower in patients with T2DM than the control cases. Another study from China demonstrated that a higher dietary intake of vitamin E and selenium seems to have a preventive effect on diabetic retinopathy [30].

The OR of our study was compatible with another study. We found that one unit increase in selenium level was associated with a unit decrease in diabetic retinopathy of 0.965 (0.939-0.991). The other study showed that one unit increase in selenium level was associated with a unit decrease in diabetic retinopathy of 0.98 (0.96, 1.00) [31].

Our findings may improve choices to prevent diabetic retinopathy. Preventive efforts are important along with intravitreous treatment options in the management of diabetic retinopathy. Good glycemic control and high blood pressure control are the cornerstones of prevention of diabetic retinopathy [31]. Besides controlling hyperglycemia and high blood pressure, we can measure blood selenium levels of patients with diabetic retinopathy and provide supplements if deficient. A recent animal study showed that sodium selenite can increase insulin secretion levels in pancreatic β cells of type 1 DM mice and improve diabetic retinopathy [32]. A recent review on the subject summarized that insufficient zinc as well as excessive copper levels are associated with increased oxidative stress levels, which can worsen microvascular lesions in DM. These abnormalities are correlated with the duration of diabetes and higher levels of HbA1C, as in our study [33].

Limitations

The main limitation of this study is that since the main setup of the study was to compare patients with and without retinopathy, and they were not divided into subgroups. The study was also conducted during the COVID-19 pandemic, so we could not reach as many patients. We suggest that future studies be done to analyze the effect of selenium deficiency on diabetic retinopathy by separating the patients according to retinopathy types. The sample size was not enough to apply these results to all the diabetic patients. We think that new studies with larger sample sizes could be more definitive to identify the relationship between the occurrence of diabetic retinopathy and selenium deficiency.

Selenium deficiency is associated with thyroid diseases, thyroid nodules, cancer, weakened immune function, and pregnancy, which could have been significant confounders to our study. For this reason malignancy, pregnancy, and immunosuppression were exclusion criteria in our study. Furthermore, patients who were taking drugs that could interfere with selenium levels were excluded. But we could not analyze whether the patients had thyroid disease or nodules. However, people receiving thyroid hormone replacement were not included in the study from the beginning as it could have been a confounding factor. Therefore, we think that excluding patients receiving thyroid-related treatment minimizes the effect of this factor.

Conclusion

In our study, a significant difference was found between patients with diabetic retinopathy and the non-retinopathy group in terms of blood selenium levels. These findings may be important for treatment or the prevention of diabetic retinopathy. Nevertheless, we still need more evidence for selenium supplementation for diabetic patients with or without microvascular complications. Future studies are needed to investigate the effect of selenium supplementation in this patient group.

