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Serum essential trace elements and toxic metals in Chinese diabetic retinopathy patients

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Abstract

Trace elements are essential for the proper functioning of proteins, enzymes, and transcriptional factors. However, toxic metals will compete with essential trace elements, and damage enzymatic activities and various physiological functions. We aimed to investigate the status of serum essential trace elements and toxic metals in Chinese diabetic retinopathy (DR) patients, and to analyze their associations.

This retrospective study included 33 normal subjects (normal group), 44 type 2 diabetes mellitus (T2D) patients with DR (DR group), and 58 T2D patients without DR (diabetes mellitus [DM] group). Serum levels of zinc (Zn), manganese (Mn), cadmium (Cd), and cesium (Cs), were measured for all participants using inductively coupled plasma-mass spectrometry.

The serum concentrations of Mn (0.0226 μg/L) and Zn (98.162 μg/L) were significantly lower in DR group, compared with both the DM group and normal group ($P < 0.05$). In contrast, the serum levels of Cs (0.0354 μg/L) and Cd (0.0149 μg/L) were significantly higher in DR group, compared with the normal group (Cs: $z = 3.136$, $P = .002$; Cd: $z = 3.766$, $P < .0001$). Similarly, the serum Cs level in the DM group was 0.0323 μg/L, which was significantly higher than that in the normal group (0.0167 μg/L, $z = 2.692$, $P = .007$). Moreover, the area under the receiver-operating characteristic curve values of Mn (0.753 [95% confidence interval, CI 0.635–0.872, $P = .002$]), and Cd (0.797 [95% CI 0.643–0.952, $P = .003$]) were significantly greater than those of Zn and Cs, for DR identification.

Our results suggest that deficient essential trace elements and accumulated toxic metals were highly associated with the presence of DR.

Abbreviations: AUROC = areas under the receiver-operating characteristic curve, BMI = body mass index, DBP = diastolic blood pressure, DM = diabetes mellitus, DR = diabetic retinopathy, HbA1c = glycosylated hemoglobin, SBP = systolic blood pressure, T2D = type 2 diabetes mellitus, TC = total cholesterol, TG = triglyceride.

Keywords: diabetes mellitus, diabetic retinopathy, inductively coupled plasma-mass spectrometry, serum toxic metals

1. Introduction

Diabetes mellitus (DM) will affect >360 million people by 2030, most of whom will be at a risk of diabetic retinopathy (DR).[1] DR is considered a leading cause of visual loss in industrialized nations, and traditionally, its pathogenesis was considered to involve the polyol pathway, glycation, protein kinase Cß1/2, hemodynamic changes, growth factors, oxidative stress, and the renin–angiotensin system.[2]

Trace elements are essential for optimal health, although they are not synthesized in the body and are mainly sourced from food. Several of these are essential for the proper functioning of proteins, enzymes, and transcriptional factors, especially in DM and DR pathogenesis. For example, insulin function can be enhanced by certain trace elements, such as zinc (Zn) and manganese (Mn).[3] In contrast, a marked decrease of serum Zn levels was found in DM cases as compared to nondiabetic subjects[4,5] and similarly, serum Zn levels also inevitably decreased in DR patients.[6] Additionally, Zn supplementation can defer cataractogenesis in diabetic rats, by downregulating the polyol pathway enzymes and exerting an antiglycating influence.[7] On the contrary, Mn is also involved in mitochondrial glycoprotein synthesis, as a cofactor of many enzymes.[8] Mn supplements decreased lipid peroxidation, and improved insulin secretion and mitochondrial function.[9]

However, populations may be at risk to high levels of toxic metals, due to industrial pollution,[8] including cadmium (Cd), and cesium (Cs), which are deposited in different tissues without degradation. In addition, Cd present in cigarette and wine also accumulates in the body. These metals remain in the tissues for a long time and compete with essential trace elements, eventually damaging enzymatic activities and various physiological functions. Likewise, such toxic metals also influence the occurrence of DM. For example, Cd level rises with increase in the duration of diabetes and hyperglycemia.[10] However, the relationship between toxic metals (Cd and Cs) and DR has not been reported until now.

