International Journal of Current Research and Review
DOI: http://dx.doi.org/10.31782/IJCRR.2020.122432

Scopus

Research Article

IJCRR
Section: Healthcare
Sci. Journal Impact Factor: 6.1 (2018)
ICV: 90.90 (2018)

Serum Magnesium, Manganese and Selenium in Diabetes Mellitus Patients and Their Association with Glyoxalase-1

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ABSTRACT

Introduction: Glyoxalase-1, an erythrocyte enzyme of the glyoxalase system catabolizes the methylglyoxal is the main precursor of advanced glycation end products (AGEs). Elevated methylglyoxal and AGEs are strongly associated with the development of diabetic complications. The association between trace elements and glyoxalase-1 in diabetic patients is not well established.

Objective: To assess the concentration of serum Mg, Se, and Mn in diabetic patients and compare these in different groups defined in terms of duration of the DM.

Methods: The present study compares the concentration of micronutrients (magnesium, selenium, and manganese) in non-complicated (group A ≤4 years) and complicated (group B >4years) diabetes mellitus type-2 patients. Sixty-four (50.8%) patients were in group A, and 62 (49.2%) patients in group B. This cross-sectional study also correlates the concentrations with glyoxalase-1 levels.

Results: Glyoxalase-1 has shown higher concentration in group B than A (50.65±5.32 vs. 41.29±3.58 ng/mL; p-value <0.001) where all the micronutrients have shown higher levels in group A. One-way ANOVA test showed a significant association between glyoxalase-1 and glycosylated Haemoglobin, magnesium, and selenium (p<0.05).

Conclusion: Glyoxalase-1 is a predictor of poor glycemic control in diabetic patients, and it is associated with the early development of a diabetic complication. The concentrations of serum magnesium, selenium, and manganese decrease with the increased duration of diabetes.

Key Words: Methylglyoxal, Advanced glycation end products, Micronutrients, Diabetic complications, Nephropathy, Retinopathy, Neuropathy

INTRODUCTION

Chronic diabetes is associated with long-term organ damage and multiple micro and macro-complications.[1] The micro-elements are an integral component of glucose homeostasis, antioxidant enzymes, lipid metabolism, and potential pro-oxidant catalysts, and these are directly or indirectly associated with diabetic complications.[2] Glyoxalase-1 and erythrocyte enzyme of the glyoxalase system catabolizes the methylglyoxal is the main precursor of advanced glycation end products (AGEs). Elevated methylglyoxal and AGEs are strongly associated with the development of diabetic complications.[3,4] Diabetic patients have shown the Magnesium (Mg) deficiency, with the incidence rate between 11-48%. Magnesium depletion is associated with insulin resistance and impaired lipid metabolism in diabetic patients, and it affects the occurrence of diabetic complications.[5] Selenium (Se), an aid to reduce the formation of peroxidases of lipoproteins and free radicals shows the reduced levels in diabetic patients and increases the risk of diabetes-related cardiac complications.[6,7] Manganese (Mn) also plays a key role in the development and progression of diabetes mellitus (DM), and it is linked with diabetic complications.[8]

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ISSN: 2231-2196 (Print) **ISSN:** 0975-5241 (Online)

Received: 17.07.2020 **Revised:** 15.09.2020 **Accepted:** 09.11.2020 **Published:** 14.12.2020

There are many studies on the comparison of micronutrients in diabetic and non-diabetic patients, but the research comparing the concentration of Mg, Se, and Mn throughout diabetes is limited. We found very few studies that assessed the association between the trace elements and glyoxalase-1, but Mg, Se, and Mn were not studied with glyoxalase-1 in diabetic patients. The study was designed to assess the concentration of serum Mg, Se, and Mn in diabetic patients and compare these in different groups defined in terms of duration of the DM. The study also measures the glyoxalase-1 levels in the pre-defined groups of diabetic patients and their association with the mentioned micronutrients.

