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ORIGINAL ARTICLE

The Impact of the Serum Level of Trace Elements on the Severity of Neuropathy in Type 2 Diabetic Patients

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ABSTRACT

Background: This study was designed in order to estimate the alterations in the levels of serum zinc, magnesium, and copper in patients with type 2 diabetes mellitus with diabetic peripheral neuropathy in comparison to those without neuropathy and their relation to duration of diabetes and glycemic control.

Method: 71 patients with Type 2 diabetes were recruited and divided into 2 groups: **Group 1:** 47 patients with diabetic neuropathy and **Group 2:** 24 patients with type 2 diabetes without diabetic neuropathy. The presence and absence of neuropathy was assessed using neuropathy disability score and neuropathy symptom score. Full history and examination were taken from all participants. Neuropathy symptom score and neuropathy disability score were assessed. A blood sample was taken for measuring A1c, serum zinc, magnesium, and copper

Results: There was a significant difference between both groups as regard duration of diabetes and serum copper level (P value< 0.05). After performing linear regression analysis, the duration of Diabetes was the significant risk factor for diabetic neuropathy symptoms. On the other hand, there was no significant difference between the 2 groups as regard serum zinc, serum magnesium, glycated hemoglobin, age, sex, and hypertension.

Conclusion: High serum CU and low serum Mg and Zn are not independent risk factors for diabetic neuropathy as previously thought, and its alterations in diabetic patients may be due to the hyperglycemia itself

Key words: Zinc, Diabetic neuropathy, Magnesium, Trace elements

INTRODUCTION

Diabetes mellitus (DM) is a cluster of metabolic disorders characterized by hyperglycemia. There are varieties of polygenic disorder caused by completely different genetic reactions and environmental factors that causing DM. Hyperglycemia is caused by either defect in insulin secretion, a reduction in glucose utilization or increase in the production of glucose resulting in end organ damage that is accompanied by different necessary issues for patients and health care system [1]. There are different complications of DM which are divided into vascular and non-vascular complications. The vascular complications of Diabetes are further sub divided into micrmicrovascularch include (retina, nerves, and glomeruli) and macromacrovascularlications [coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular disease]. The accepted theory that proves these complications is the decrease in intra cellular zinc and zinc dependent antioxidant enzymes and increase in intracellular oxidants and free radicals [2]

Hayee et al. conducted a double-blind study and showed that serum zinc levels at the start of the study were significantly lower in diabetic patients with peripheral nerve dysfunction in comparison to healthy subjects. After 6 weeks of zinc supplementation there was an improvement of fasting plasma glucose, 2-hour (2-h) post prandial blood glucose plasma glucose and improvement in motor nerve conduction velocity in comparison to those receiving placebo. They concluded that zinc therapy may lead to better glycemic control and improvement in Diabetic peripheral neuropathy (DPN) [3]The relation between decreased magnesium level and diabetic retinopathy was reported in two cross-sectional studies that involved both type 1 and type 2 diabetic subjects and revealed that diabetic patients had lower serum Mg levels in comparison to those without diabetes and the degree of retinopathy was inversely related to the serum magnesium level. The kidney plays a

major role in magnesium homeostasis and in maintenance of magnesium concentration [4]

The increase in Copper (Cu) ion levels in patients with diabetes mellitus may be attributed to hyperglycemia that may stimulate glycation and release of copper ions and this accelerates the oxidative stress resulting in formation of Advanced Glycation End products, that are involved in the pathogenesis of diabetic complications [5]

Thus, there may be a change in the levels of serum zinc, magnesium, and copper in patients with type 2 DM [6].The aim of this study was to investigate the correlation between the levels of some trace elements such as (Serum Zinc, serum Magnesium and serum Copper) and the severity of diabetic neuropathy in type 2 diabetic patients.

