ORIGINAL RESEARCH

Role of serum magnesium and serum zinc in diabetes type 2 patients with or without microvascular diseases

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ABSTRACT
Diabetic patients' life expectancy has increased as a result of advances in therapies and research, as has the elderly population as a whole. Trace element-associated antioxidant enzymes are changed in diabetes. Many cohort studies have indicated that diabetes can disrupt trace element homeostasis.Early abnormalities in particular components may have a significant effect in altering insulin metabolism.
The present study was conducted in the Department of Biochemistry. It was designed to estimate the alterations in the levels of serum Magnesium and Serum Zinc in type 2 diabetes mellitus patients with microangiopathic complications in comparison to those without these complications and their relation with oxidative stress, insulin resistance, and glycaemic control. The aim of this study is to correlate the serum Magnesium and Serum zinc andinsulin resistance levels in patients of type2 diabetes mellitus with and without micro vascular complications.
The study was permitted by the Ethical Committee of Institution. A written informed consentwas attained from all the 600 participants, upon fulfilling the inclusion criteria.
We concluded that mean blood magnesium and zinc levels were considerably lower in type 2 diabetics with problems compared to those without difficulties and the control group. These levels declined considerably during the course of diabetes. A momentous negative correlation was showed by Serum levels of Magnesium and Zinc with FBS,HbA1c,HOMA-IRandMDA.
Current observations recommend that Screening panel in the risk detection and progression of diabetes should include the estimation of serum zinc, magnesium and copper estimation. Supplementation of Mg and Zn and recurrent one-to-one care of levels of both minerals in type 2 diabetic patients.
Key words: Magnesium, zinc, diabetes, fasting blood sugar, HbA1c

INTRODUCTION

Miners and traces of elements are critical micronutrients that the body needs to operate properly. These elements are especially useful for physiological processes.[1] Minerals and trace elements are required for many biological reactions, operate as stabilising components of enzymes and proteins, and serve as cofactors for several enzymes. Certain trace elements control critical biological processes by attaching to the receptor location of the cell membrane or altering the shape of the receptor to prevent specific molecules from entering the cell.[2] Micronutrients perform two functions: they keep cellular structures stable at appropriate levels, while deficiency leads to other routes and may cause illness.[3]These vital micronutrients have significant physiological significance and show direct connections with diabetes.[4,5]

The scientific literature and medical data from diabetes studies provide reasonable estimates of crucial micronutrient deficiency/overload. However, the numerous inconsistencies in research make it difficult for physicians to formulate dietary advice for diabetics.[6]Diabetic patients' life expectancy has increased as a result of advances in therapies and research, as has the elderly population as a whole. Trace element-associated antioxidant enzymes are changed in diabetes.[7] Many cohort studies have indicated that diabetes can disrupt trace element homeostasis.[8]Early abnormalities in particular

components may have a significant effect in altering insulin metabolism [9,10,11]. The bulk of cohort studies concentrate on a single aspect or a small set of components exclusively.

Micronutrients have been recognised as essential nutrients that are required in trace levels for homeostasis, enzyme control, and function.[12,13]

ASSOCIATION BETWEEN DIABETES MELLITUS AND ALTERATIONS IN THE METABOLISM OF TRACE ELEMENTS

Numerous human and animal studies have shown a clear link between mineral metabolism and human health. A change in the metabolism of these elements has frequently been seen. The micronutrient status of patients with diabetes mellitus has changed over the past 60 years, according to numerous studies, and in a few of these studies, a mineral deficiency has been linked to the prevalence of diabetic complications. Contradictory results and opposing conclusions have been produced as a result of methodological flaws and variations in the patient population under study.