MATERIAL AND METHODS

Study design, participants and grouping

The present study is a prospective cross-sectional analysis that was performed at Shree Guru Gobind Singh Tricentenary University, India. It included 126 diabetes mellitus patients age between 35 and 65 years with different duration of onset and divided into two groups. The first group had the patients with a duration of ≤4 years (group A), and the second group had the patients with a duration of >4years (group B). The groups were made after an appropriate literature research and experts' consultation in the field to define the effect of short-term (recent) and long-term (chronic) diabetic status on serum trace elements and glyoxalase-1 levels.

Pregnant and lactating women, patients on hormone therapy, micronutrients, and steroids, and patients with chronic kidney disease except for diabetic nephropathy, liver disease, and thyroid dysfunction were excluded from the study. Patients with obesity, anaemia, hepatic carcinoma and other conditions which can influence the concentrations of trace elements were also excluded from the study.

Ethical consideration

The study was approved by the Institutional Ethics Committee (IEC) of Shree Guru Gobind Singh Tricentenary University, Haryana, to be conducted between September 2013 and August 2018. All the participants were included after obtaining a written consent form to conduct a clinical examination and to collect blood samples for analysis.

Study Parameters

Age, gender, and social habits were documented under sociodemographic parameters. Clinical characteristics include family history, the average duration of diabetes, body mass index (BMI), systolic, and diastolic blood pressure were observed and analysed. Diabetic complications i.e., diabetic nephropathy, neuropathy and retinopathy were compared in both the group to evaluate the impact of the diabetic duration on these complications. Glycated Haemoglobin (GlyHb) is the major biomarker to diagnose diabetes and it has an impact on the long-term complications. We have measured GlyHb levels for all the patients along with the lipid levels (high and low-density lipids) under biochemistry investigations and analysed appropriately to associate with the objective of the study.

Trace elements and glycated Haemoglobin analysis

Five millilitres (mL) blood was taken from the antecubital vein after at least 8 hours fasting for all participants. The serum was separated by centrifuging the blood sample at 4000 rpm for 10 minutes and stored at -80 °C in the deep-freezer. Each sample was diluted with glycerol immediately after separating plasma. An auto-analyzer atomic absorption spectrometer was used to assess the concentration of Mg, Mn, and Se in serum. The serum level of trace elements in each sample was obtained by the calibration curve. Immunoturbidity Enhance Enzymatic (IEE) method was used to analyze GlyHb and was done in a fully automated analyzer (BS-300).

Glyoxalase-1 testing

The ELISA assay was performed to determine the in vitro concentration of human glyoxalase-1 in serum. Five ml of the blood sample was collected in a disposable and non-endotoxin tube from the antecubital vein and allowed to clot for 2 hours at room temperature. After clotting, the sample was centrifuged for 15 minutes at 1000*g at 2~8^{o} C. Supernatant was used for ELISA assay analysis.

Statistical Analysis

Categorical and continuous variables were reported in the form of proportions and mean ± standard deviation (SD), respectively, for the baseline characteristics. Statistical differences between each parameter of both the groups were estimated for significance by Independent T-test and Chi-square test. Concentration differences of serum trace elements between both the groups (≤4 years and >4 years) were presented using box-plots. One-way ANOVA was run to find the correlation/association between glyoxalase-1 and GlyHb and three trace elements. Scatter-plots are used to present the correlation. The p-value < 0.05 was considered for significant results. The data were analyzed using SPSS 22.0 statistical analysis software.

RESULTS

Baseline characteristics

Out of a total of 126 patients, 64 (50.8%) patients had the duration of diabetes onset ≤4 years, and 62 (49.2%) patients had a duration of >4 years. Seventy-seven (61.1%) patients were male, in those 39 were in group A, and 38 were in group

B. The Mean age of the patients and proportions of complications were significantly high in group B than A. Other parameters were comparatively matched in both the groups. The biochemistry investigations were measured and compared between groups A and B [Table 1].

Comparison of trace elements and glyoxalase-1 in different groups

All three trace elements and glyoxalase-1 showed a significant difference in both the tested groups of diabetic patients. Magnesium, Mn and Se concentration were high in group A versus group B ($p < 0.001$) [Table 2]. Glyoxalase-1 has shown higher concentration in group B than A (50.65±5.32 vs. 41.29±3.58 ng/mL; $p < 0.001$) where all the micronutrients have shown higher concentration in group A [Figure 1].