In this study, we measured and compared the serum levels of both essential trace elements and toxic metals in DM patients

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with DR, DM patients without DR, and normal subjects, in an effort to comprehensively evaluate the risk predictive capacity of these elements for the presence of DR in China.

2. Methods

2.1. Study design

This retrospective study enrolled 112 type 2 DM (T2D) patients including 44 DR cases (DR group), 58 non-DR cases (DM group). Patients with chronic or infectious diseases, such as diabetic nephropathy, and taking medications of vitamin or mineral supplements, were excluded. The diagnosis of DR (nonproliferative and proliferative types) and T2D was made according to the Early Treatment Diabetic Retinopathy Study standards[11] and 2014 American Diabetes Association criteria,[12] respectively. The mean ages of the subjects in the DR and DM groups were 60 (42–79) and 63 (49–81) years, respectively. Additionally, a small group of 33 healthy volunteers (normal group) with a mean age of 58 (43–94) years, were recruited as controls for the determination of trace elements in healthy people. The Institutional Review Board of China Medical University approved the protocol, and all participants were treated in accordance with the tenets of the Declaration of Helsinki. All participants signed the informed consents.

2.2. Measurements of trace elements, toxic metals, and other indices

We used inductively coupled plasma-mass spectrometry (ICP-MS, Series 7700, Agilent Technologies Inc., USA) to measure the isotopes ^{55}Mn, ^{66}Zn, ^{111}Cd, and ^{133}Cs in serum samples carefully, according to Rosewell et al's study,[13] to avoid metal/metalloid contamination.

In addition, the systolic blood pressure (SBP), diastolic blood pressure (DBP), triglyceride (TG), total cholesterol (TC), glycosylated hemoglobin (HbA1c), body mass index (BMI, kg/m^2), and waist circumference were also recorded for all the subjects included in this study. The smoking history, DM family history, and DM duration were recorded simultaneously (Table 1).

2.3. Statistical analyses

Statistical analyses were performed by SPSS (version 19.0, Inc., Chicago, IL). The data were expressed as median (min–max). The differences in the serum concentrations of essential trace elements and toxic metals among DR group, DM group, and normal group were analyzed using Mann–Whitney Wilcoxon tests. The areas under the receiver-operating characteristic curve values (AUROC) for trace elements and toxic metals were performed for DR identification. P value $< .05$ was considered statistically significant.

3. Results

No significant differences were found in sex, smoking, and DM family history (Pearson χ^2, $P > .05$) among the 3 groups. Additionally, there were also no differences in BMI, waist circumference, SBP, DBP, TG, and TC among the three groups (Mann–Whitney Wilcoxon tests, $P > .05$). However, the DM duration in the DR group was significantly longer than that in the DM groups (Mann-Whitney-Wilcoxon tests, $P < .05$). Similarly, HbA1c in the DR groups was significantly higher than that in the DM and normal groups (Mann-Whitney-Wilcoxon tests, $P < .05$, Table 1).

The serum concentrations of trace elements and toxic metals for the subjects in DM, DR, and normal groups are shown in Table 2.

A lower Mn serum concentration was detected in the DR group (0.0226 μg/L), compared to the DM (0.0468 μg/L, z = 3.698, $P < .0001$) and normal groups (0.0363 μg/L, z = 3.302, $P = .001$). Additionally, the serum concentration of Zn decreased gradually from the normal group (112.936 μg/L) to the DM group (107.430 μg/L) and finally, to the DR (98.162 μg/L) group, with statistical significance ($P < .05$).

In addition, the serum concentration of Cd in the DR group (0.0149 μg/L) was higher than that in the DM group (0.0101 μg/L, z = 3.004, $P = .003$) and the normal group (0.00720 μg/L, z = 3.766, $P < .0001$) obviously, but no significant difference was observed between the DM and normal group (z = 1.727, $P = .084$). Compared to normal group, the serum concentration

Table 1

Baseline data of the DM, DR, and normal group patients included in this study.