Magnesium is the body's fourth most abundant cation and the second most prevalent intracellular cation after potassium. By acting as a cofactor for the various enzymes involved at multiple stages in insulin secretion, binding, and activity, magnesium plays a crucial role in glucose metabolism and the transport of glucose by the cell.[14]

The only transition metal that is present in every type of enzyme is zinc, which is the second most prevalent transition metal in organisms after iron. Zinc, a crucial trace element, is crucial for the synthesis, storage, and release of insulin as well as other tissue-maintaining processes. Additionally, it keeps insulin's structural structure intact. *In vitro* studies have shown that zinc increases the efficiency of insulin, and it has been suggested that a zinc deficit may make non-insulin-dependent diabetes mellitus patients more resistant to insulin.[15]

Since, zinc is a requirement for the enzyme's superoxide dismutase, catalaseand peroxidase, changesin zinc metabolism that render sufficient zinc insufficient for these enzymes may be expected to add to the tissue damage seen in diabetes. A higher chance of developing diabetes appears to be associated with low dietary zinc intake. According to a recent meta-analysis, zinc supplementation in diabetic individuals enhances glycaemic control and supports healthy lipid parameters.[16]

The present study is designed in order to estimate the alterations in the levels of serum Magnesium and Serum Zinc in type 2 diabetes mellitus patients with microangiopathic complications in comparison to those without these complications and their relation with oxidative stress, insulin resistance, and glycaemic control. The aim of this study is to correlate the serum Magnesium and Serum zinc andinsulin resistance levels in patients of type2 diabetes mellitus with and without micro vascular complications.

MATERIAL AND METHODS

SUBJECTS AND STUDY DESIGN: The present study was conducted in the Department of Biochemistry. The study was permitted by the Ethical Committee of Institution. A written informed consentwas attained from all the participants, upon fulfilling the inclusion criteria.

SELECTION OF SUBJECTS: In all 600 subjects were in the present study.

CONTROL GROUP: 200 healthy age and gender-matched participants were chosen from the workplace. These participants would be non-diabetic, physically active, and had fasting blood glucose levels ranging from 70 to 100 mg%. All patients would be between the ages of 35 and 70. Both sex groups will be included. They will be clear of any serious illnesses that might impact the parameters being studied.

TEST GROUP-1: Include 200 type 2 diabetic patients with a duration of less than 5 years. They have no clinical indications of microvascular consequences from diabetes mellitus. Diabetes will be diagnosed using the same criteria established by the World Health Organisation.Diabetes mellitus symptoms include fasting plasma glucose levels of 126 mg/dL or above.

OR test for postprandial plasma glucose levels of 200 mg/dL or above.

TEST GROUP-2: The study included 200 type 2 diabetes mellitus patients who had one or more microvascular manifestations of diabetes mellitus. Diabetes lasts five or more years.

To detect microvascular problems, the physician will use the following procedures.

DIABETIC RETINOPATHY:A thorough fundus examination was done to look for retinal vascular micro aneurysms, blot and cotton wool spots (non-proliferative diabetic retinopathy) and appearance of neovascularisation (proliferative diabetic retinopathy).

DIABETIC NEUROPATHY:A complete motor and sensory examination was carried out to any polyneuropathy, radiculopathy or mono-neuropathy. Diabetic Nephropathy: Urinary micro protein estimation along with serum creatinine was carried out.

EXCLUSION CRITERIA:1. Patients with type I Diabetes Mellitus. 2. Pregnant and lactating females3.Patients taking diuretics, lipid-lowering, and multivitamins drugs. 4.Patients with disease unrelated to diabetes which may alter chosen parameters

i.e.AIDS, thyroid disease, tuberculosis, and cancer patients.

PARAMETERS PERFORMED

1. **BIOCHEMICAL PARAMETERS:**Fasting Blood glucose, HbA1C, Serum Magnesium, Serum Zinc.
2. **SPECIAL PARAMETERS:**Serum insulin, Insulin Resistance.

ESTIMATION OF SERUM MAGNESIUM

METHOD: Calmagite indicator method (Colorimetric; kits by tulips Diagnostic).

PRINCIPLE: Magnesium reacts with calmagite in an alkaline solution to generate a crimson complex. The use of particular chelating agents and detergents eliminates calcium-protein interference. The intensity of the colour produced is exactly proportional to the quantity of magnesium in the sample.