Correlation between glyoxalase-1 and trace elements

One-way ANOVA test showed the significant association/correlation between glyoxalase-1 and GlyHb, Mg, and Se (p-values of 0.008, <0.001, and 0.026, respectively) [Table 3]. The glyoxalase-1 level was increasing with the increased levels of GlyHb where the glyoxalase-1 level was decreasing with the increased levels of Mg, Se, and Mn [Figure 2].

DISCUSSION

Primary findings of the present study based on the assessment of serum concentration of micronutrients and glyoxalase-1 are that;

1. The glyoxalase-1 level increases with the increased duration of diabetes
2. The concentrations of serum Mg, Se, and Mn decrease with the increased duration of diabetes, and it may associate with diabetic complications.
3. Glyoxalase-1 was significantly associated with Mg and Se levels in diabetic patients. Glyoxalase-1 level decreases with the increased levels of Mg, Se, and Mn, or in another term, we can define in the manner that elevated glyoxalase-1 levels are associated with decreased Mg and Mn levels in diabetic patients.

Further, we found that the glyoxalase-1 levels increase with the escalated levels of Hb. The above findings suggest that increased glyoxalase-1 levels could be a predictor or indicator of poor glycemic control and early occurrence of diabetic complications in chronic patients. It also correlates with Mg and Se deficiency with the aging of diabetes.

The prevalence of diabetes is rapidly increasing across the world, and poor glycemic control leads to diabetic complications. Literature suggested that the alteration in micronutrient metabolism contributes to the pathogenesis of diabetes by adversely affecting the pancreatic islet.[9,10] Micronutrients are being involved in the development of secondary complications of DM by generating reactive oxygen species (ROS) and oxidative stress.[11] Diabetic patients develop impaired metabolism of calcium, zinc, copper, and magnesium, and other micro-elements, which lead to increased sensitivity to oxidative damage and development of diabetic complications.[12] Proteins glycosylation increased oxidative stress, and free radicals production are the responsible factors for the occurrence of complications in diabetic patients.[13]

Magnesium (Mg) is known for its critical roles in the enzymatic reactions in the human body and had an inverse correlation with the DM incidences.[14] Magnesium concentration decreases with increased duration of diabetes and associated with poor glycemic control.[15,16] It also triggers the early occurrence of diabetic complications, especially nephropathy as Mg depletion is strongly associated with microalbuminuria, which is a strong predictor of insulin resistance and hyperglycemic state.[17] At the same time, it is accepted as a predictor of other macro and micro-vascular complications of diabetes, i.e., cardiovascular morbidity, diabetic retinopathy and diabetic neuropathy.[18-21] Cellular Mg deficiency alters the membrane-bound sodium-potassium adenosine triphosphatase enzyme activity that again alters the glucose transport, reduces insulin secretion from the pancreas, defects insulin signalling, and alter insulin-insulin receptor interactions.[22] The study comparing Mg levels in non-diabetic, diabetic patients with and without retinopathy showed that the serum levels were low in diabetic patients than non-diabetics and lowest in patients with diabetic retinopathy (2.62, 2.02 and 1.38 mg/dL; p-value <0.001 for the concentration difference between groups.[23]

Both the third National Health and Nutrition Examination Survey (NHANES III) and report of 2003–2004 show a positive association between the prevalence of DM, insulin resistance, and serum Se levels.[24,25] In the present study, serum Se levels were significantly low in patients with DM duration >4 years than the comparative group. Selenium deficiency was seen in patients with uncomplicated diabetes than nondiabetic patients[11]. Another study comparing Se levels in diabetic and nondiabetic patients showed lower levels of Se in the diabetic group.[26] A significant positive association between serum Se level and Se dietary intake was seen in diabetic patients in a study compared to the correlation between dietary Se and diabetes in middle-aged and elderly adults.[6,27]

Serum Mn concentration decreases in patients with DM in comparison to nondiabetic patients. Manganese deficiency has associated with the early occurrence of diabetic complications.[8] Other studies evaluating trace elements in diabetic patients suggested that the diabetic patients show a lower level of Mn than in the patients without diabetes, and patients with chronic DM showed the lower levels of Mn than in acute-onset diabetes.[28,29] This study also indicates

the decreased level of Mn in DM with the duration >4 years than the DM with a duration of ≤4 years.