METHODS

Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Mansoura University. IRB Code Number: R/ 18.03.99. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. This is a Cross Sectional Study that was done in Mansoura Specialized Medical Hospital, Mansoura University, Egypt. Subjects were 71 patients, after taking informed consents, with type 2 diabetes recruited from diabetes and diabetic neuropathy clinic in Mansoura Specialized Medical Hospital, from May 2017 to December 2017 divided into 2 groups. **Group 1**: Patients with type 2 diabetes and diabetic neuropathy including 47 patients and **Group 2**: Patients with type 2 diabetes without diabetic neuropathy including 24 patients.

Inclusion criteria: Type 2 diabetes patients from 18 to 70 years with and without diabetic neuropathy

Exclusion criteria: Critically ill diabetic patients and diabetic patients on intra venous fluids or total parenteral nutrition were excluded from the study.

Data was collected as full history and examination including (age, sex, Body mass index, duration of diabetes and its regimen of treatment and presence or absence of Hypertension), presence or absence of neuropathy was assessed using Neuropathy Disability Scoring System) and Neuropathy Symptom Score System [7]

Neuropathy symptom score (NSS)

The Neuropathy symptoms score (NSS) consists of five questions. The total maximum abnormal symptoms score are 9 points [7]

- Aching /cramping/ fatigue (1p) or tingling /numbness / Burning (2p) feeling in the lower limb.
- Presence of the Symptoms in the calf (1p) or in the feet(2p).
- Symptoms exacerbation during night (2p) or present similarly at night and day (1p).
- The case awakes from sleep because of The symptoms (1p).
- Standing (1p) or Walking (2p) maneuvers reduces the symptoms.

A total symptom score of:

- 3-4points is regarded as mild symptoms.
- 5-6 points is regarded as moderate.
- 7-9 points as severe symptoms

Neuropathy disability score (NDS:[7]

- Tendon Achilles reflex: the reflex hammer broad end is applied at the tendo Achilles. Jerk with re information (1p), no jerk (2p).
- Perception of Vibration: A 128-Hz vibration fork is applied longitudinally on the 1st toe 3 times. The case is asked to tell whether he felt the vibration of the fork or not. 2 of 3 wrong answers are regarded as an incorrect answer(1p) or ٢ of 3 right answers are regarded as a correct answer (0p),
- Cold sponge Thermal sensation: 1 room temperature and 1cold sponge is put on the dorsal surface of the foot. The cases are asked to tell which of them is normal or cold, incorrect answer (1p. correct answer (0p).
- Pinprick (Tactile sensation): The reverse end of the tendon hammer and turning fork, dull or sharp, incorrect answer (1p), correct answer (0p).

A total symptom score of

- 3-5 points is regarded as mild disability.
- 6-8 points is regarded as moderate disability.
- 9-10 points is regarded as severe disability.

All the 71 patients were subjected to a non-fasting blood sample and the following was measured by end point spectrophotometry (Serum Zinc, Serum Magnesium, Serum copper and Glycated hemoglobin)

- **HbA1c level detection** [8]

Assay principal:

This method utilizes the interaction of antigen and antibody to determine the HbA1c in whole EDTA blood. HbA1c in test samples is absorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction) and gives agglutination. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

Assay procedure: Two ml hemolysis reagent was dispensed into a test tube. Then 20 ul of well mixed whole EDTA blood (Samples, Standards and Controls) were placed into the test tube and mixed until complete lysis is evident.

375 ul of R1 reagent was added to 5 ul of sample and standard and then was mixed and incubated for 2 minutes. Then 75 ul of R2 reagent was added to each tube.

Mix and read absorbance (A1), Incubate for 5 minutes, and read absorbance (A2

Samples and standard were read at wavelength 650 nm, 37º c using Ribonic spectrophotometer.

Calculation: Reference curve was generated using HbA1c standard set. Determine D absorbance of the sample and each standard as following: D absorbance of sample = (A2 - A1) sample D absorbance of each standard = (A2 - A1) for each Standard Plot the calibration curve and obtain the result.