ESTIMATION OF SERUM ZINC

Method: Nitro-PAPS method(Colorimetric; kits by tulips Diagnostic).

PRINCIPLE: Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple-coloured complex. The intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

RESULTS

Out of 600 study population in the age group of 35-70 years, 200 healthy volunteers were chosen as control while 200 T2DM patients without microvascular complications and 200 T2DM patients with microvascularcomplications were distributed asstudygroup-Iand study group-II. Males and females were enrolled in this study in greater proportion than females. Group I had 121 male and 79 female members, Group II had 120 male and 80 female members whereas Group III had 125 male and 75 female members. Within each group, there was a notable distinction between the men and females.TheControlgrouphadameanageof47.05 was significantlylower than other groups. There was a significant difference compared mean age between the groups.

The control group had normal glucose levels. Both research groups had significantly elevated glucose levels relative to the control group. Group III had higher glucose levels than Groups I and II. The differences between groups were statistically significant.Hba1C levels increased significantly in both diabetes groups as compared to the control group. Group II exhibited a substantial difference from Group III. Group-III had the greatest rise in HbA1C compared to Groups II and I. Group-I showed a significant difference compared to other groups. Group-III showed maximum insulin level compared to other groups. Both diabetes groups showed a considerable rise in HOMA-IR as compared to control participants. HOMA-IR increased significantly in Group III when compared to the other groups.

Maximum magnesium levels were seen in the control group. Diabetic individuals had significantly lower magnesium levels compared to the control group. A considerable difference was discovered between Groups II and III.Group-Ishowedhighlevelsof zinc compared to Group-II and III. Control group showed significant difference compared Group-II and III. Least Zinc value was noticed in Group-III. FBS, Zn and PCR shown a significant increase with the duration of onset of diabetes. Highest values of FBS and PCR were noticed in above 3 years duration.While zinc significantly declines with the duration of diabetes.

HbA1C, HOMA-IR, MDA values shown a significant increase with the duration of onset of diabetes. Highest values of HbA1C, HOMA-IR and MDA were, noticed in above 3 years duration.While Magnesium significantly declines with the duration of diabetes.

Thedistributionofmagnesium incontrolsandtestgroups. Hypo-magnesiumwas significantly higher in both study groups compared to control groups. Zinc deficiencywassignificantlyhigherintype2diabeticswith microvascularcomplicationgroup when compare to control and groupII.

Linearregressionanalyseswereperformedbetweenmagnesiumwithothervariables,inwhich serummagnesium was the dependent variableandFBS,HbA1C,HOMA-IR,serum Zinc,MDAandPCRweretheindependentvariables.RevealedthatonlySerumZincwaspositivelycorrelatedwhileFBS,HbA1C,HOMA-IR, and MDA showed inverse relationships with serum Magnesium. (Table 9)

Linear regression analyses were performed between zinc and other variables, in which serumzincwasthedependentvariableandFBS,HbA1C,HOMA-IR,serumMg, MDA, and PCR were the independent variables. Revealed that only SerumMagnesiumandVitaminDwerepositivelycorrelatedwhileFBS,HbA1C,HOMA-IR and MDA showed a negative correlation with serum Zinc. (Table 10)

Table1: Comparison of fasting blood glucose (mg/dl), HbA1C, Insulin and HOMA-IR levels between the groups

Groups	Description of group	Fasting blood glucose (mg/dl) (MEAN±SD)	HbA1C (MEAN±SD)	Insulin (MEAN±SD)	HOMA-IR (MEAN±SD)
Groups-I	Control	86.17±7.35	5.23±0.51	5.72±0.98	1.33±0.19
Groups-II	T2DM without microvascular complications	140±30.75*	6.87±0.75*	9.98±7.5*	3.89±2.2*
Groups-III	T2DM with microvascular complications	172.56±52.9*#	7.96±1.83*#	11.5±8.55*	5.18±2.6*

(*p<0.05 significant compared Group-I with other groups, #p<0.05 significant compared Group-II with other Groups).