The methylglyoxal modifies amino acid residues of deoxyribonucleic acid (DNA), proteins, and lipids and works as a precursor of AGEs. It detoxifies by the glyoxalase system, and glyoxalase-1 converts methylglyoxal into S-D-lactoylglutathione with the help of cofactor glutathione.[3] Glyoxalase-1 also contributes to the impairment of endothelial function that again leads to diabetes-related vascular damage[30]. Endothelial dysfunction is the initiation and progression of vascular complications, i.e., retinopathy, neuropathy, macroangiopathy, and nephropathy.[31-33] The present study establishes the strong association between hyperglycemia (GlyHb), serum Se and Mg levels. Glyoxalase-1 levels were increasing with comparable Diabetic patients show the Mg and Se depletion with a chronic course of diabetes and increased glyoxalase-1 levels. Manganese levels decrease with increased glyoxalase-1 levels but not significantly.

CONCLUSION

Glyoxalase-1 was seen high in chronic diabetic patients and has increased proportionally with the duration of diabetes. It could be a predictor of poor glycemic control in diabetic patients as it is associated with the early development of diabetic complications. The concentrations of serum Mg, Se, and Mn decrease with the increased duration of diabetes. Increased glyoxalase-1 levels also correlate to serum micronutrient deficiency.

ACKNOWLEDGMENT

The study team would like to thank the laboratory facilities of King George's Medical University, Lucknow, Uttar Pradesh, for their support in analyzing trace elements.

Conflict of interest: The authors do not declare any conflict of interest and study did not get any external funding.

Financial Support: The study did not get any external funding.

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Table 1: Baseline socio-demographic and clinical characteristics of diabetic patients with different durations (≤4 years and >4 years).

Parameters	Group A; ≤4 Years (n, 64)	Group B; >4 Years (n, 62)	p-value
Age	49.36±7.50	58.66±4.75	<0.001
Gender			
Male	39 (60.9%)	38 (61.3%)	0.557
Female	25 (39.1%)	24 (38.7%)	
Social Habits			
Smoking	27 (42.2%)	21 (33.9%)	0.219
Alcoholic	8 (12.5%)	13 (21.0 %)	0.150
Clinical history			
Family history of diabetes	15 (23.4%)	11 (17.7%)	0.285
Dyslipidemia*	50 (78.1%)	50 (80.6%)	0.449
Diabetic Complications			
Neuropathy	7 (10.9%)	23 (37.1%)	<0.001
Retinopathy	3 (4.7%)	15 (24.2%)	
Nephropathy	2 (3.1%)	10 (16.1%)	
Average Duration (years)	2.20±0.96	6.81±1.94	--
Body mass index	24.1±3.3	23.8±3.3	0.725
Systolic blood pressure	123.8±6.2	128.7±6.4	<0.001
Diastolic blood pressure	82.8±3.4	85.7±3.7	<0.001
Lab Investigations			
Glycated Haemoglobin	8.7±2.5	9.7±2.7	0.029
Total cholesterol	195.6±22.9	196.6±27.2	0.834
Triglycerides	220.4±44.5	227.3±50.8	0.421
High density lipids	42.2±5.9	41.9±6.3	0.771
Low density lipids	94.0±22.5	92.9±20.4	0.779
Very low density lipids	43.6±10.3	43.3±9.8	0.889

*Dyslipidemia defined as total cholesterol >250 mg/dL, Low density lipids (LDL) >130 mg/dL, and High density lipids (HDL) <40 mg/dL (<50 mg/dL for women) in the fasting state

Table 2: Comparison of trace elements and glyoxalase-1 between both the groups of diabetic patients with different durations (≤4 years and >4 years)