Measurements	Normal subjects	DM patients	DR patients
N	33	58	44
Sex (n)*	(males: n = 13, females: n = 20)	(males: n = 23, females: n = 35)	(males: n = 21, females: n = 23)
Age, y†	58 (43–94)	63 (49–81)	60 (42–79)
Smoking (n)*	4	13	8
Diabetes mellitus duration, y	0 (0–0)	3.5 (0–30)‡	10 (0–36)‡
Diabetes mellitus family history (n)*	8	14	18
Body mass index, kg/m^2†	25.0 (20.3–31.3)	24.1 (20.6–30.8)	23.7 (15.4–30.5)
Waist circumference, cm†	85.5 (73–92)	84 (75–120)	82 (63–105)
Systolic blood pressure, mmHg†	140 (120–160)	140 (100–172)	140 (105–170)
Diastolic blood pressure, mmHg†	80 (68–100)	80 (63–103)	83.5 (65–100)
Triglyceride, mg/dL†	6.8 (5.0–9.1)	7.2 (3.9–19.2)	7.3 (4.7–14.8)
Total cholesterol, mg/dL†	5.40 (2.01–6.60)	5.60 (3.50–7.85)	5.19 (3.94–7.88)
Glycosylated hemoglobin (HbA1c) (%)	6.8 (4.5–10.2)	6.2 (4.4–10.7)	7.95 (4.6–12.0)§

DM = diabetes mellitus, DR = diabetic retinopathy.

* No significant differences among DR group, DM group, and normal group in sex, smoking and diabetes mellitus family history (Pearson χ^2, $P > .05$).

† No significant differences among DM group, DR group, and normal group in age, body mass index, waist circumference, systolic blood pressure, and diastolic blood pressure, triglyceride, and total cholesterol (Mann-Whitney-Wilcoxon tests, $P > .05$).

‡ Significant differences between DM and DR groups in diabetic mellitus duration (Mann-Whitney-Wilcoxon tests, $P < .05$).

§ HbA1c in DR groups was significantly higher than that in DM group and normal group (Mann-Whitney-Wilcoxon tests, $P < .05$).

Table 2

Serum concentration of trace elements in the DM group, DR group, and normal group included in this study.

Measurements	Normal group (n = 33)	DM group (n = 58)	DR group (n = 44)
^{55}Mn, μg/L[*,‡]	0.0363 (0.000–1.898)	0.0468 (0.000–3.071)	0.226 (0.0455–1.500)
^{66}Zn, μg/L[*,†,‡]	112.936 (82.539–160.986)	107.430 (52.957–202.538)	98.162 (35.214–131.142)
^{111}Cd, μg/L[*,‡]	0.00720 (0.00133–0.0101)	0.0101 (0.00160–0.0203)	0.0149 (0.00613–0.0215)
^{133}Cs, μg/L[†,‡]	0.0167 (0.0106–0.0999)	0.0323 (0.0114–0.229)	0.0354 (0.00773–0.199)

DM = diabetes mellitus, DR = diabetic retinopathy.

[*] Significant differences between DR and DM groups in trace elements (Mann-Whitney-Wilcoxon tests, $P < .05$).

[†] Significant differences between DM group and normal group in trace elements (Mann-Whitney-Wilcoxon tests, $P < .05$).

[‡] Significant differences between DR group and normal group in trace elements (Mann-Whitney-Wilcoxon tests, $P < .05$).

of Cs was significantly higher in the DR (0.0354 μg/L, z = 3.136, $P = 0.002$) and DM groups (0.0323 μg/L, z = 2.692, $P = 0.007$). In contrast, there was no difference in Cs serum concentration between the DR and DM groups (z = 0.926, $P = .355$).