- **Measurement of serum Copper, Zinc and Magnesium**

- **Serum Magnesium by Biomed Kits**

Samples and standard were read at wavelength 520 nm, 37º c using Riboa nic spectrophotometer.

Calculation:

Serum Magnesium mg/dL = ((A) Sample / (A) Standard) × 2.5 (2.5 is the standard value)

Reference Range: 1.9-2.5mg/dl

- **Serum Zinc by Spectrum Kits**

Samples and ststandardsere read at wavelength 560 nm, 37º c using Ribonic spectrophotometer.

Calculation: Serum Zinc mg/dL = ((A) Sample / (A) Standard) × 200 (200 is the standard value)

Reference Range: 72.3-127ug/dl

- **Serum Copper by Biomd Kits**

Samples and sstandardswere read at wavelength 580 nm, 37º c using Ribonic spectrophotometer.

Calculation:

Serum Copper µg/dL= ((A) Sample / (A) Standard) × 100 (100 is the standard value)

Reference Range: Adult male 70-140 µg/dL Adult Female 76-152 µg/dL

STATISTICAL ANALYSIS

The collected data were analyzed by using the statistical package for social sciences (SPSS/PC/VER 20).

A 2 tailed P values less than 0.05 were considered significant. Continuous data were presented as mean ± standard (SD deviation for parametric measures and median (minimum-maximum) and inter quartile range (IQR)for non-parametric measures and categorical data were presented as number and (%).All correlations were done using **Pearson** for parametric data and **Spearman** for non-parametric data correlation method.

RESULTS

Seventy-one patients with type 2 diabetes recruited from diabetes clinic and diabetic neuropathy clinic in Specialized Medical Hospital, from May 2017 to December 2017 were enrolled in our study. They were divided into 2 groups. **Group 1**: Patients with type 2 diabetes and diabetic neuropathy including 47 patients and **Group 2**: Patients with type 2 diabetes without diabetic neuropathy including 24 patients as control group.

Analysis of the demographic data showed that the mean age of type 2 diabetic patient with neuropathy was (56.93±7.45) and type 2 diabetic patient without neuropathy was (54.62±9.14). In type 2 diabetic patient with neuropathy (23.4%) were male and (76.6%) were female. In diabetic patient type 2 without neuropathy (16.7%) were male and (83.3%) were female as presented in (Table 1). There was a significant difference between both Groups according to the duration of diabetes (P value =0.001) and serum copper level (P=0.025 value). On the other hand, there was no significant statistical difference between both groups according to Age, sex, BMI, hypertension, serum Zinc, serum Magnesium and Glycated hemoglobin (p value > 0.05) (Table 1).

After Multivariate Logistic regression analysis only the duration of diabetes was the significant risk factor for the diabetic neuropathy symptoms **OR =1.15 (p value = 0.004)**. (Table 2). After doing correlation between duration of diabetes and trace elements (Serum CU, Serum Zn and Serum Mg) the results were non-significant **(p value >0.05).** On the contrary there was significant positive correlation between duration of diabetes and Glycated hemoglobin **(p value<0.001)** (Table 3). Also Glycated hemoglobin was positively correlated to Serum CU **(p value =0.029)** and negatively correlated to Serum Zn **(p value =0.041)** (Table 4).

The area under receiver operating characteristic curve (Roc) for prediction of Diabetic neuropathy by Serum CU is 0.664 (Table 5) (95% confidence interval): 0.522-0.806. By using Roc curve Sensitivity, Specificity. PPV, NPV and accuracy at cutoff value of Serum CU>201 are (87.2%,50%,77.4%,66.7 and 74.6% respectively) (Table 5) (Figure 1)

Table (1): Demographic and clinical data among the studied groups

Items	Diabetic neuropathy (n=47)		No diabetic neuropathy (n=24)		Test of significance	p-value
	No	%	No	%		
Sex					χ^2=0.433	0.511
Male	11	23.4%	4	16.7%		
Female	36	76.6%	20	83.3%		