Table2: Comparison of magnesium levels between the groups

Groups	Descriptionofgroup	Magnesium (Mean±SD)
Group-I	Control	2.13±.27
Group-II	T2DMwithoutmicrovascular complications	1.77±.25*
Group-III	T2DMwithmicrovascular complications	1.61±.20*#

(*p<0.05significantcomparedGroup-Iwithothergroups,#p<0.05significantcompared Group-II with other Groups)

Table3: Comparison ofZinclevelsbetweenthegroups

Groups	Descriptionofgroup	Zinc(Mean ±SD)
Group-I	Control	99.85±11.35
Group-II	T2DMwithoutmicrovascular complications	85.59±12.71*
Group-III	T2DMwithmicrovascular complications	73.77±12.37*,#

(*p<0.05significantcomparedGroup-Iwithothergroups,#p<0.05significantcompared Group-II with other Groups)

Table4: Comparison of mean fasting blood glucose,zinc,PCRwithinthe Group-II and III based on the duration of diabetes

Observation	Lessthan1year (Mean ±SD)	1-3 years (Mean ±SD)	Above3years (Mean ±SD)
Fastingblood glucose	105.02±1.02	115.12±0.23*	138.20±0.86*,#
Zinc	95.86±11.32	89.27±11.87*	75.19±11.88*,#
PCR	17.20±4.09	22.89±8.74*	165.49±90.66*,#

(*p<0.05significantcompared lessthan1yearwithothertimeperiods,#p<0.05 significant compared 1-3 years with other time periods)

Table5: Comparison of mean HbA1C, insulin, HOMA-IR, magnesium, MDAwithintheGroup-IIandIIIbasedonthedurationofdiabetes

Observation	Lessthan1year (Mean±SD)	1-3 years (Mean±SD)	Above3years (Mean±SD)
HbA1C	6.19±0.87	7.90±1.34*	8.87±0.95*,#
HOMA-IR	1.57±0.34	3.49±0.43*	4.11±0.45*
Magnesium	1.98±.43	1.70±.58	1.67±0.89*
MDA	1.20±0.32	2.02±0.73	2.49±0.96*

(*p<0.05significantcomparedlessthan1yearwithothertimeperiods,#p<0.05 significant compared 1-3 years with other time periods)

Table6: Comparisonofmeanmagnesium and zinc within the Group-III based on the complications

Observation	Retinopathy(n=73) (Mean±SD)	Nephropathy (n=55) (Mean±SD)	Neuropathy(n=23) (Mean±SD)	Multiple complications (n=49)
Magnesium	1.64±0.19	1.71±0.44*	1.70±0.33*	1.50±0.15*
Zinc	78.40±11.65#	68.78±7.66	83.50±8.86*#	70.29±11.72

(*p<0.05significantcomparedretinopathywithothers,#p<0.05significantcompared nephropathy with others)

Table7: Comparison ofMagnesiumDistributionbetweenthegroups

Magnesiumlevels	ControlNumber%		Group-IINumber%		Group-IIINumber%	
1.0-1.8	25	(12.5%)	105	(52.5%)	125	(62.5%)
1.8-2.5	168	(84%)	90	(45%)	75	(37.5%)
>2.5	7	(3.5%)	5	(3%)	0	(0%)

Table8: ComparisonofZincDistributionbetweenthe groups

Zinc Levels	Control Number	(n=200) %	Normodiabetic(n=200) Number%		Group-III(n=200) Number%	
<60.	0	0%	06	3%	21	10.5%
60-120	195	97.5%	194	97%	179	89.5%
>120	05	2.5%	00	0%	00	0%