Elements and enzyme	Group A; ≤4 Years (n, 64)	Group B; >4 Years (n, 62)	p-value
Glyoxalase-1 (ng/mL)	41.29±3.58	50.65±5.32	<0.001
Magnesium (mg/dL)	1.689±0.332	1.342±0.168	<0.001
Manganese (mcg/L)	0.221±0.038	0.189±0.018	<0.001
Selenium (ng/dL)	78.99±16.59	52.24±5.44	<0.001

Table 3: One-way ANOVA table shows the association between trace elements, glycated Haemoglobin and glyoxalase-1 levels

	F Value	p-value
Glycated Haemoglobin	1.988	0.008
Magnesium	3.581	<0.001
Selenium	1.738	0.026
Manganese	0.89	0.740

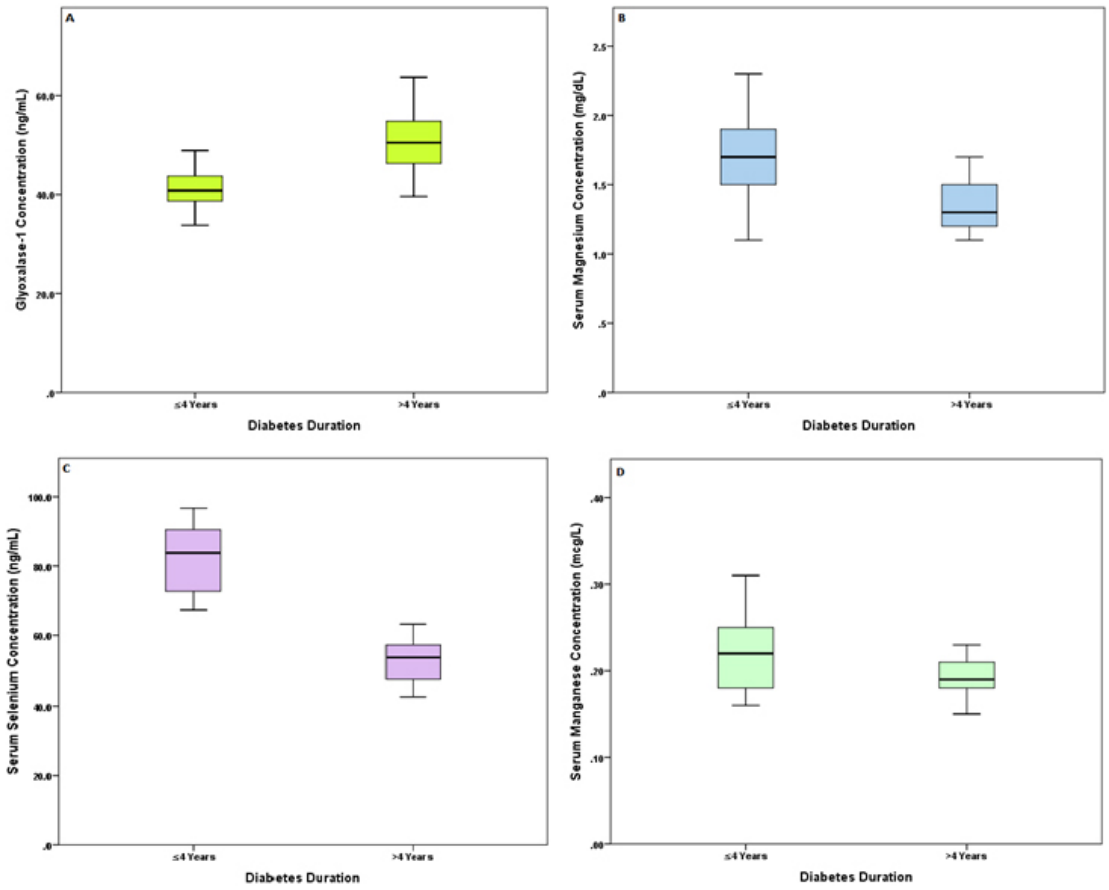

Figure 1: The box plots show the concentration differences of trace elements and glyoxalase-1 between both the groups- (A) for glyoxalase-1; (B) for magnesium; (C) for selenium; (D) for manganese.