Items	Diabetic neuropathy (n=47)		No diabetic neuropathy (n=24)		Test of significance	p-value
	No	%	No	%		
Age/years Mean ± SD	56.93±7.45		54.62±9.14		t=1.14	0.257
BMI					χ^2=0.33	0.560
>30	28	59.6%	16	66.7%		
≤30	19	40.4%	8	33.3%		
Duration of DM Median (IQR)	10 (5-16)		4 (1-10)		Z=3.38	0.001*
DPN					χ^2=11.41	0.001*
yes	17	36.2%	0	0.0%		
no	30	63.8%	24	100.0%		
HTN					χ^2=1.32	0.250
yes	32	68.1%	13	54.2%		
no	15	31.9%	11	45.8%		
Serum ZN Mean ± SD	102.80±16.65		95.62±27.19		t=1.37	0.173
Serum Mg Median (IQR)	3 (1.9-3.6)		2.4 (1.9-3.77)		Z=0.128	0.898
Serum CU Median (IQR)	400 (272-511)		234 (176-429)		Z=2.24	0.025*
HA1c Mean ± SD	9.65±1.97		9.52±2.11		t=0.254	0.800

t: student t-test, χ^2: chi square test, Z: Mann Whitney test

Table (2): Multivariate Logistic regression analysis of independent predictors of Diabetic neuropathy

Independent predictors	Logistic regression analysis		
	B	OR (95% CI)	p-value
Duration of DM	0.144	1.15 (1.04-1.27)	0.004*
Serum CU	0.00	1.0 (0.99-1.01)	0.788

Table (3): Correlation between Duration of DM, trace elements and Glycated hemoglobin

	Duration of DM	
	R	p-value
Serum CU	0.057	0.637
Serum Zn	0.135	0.262
Serum Mg	-0.177	0.139
Glycated hemoglobin	0.461	<0.001*

Table (4): Correlation between Glycated hemoglobin and trace elements

	Glycated hemoglobin	
	R	p-value
Serum CU	0.260	0.029*
Serum Zn	-0.243	0.041*
Serum Mg	-0.002	0.984

Table (5): Roc curve for prediction of Diabetic neuropathy by Serum CU

AUC	95% CI		Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
	Lower	Upper						
0.664	0.522	0.806	>201	87.2%	50%	77.4%	66.7%	74.6%

AUC :area under curve PPV: positive predictive value NPV :negative predictive value CI: confidence interval.

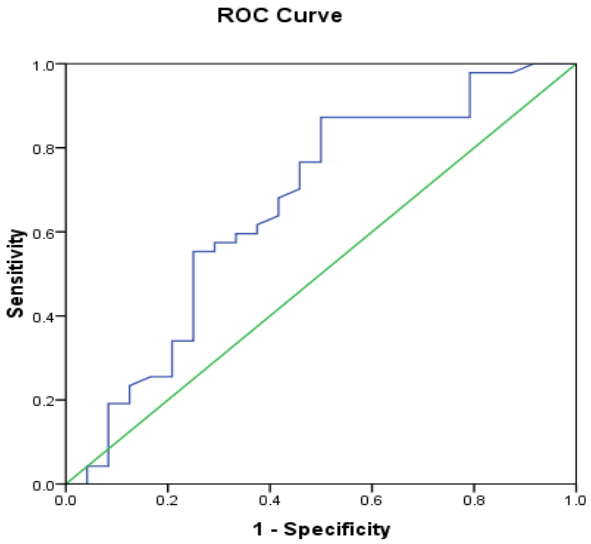

Fig. (1): Roc curve for prediction of Diabetic neuropathy by Serum CU

DISCUSSION

This cross-Sectional Study was designed to estimate the alterations in the levels of the trace elements; serum zinc, magnesium, and copper in patients with type 2 diabetes with diabetic neuropathy in comparison to those without neuropathy and their relation to the duration of diabetes and glycemic control. It was done in Mansoura Specialized Medical Hospital, Mansoura University, Egypt.