Table9: CorrelationofMagnesiumwithother observations

X-axis numbers	Observation	Pearsoncorrelation	P-value
1	Fastingblood glucose	-0.218*	0.001
2	HBA1C	-0.182*	0.010
3	HOMA-IR	-0.167*****	0.014
4	Zinc	0.155*****	0.026
5	Copper	-0.162*****	0.018
6	Vitamin-D3	0.164*	0.017
7	MDA	-0.289#	0.001
8	PCR	-0.141*****	0.03

(**p*<0.05significant,#*p*<0.001 significant)

Table10: CorrelationofZincwithother observations

X-axis numbers	Observation	Pearsoncorrelation	P-value
1	Fastingblood glucose	-0.192*	0.006
2	HBA1C	-0.145*	0.039
3	HOMA-IR	-0.171*****	0.014
4	Magnesium	0.155*	0.026
5	Copper	-0.165*	0.018
6	Vitamin-D3	0.242#	0.001
7	MDA	-0.260#	0.001
8	PCR	-0.166#	0.002

(**p*<0.05significant,#p<0.001 significant)

DISCUSSION

A series of events leading to micro and macrovascular problems in diabetes, including retinopathy, nephropathy, neuropathy, cardiovascular illnesses, and peripheral vascular diseases, are set off by chronic hyperglycemia. These consequences impact numerous tissues and organs.[17]The etiology of type 2 diabetes is complicated and still unknown. Diabetes and associated consequences might develop more quickly for a variety of reasons. Genetics, age, weight, food, exercise, and pregnancy are a few of them.[18]

Magnesium is a necessary element that is vital to both the action of insulin and the metabolism of carbohydrates in general. It contributes to insulin binding, activity, and secretion at several levels. Tyrosine kinase activity is influenced by magnesium, which affects how insulin and glucose are metabolized.[19] It is crucial to the actions of several enzymes involved in the oxidation of glucose.

In this investigation, we discovered that the serum magnesium level in both diabetes groups was substantially lower (P<0.001) than in the control group, and hypomagnesemia was reported in 63% of the study patients compared to 12% of the control group, suggesting that hypomagnesemia is more common in diabetics.This is in line with earlier research that shown type 2 diabetes patients had lower mean plasma magnesium levels and a greater incidence of hypomagnesemia.[20,21]

Earlier studies have reported decreased magnesium levels in diabetesbydemonstrating reduced levels of Mg in plasma, serum, and erythrocytes.[22,23] The precise cause of behind hypomagnesemia in type-2 diabetes mellitus is not completely clear, but possible causes may include osmotic diuresis causing high renal excretion of magnesium,impaired magnesium absorption and distributionof magnesium from plasma to redblood cells caused by insulin effect. [24]

Type 2 diabetes has been attributed to hypomagnesemia, and glycemic control and FBS seem to be negatively impacted by magnesium shortage. Plasma magnesium levels were shown to be statistically significantly correlated negatively with fasting plasma glucose (r=-0.229; P=<0.0001) and HbA1c (r=-0.193; P=0.010).

In addition, studies also imply a link between magnesium deficiency and insulin resistance. Mg deficiency decreasesinsulin sensitivity viaanalteration oftheinsulin-receptor associated tyrosine kinase intype-2diabetes.[14]Thisissupportedby present results when serum mg levelswere negatively correlatedwithHOMA-IR(r=-0.172.P=0.014).

According to our research, type 2 diabetes patients with microvascular difficulties had serum magnesium levels that were considerably lower than those of T2DM patients without microvascular issues (P=0.025 and Mean difference 0.67; Table 4.24& Figure 4.8). Similar findings were reported by Baig *et al*. (2012), who hypothesized that hypomagnesemia would cause microvascular problems by inhibiting the action of the prostacyclin receptor, resulting in an imbalance between the effects of prostacyclin and thromboxane. This imbalance has a strong atherogenic potential.