References

1. IDF Diabetes Atlas 9th Edition .https://www.diabetesatlas.org (accessed on November 11, 2022)
2. Prokofyeva E, Zrenner E. Epidemiology of major eye diseases leading to blindness in Europe: a literature review. Ophthalmic Res. 2012;47(4):171-88. doi: 10.1159/000329603. Epub 2011 Nov 26. PMID: 22123077.
3. Zhang L, Krzentowski G, Albert A, Lefebvre PJ. Risk of developing retinopathy in Diabetes Control and Complications Trial type 1 diabetic patients with good or poor metabolic control. Diabetes Care. 2001 Jul;24(7):1275-9. doi: 10.2337/diacare.24.7.1275. PMID: 11423515.
4. Yeh PT, Yang CM, Huang JS, Chien CT, Yang CH, Chiang YH, et al. Vitreous levels of reactive oxygen species in proliferative diabetic retinopathy. Ophthalmology. 2008 Apr;115(4):734-7.e1. doi: 10.1016/j.ophtha.2007.05.041. Epub 2008 Jan 4. PMID: 18177940.
5. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis. 2018 Jan 25;9(2):119. doi: 10.1038/s41419-017-0135-z. PMID: 29371661; PMCID: PMC5833737.
6. Heng LZ, Comyn O, Peto T, Tadros C, Ng E, Sivaprasad S, et al. Diabetic retinopathy: pathogenesis, clinical grading, management and future developments. Diabet Med. 2013 Jun;30(6):640-50. doi: 10.1111/dme.12089. PMID: 23205608.
7. Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct. 2012;2012:646354. doi: 10.1155/2012/646354. Epub 2011 Oct 2. PMID: 21977319; PMCID: PMC3184498.
8. Ishida S, Usui T, Yamashiro K, Kaji Y, Ahmed E, Carrasquillo KG, Amano S, Hida T, Oguchi Y, Adamis AP. VEGF164 is proinflammatory in the diabetic retina. Invest Ophthalmol Vis Sci. 2003 May;44(5):2155-62. doi: 10.1167/iovs.02-0807. PMID: 12714656.
9. Aiello LP, Gardner TW, King GL, Blankenship G, Cavallerano JD, Ferris FL 3rd, et al. Diabetic retinopathy. Diabetes Care. 1998 Jan;21(1):143-56. doi: 10.2337/diacare.21.1.143. PMID: 9538986.
10. Vural P, Kabaca G, Firat RD, Degirmencioglu S. Administration of Selenium Decreases Lipid Peroxidation and Increases Vascular Endothelial Growth Factor in Streptozotocin Induced Diabetes Mellitus. Cell J. 2017 Oct;19(3):452-60. doi: 10.22074/cellj.2017.4161. Epub 2017 Aug 19. PMID: 28836407; PMCID: PMC5570410.
11. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41:13-27.
12. Dhand NK, Khatkar MS. Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Independent Means. Accessed 8 November 2021 at http://statulator.com/SampleSize/ss2M.html
13. American Academy of Ophthalmology Retina/Vitreous Panel Preferred Practice Pattern Guidelines. Diabetic Retinopathy. San Francisco, CA: American Academy of Ophthalmology; 2016. November 2016, Accessed 8 November 2021 at http://www.aao.org/ppp
14. Leasher JL, Bourne RR, Flaxman SR, Jonas JB, Keeffe J, Naidoo K, et al; Vision Loss Expert Group of the Global Burden of Disease Study. Global Estimates on the Number of People Blind or Visually Impaired by Diabetic Retinopathy: A Meta-analysis From 1990 to 2010. Diabetes Care. 2016 Sep;39(9):1643-9. doi: 10.2337/dc15-2171. Erratum in: Diabetes Care. 2016 Nov;39(11):2096. PMID: 27555623.
15. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care. 2012 Mar;35(3):556-64. doi: 10.2337/dc11-1909. Epub 2012 Feb 1. PMID: 22301125; PMCID: PMC3322721.
16. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019 Jun 15;11(3):45-63. PMID: 31333808; PMCID: PMC6628012.