After adjusting the DM duration and the HbA1c value, the AUROC value was 0.753 (95% confidence interval [CI] 0.635–0.872, $P = .002$) for Mn and 0.797 (95% CI 0.643–0.952, $P = .003$) for Cd. The areas under these curves were significantly different, which was not the case for Cs and Zn. The AUROC value was 0.563 (95% CI 0.427–0.698, $P = .355$) for Cs and 0.489 (95% CI 0.357–0.622, $P = .89$) for Zn.

4. Discussion

Hyperglycemia induces retinal damage by activating and dysregulating several metabolic pathways. However, it has been suggested that the cellular and functional changes were not entirely the result of excess plasma glucose, in the progression of DR.[14] Abnormal trace elements and toxic metal levels may lead to diabetic microvascular complications in DR. In the present study, serum levels of Mn, Zn, Cd, and Cs in DR patients were measured and compared with both DM patients and healthy individuals. In particular, deficient essential trace elements (Mn, Zn) and accumulated toxic metals (Cd, Cs) were found in the DR group. As we know, DR is involved in glycation and oxidative processes, which impacted the concentrations of Cd and Zn.[10]

The present study revealed that DR group had a lower Mn level than the DM group and normal group. Previously, Yazigi et al[15] and Forte et al[16] also observed that the level of serum Mn was lower in DM patients than in normal subjects. Mn can influence β-cell function by enhancing the secretion of insulin and improving glucose tolerance,[9] and change various noncarbohydrate contents into glucose via gluconeogenesis, as a cofactor of pyruvate carboxylase.[17] Therefore, we suspected that the deficiency of Mn may account for the progression of DR in DM patients. As we know, a diagnostic test with the AUROC closest to 1.0 has the best discriminatory power. The AUROC value of Mn was also demonstrated to be a reliable risk predictor for DR in this study.

Zn is essential for insulin in pancreatic β cells, including the synthesis, storage, and secretion, which subsequently increases the uptake of glucose.[18] Moreover, the mutation of Zn transporter, also involved in T2D.[19] Therefore, decreased serum levels of Zn negatively affect the production of insulin in islet cells.[20] Lower Zn levels are more likely related to prolonged DM duration, worse glucose control, and β-cell function, as well as a higher occurrence of diabetic microvascular complications.[6] In our study, a lower serum Zn level was found from normal group, to DM group and to DR group significantly, which may be as a result of increased urinary depletion.[17] As we know, Zn is a retinal protective factor, and by stabilizing the membrane structure, activating metallothionein, clearing free radicals, and inhibiting lipid peroxidation, it may reduce the expression of vascular endothelial growth factor and inhibit neovascularization and exudation.[21] Hence, the deficient serum Zn may aggravate the development of DR.

The accumulation of toxic metals has also been found in DM patients, disrupting glucose uptake and regulation.[8] We found that the serum levels of Cs and Cd were significantly higher in the DR groups, compared to the normal group. Moreover, our AUROC value of Cd was also demonstrated as a reliable risk predictor for DR.

Cd is a source of mitochondrial ROS in DR, and it can downregulate GLU T4 translocation by disrupting pancreatic β cells.[14] In addition, Cd exposure will elevate ROS levels and deplete mitochondrial membrane potential.[22] Meanwhile, the serum concentration of Cs in the DM group was significantly higher than that in the normal group, according to our results. Like Cd, Cs also causes mitochondrial dysfunction and leads to increased superoxide levels.[23] We considered, in the end, that all these biochemical effects of toxic metals aggravate the progression of DR.

There are also some limitations in the present study. For example, trace elements in the urine were not correspondingly analyzed; similar toxic trace metals such as lead and mercury are not examined here along with Cd and Cs. These should be investigated in further research.

In conclusion, this study firstly investigated the potential associations between serum essential trace elements and toxic metals and DR in Chinese T2D patients. Our results suggest that deficient essential trace elements (Mn and Zn) and accumulated toxic metals (Cd and Cs) were highly associated with the presence of DR, which may beneficial for its prevention and treatment.

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Author contributions

XMZ performed the experiment and data collection and drafted the paper. RH designed the study protocols, graded the fundus photographs, and drafted the paper.

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