We found a statisticallysignificant negative correlation between serum Mg level with urinary albumin/creatinine ratio (r= -0.140; P=0.004) This is consistent with the results of Arpacietal,2015andCoricaF*etal*.,2006.[25,26]

A highly significant correlation was found between the variables in our study, which included malondialdehyde as the dependent variable and magnesium as the independent variable. This suggests that a deficiency in magnesium may contribute to increased oxidative stress and decreased antioxidant potential. It is consistent with the prior research conducted in 2002 by Hans CP *et al*.[27]

In their investigations, Deng *et al*. (2006) and Kelishadi R *et al*. (2014) examined the possible roles that magnesium consumption, either on its own or in combination with vitamin D intake, may play in maintaining vitamin D status. Additionally, we discovered in this investigation that serum magnesium and vitamin D3 positively correlated.[28,29]

Zinc is a necessary trace element that aids in the production, storage, and secretion of insulin as well as maintaining the hexameric form of insulin's structure.[30]

Furthermore, zinc has a dynamic function as a "cellular second messenger" in the regulation of glucose homeostasis and insulin signalling, as demonstrated by recent investigations. The human body has a strictly controlled concentration of zinc, and disruptions in zinc homeostasis have been linked to a number of illnesses, including diabetes mellitus.[31]

Numerous studies have documented the relationship between zinc metabolism and the metabolic and biochemical elements implicated in the pathophysiology of type 2 diabetes mellitus. The majority of them stated that low zinc status had accelerated the development of diabetes.[20]

The result of the present study explain zinc levels in diabetics(both with complicationsand without complications) werelowerthan the control group. This finding wasin agreement withthe findingsof MeenakshiT,2016;Puri*etal*.,2013,Walter*et al*.,1991.[32,20,33]The fact that hyperglycaemia in diabetes is typically linked to hyperzincuria and increased urine zinc loss, which causes reductions in total body zinc, might be one explanation for this. Disrupted zinc metalloenzyme metabolisms and aberrant zinc binding to tissue proteins, which results in hyperzincuria, are two more potential reasons. However, contradictory results were also noticed in other studies according to which there was no significant difference in serum zinc levels among the type 2 diabetic patients.[34]

Zinc demonstrated a strong negative correlation with FBS, HbA1c, and HOMA-IR. These results are consistent with those of Kamal *et al*. (2009). This is explained by zinc's function in regulating insulin synthesis through pancreatic tissue and glucose utilization through muscles and fat cells. Hyperglycemia results from zinc deficiency, and the ability to synthesize and produce insulin, as well as consume glucose, is compromised. [35]

Our investigation revealed a significant negative connection between Zn and MDA ($r < 0.001$). This conclusion is consistent with that of Kamal *et al*. (2009) who concluded that Zn metabolism plays a role in antioxidant mechanisms. It might also be supported by the presence of Zn in numerous antioxidant enzymes, including catalase, peroxidase, and dismutase. As a result, zinc deficiency may cause oxidative stress, which is responsible for the majority of diabetic problems.[35]

Zinc is a retinal protective factor, 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 the neovascularization and exudation(MiaoX*etal*.,2014).Thisissupportedbyourpresentresults wherewefoundlowerzinclevelsinDRpatientsthaninthosewithoutDR.Suggestingthatzinc might play an important role in the development of DR.[36]

Our study also found that individuals with increased urine ACR had considerably lower zinc levels, with those with an ACR greater than 300 mg/g having the lowest zinc levels in serum. Our findings also indicated that serum zinc levels were an independent risk factor for DN.Our study also found that zinc levels were considerably lower in diabetic neuropathy patients. Diabetic neuropathy was mostly caused by oxidative stress.

Conclusion

The mean blood magnesium and zinc levels were considerably lower in type 2 diabetics with problems compared to those without difficulties and the control group. These levels declined considerably during the course of diabetes. A momentous negative correlation was showed by Serum levels of Magnesium and Zinc with FBS,HbA1c,HOMA-IR, andMDA. Current observations recommend that Screening panel in the risk detection and progression of diabetes should include the estimation of serum zinc, magnesium and copper estimation. Supplementation of Mg and Zn and recurrent one-to-one care of levelsof both minerals in type 2 diabetic patients.

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