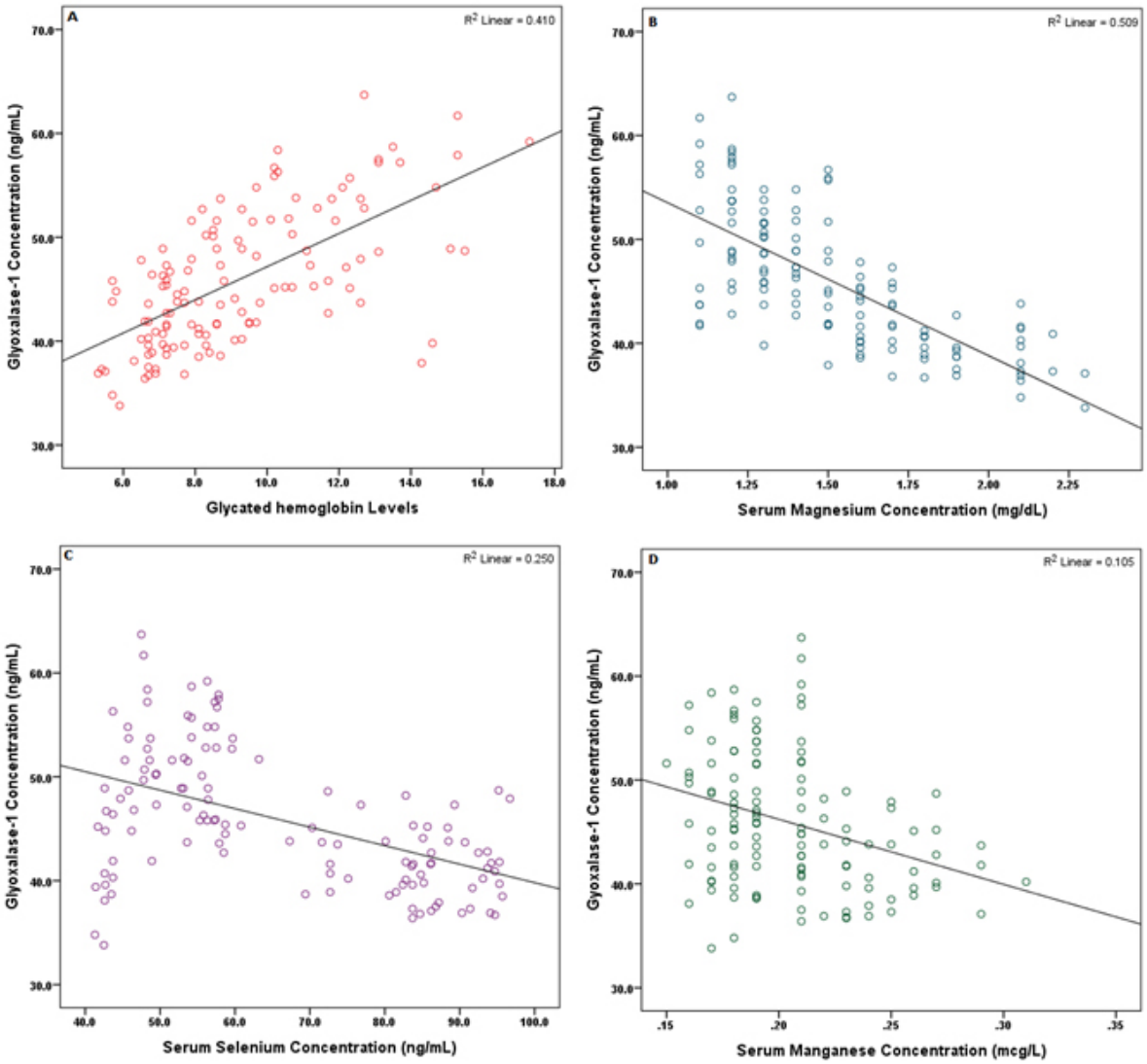

Figure 2: The scatter plots show the correlation graph between- (A) glyoxalase-1 and glycated Haemoglobin; (B) glyoxalase-1 and magnesium; (C) glyoxalase-1 and selenium; (D) glyoxalase-1 and manganese.