17. Longo-Mbenza B, Mvitu Muaka M, Masamba W, Muizila Kini L, Longo Phemba I, Kibokela Ndembe D, et al. Retinopathy in non diabetics, diabetic retinopathy and oxidative stress: a new phenotype in Central Africa? Int J Ophthalmol. 2014 Apr 18;7(2):293-301. doi: 10.3980/j.issn.2222-3959.2014.02.18. PMID: 24790873; PMCID: PMC4003085.
18. Kowluru RA, Koppolu P. Diabetes-induced activation of caspase-3 in retina: effect of antioxidant therapy. Free Radic Res 2002 Sep;36:993-9.
19. Kähler W, Kuklinski B, Rühlmann C, Plötz C. Diabetes mellitus--eine mit Freien Radikalen assoziierte Erkrankung. Resultate einer adjuvanten Antioxidantiensupplementation [Diabetes mellitus--a free radical-associated disease. Results of adjuvant antioxidant supplementation]. Z Gesamte Inn Med. 1993 May;48(5):223-32. German. PMID: 8390768.
20. González de Vega R, García M, Fernández-Sánchez ML, González-Iglesias H, Sanz-Medel A. Protective effect of selenium supplementation following oxidative stress mediated by glucose on retinal pigment epithelium. Metallomics. 2018 Jan 24;10(1):83-92. doi: 10.1039/c7mt00209b. PMID: 29119175.
21. Gebre-Medhin M, Ewald U, Plantin LO, Tuvemo T. Elevated serum selenium in diabetic children. Acta Paediatr Scand. 1984 Jan;73(1):109-14. doi: 10.1111/j.1651-2227.1984.tb09907.x. PMID: 6702438.
22. Vaquero MP. Magnesium and trace elements in the elderly: intake, status and recommendations. J Nutr Health Aging. 2002;6(2):147-53. PMID: 12166371.
23. Zhu X, Hua R. Serum essential trace elements and toxic metals in Chinese diabetic retinopathy patients. Medicine (Baltimore) 2020;99:e23141.
24. Hasan NA. Effects of trace elements on albumin and lipoprotein glycation in diabetic retinopathy. Saudi Med J. 2009 Oct;30(10):1263-71. PMID: 19838431.
25. Ulas M, Orhan C, Tuzcu M, Ozercan IH, Sahin N, Gencoglu H, et al. Anti-diabetic potential of chromium histidinate in diabetic retinopathy rats. BMC Complement Altern Med. 2015 Feb 5;15:16. doi: 10.1186/s12906-015-0537-3. PMID: 25652875; PMCID: PMC4321702.
26. Temurer Afşar Z, Ayçiçek B, Tütüncü Y, Çavdar Ü, Sennaroğlu E. Relationships between microvascular complications of diabetes mellitus and levels of macro and trace elements. Minerva Endocrinol. 2020 Jul 3. doi: 10.23736/S0391-1977.20.03139-9. Epub ahead of print. PMID: 32623842.
27. Erdoğan A, Şeker ME, Kahraman SD. Evaluation of Environmental and Nutritional Aspects of Bee Pollen Samples Collected from East Black Sea Region, Turkey, via Elemental Analysis by ICP-MS. Biol Trace Elem Res. 2022 Apr 1. doi: 10.1007/s12011-022-03217-3. Epub ahead of print. PMID: 35362937.
28. Öztürk DK. Element concentrations of cultured fish in the Black Sea: selenium-mercury balance and the risk assessments for consumer health. Environ Sci Pollut Res Int. 2022 Dec;29(58):87998-8007. doi: 10.1007/s11356-022-21914-3. Epub 2022 Jul 12. PMID: 35819669.
29. Sonkar SK, Parmar KS, Ahmad MK, Sonkar GK, Gautam M. An observational study to estimate the level of essential trace elements and its implications in type 2 diabetes mellitus patients. J Family Med Prim Care. 2021 Jul;10(7):2594-9. doi: 10.4103/jfmpc.jfmpc_2395_20. Epub 2021 Jul 30. PMID: 34568141; PMCID: PMC8415681.
30. She C, Shang F, Cui M, Yang X, Liu N. Association between dietary antioxidants and risk for diabetic retinopathy in a Chinese population. Eye (Lond). 2021 Jul;35(7):1977-1984. doi: 10.1038/s41433-020-01208-z. Epub 2020 Oct 2. PMID: 33009517; PMCID: PMC8225784.
31. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. JAMA. 2007 Aug 22;298(8):902-16. doi: 10.1001/jama.298.8.902. PMID: 17712074.
32. Wang X, Sui H, Su Y, Zhao S. Protective effects of sodium selenite on insulin secretion and diabetic retinopathy in rats with type 1 diabetes mellitus. Pak J Pharm Sci. 2021 Sep;34(5):1729-35. PMID: 34803009.
33. Dascalu AM, Anghelache A, Stana D, Costea AC, Nicolae VA, Tanasescu D, et al. Serum levels of copper and zinc in diabetic retinopathy: Potential new therapeutic targets (Review). Exp Ther Med. 2022 May;23(5):324. doi: 10.3892/etm.2022.11253. Epub 2022 Mar 11. PMID: 35386624; PMCID: PMC8972839.

The National Library of Medicine (NLM) citation style guide has been used in this paper.