Diabetes mellitus is a metabolic disorder characterized by presence of oxidative stress which has a great impact on the pathogenesis of the complications of Diabetes [9]. Trace elements are required in small quantities for specific functions in the body, moreover a link was shown between trace elements and glucose homeostasis. It was suggested that imbalances of several trace elements may affect glucose and insulin metabolism. On the other hand, chronic hyperglycemia may cause significant alterations in the status of some trace elements [10]. In addition, development of diabetic complications was related to disturbances in trace element status and increased oxidative stress [11]

In the current study, Serum copper was higher than reference range in both groups this may be explained by that a Transition metal like copper has the greatest binding capacity to glycated proteins. Moreover, there is an elevation of serum concentrations of copper and ceruloplasmin in patients with type 2 DM [12] However, Serum copper was significantly higher in diabetic patients with neuropathy than those without neuropathy (P=0.025), this result was supported by ***Meenakshi et al. [6]*** who revealed that serum copper levels was higher in type2 diabetes mellitus patients with microvascular complications than who without microvascular complications (p<0.001).This may be due to the fact that incubation of ceruloplasmin with glucose which causes fragmentation and time dependent release of its bound Cu2+ , which then appears to participate in a Fenton type reaction to produce hydroxyl radicals [13]

The redox active metal ions (Cu2+ and Fe3+) have been implicated in catalyzing the autoxidation of glycolaldehyde and generation of hydroxyl radical, leading to production of glyoxal and associated α -oxoaldehyde derived AGE (Advanced glycosylation end products) formation [4] Which have an etiological role in the development of diabetic complications and other diseases [15]

Regarding serum Mg, it was slightly higher than reference range in both groups (Median (IQR): 3 (1.9-3.6), 2.4 (1.9-3.77) in patients with and without diabetic neuropathy respectively) this may be attributed to monthly prescribed laxatives or antacids from our clinic ,However we found no significant difference in Mg level between those with neuropathy and those without(p=0.898) ,which is in agreement with ***Xuet et al.*** who found that in patients with diabetic complications, the serum Mg level was independent of the presence of either diabetic nephropathy, retinopathy, or peripheral neuropathy [16] but in contrast to ***Sales***

et al. who suggested an association between hypomagnesemia and other diabetic complications including neurological abnormalities and dyslipidemia [17]

For serum Zn, it was within reference range in both groups with no significant difference found in those with and without diabetic neuropathy (p=0.173) which was like ***Diwan et al.*** results [18] and may be explained by that lower levels of Zn were linked to increased urinary excretion of Zn, especially in patients with diabetic nephropathy [19]. However, our findings were contradictory to ***Walter et al. and Meenakshi et al.*** findings [6,20].

Glycated hemoglobin in our study was positively correlated to Serum CU **(p value =0.029)** and negatively correlated to Serum Zn **(p value =0.041)** in agreement with ***Farid and Abulfaraj*** study done in Saudi Arabia who found Positive correlations between levels of HbA1c and Cu (P= 0.002), and negative correlations with Zn (P = 0.01) [2١]. On the other hand, Glycated hemoglobin in our study was not correlated with serum Mg ***(p value > 0.05)*** which is contrary to ***Yossef et al.*** study of the relation of serum magnesium level to microvascular complications in patients with type 2 diabetes mellitus that showed statistically significant negative correlation between serum Mg level and HbA1c (P=0.0001) [22]

In the current study, there was no significant correlation between duration of diabetes and trace elements (serum CU, serum Zn and serum Mg) **(p value >0.05), matching** with a study undertaken by a group of investigators, in which no statistically significant change was found in level of (Zn, Mg, and Cu) with the duration of diabetes [23]

Future research on larger sample size and a different category population will be needed**.**

CONCLUSION

High serum CU and low serum Mg and Zn are not independent risk factors for diabetic neuropathy as previously thought, and its alterations in diabetic patients may be due to the hyperglycemia itself.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest.

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