

# Evaluation of serum ferritin and some metal elements in type 2 diabetes mellitus patients: comparative cross-sectional study

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**Background:** The chronic hyperglycemia of diabetes has been associated with an imbalance of some trace metal elements in the blood sample of type 2 diabetes patients.

**Aim:** To evaluate the status of serum ferritin and some selected metal elements among type 2 diabetes mellitus (T2DM) patients.

**Methods:** Facility-based comparative cross-sectional study was conducted from February 15, 2015 to October 30, 2015, at Jimma University Specialized Hospital, Ethiopia. A total of 428 type 2 diabetes and nondiabetes study subjects were recruited to the study. After overnight fasting, 10 mL of venous blood samples were taken for biochemical and trace metal element analysis. Data were entered into EpiData version 3.5.1 and exported to SPSS version 20 for Windows for analysis.

**Results:** Serum concentration of Zn<sup>+2</sup>, Mg<sup>+2</sup>, Cr<sup>+3</sup>, ferritin, and Fe<sup>+3</sup> in patients with type 2 diabetes was significantly lower ( $p < 0.0001$ ) than nondiabetes patients. In contrast, serum Cu<sup>+2</sup> was significantly higher ( $p < 0.0001$ ) in type 2 diabetes patients than nondiabetics. In addition, significant differences were not seen in both groups with regard to serum Mn<sup>+2</sup>, Ca<sup>+2</sup>, and Po<sub>4</sub><sup>-3</sup>. Waist-to-hip ratio (WHR), serum Fe<sup>+3</sup>, ferritin, and Mn<sup>+2</sup> were significantly higher among oral hypoglycemic agent users of type 2 diabetes patients than the injectable insulin users. Serum Zn<sup>+2</sup> had significant positive correlation with serum Mg<sup>+2</sup> ( $r = 0.738$ ), Cr<sup>+3</sup> ( $r = 0.233$ ), Ca<sup>+2</sup> ( $r = 0.238$ ), and Po<sub>4</sub><sup>-3</sup> ( $r = 0.222$ ). In addition, serum Zn<sup>+2</sup> had shown significant and negative correlation with body mass index (BMI,  $r = -0.331$ ), WHR ( $r = -0.340$ ), and fasting blood glucose (FBG,  $r = -0.186$ ). Likewise, serum Mg<sup>+2</sup> and Po<sub>4</sub><sup>-3</sup> are significantly and negatively correlated with BMI, WHR, and FBG.

**Conclusion:** The imbalance of trace metal elements in the blood sample of diabetes is uncertain. Thus, we recommend a prospective cohort study to find out the principal factors behind the problem.

**Keywords:** diabetes, ferritin, trace metal elements, hyperglycemia, insulin, metal

## Background

Trace metal elements are naturally occurring inorganic elements and have been identified as potential candidates for improving metabolic disorders, because they are involved in prosthetic groups of many proteins and cofactors of many enzymes.<sup>1</sup> In the last decades, many research studies have reported frequently the direct correlation of diabetes and an imbalance of some trace metal elements.<sup>2,3</sup> The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.<sup>4</sup> Mortality attributable to diabetes accounts for 2%–3% of deaths in poor countries and by 2030, ~8% of the adult population is estimated to have diabetes.<sup>5</sup> According to the International Diabetes Federation, there were over 1.33 million

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417

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cases of diabetes in Ethiopia during 2015, and the number of deaths due to diabetes was 23,145.<sup>6</sup> Owing to the increasing prevalence of diabetes, multidisciplinary study aimed at preventing and treating diabetes is one of the worldwide research priorities.<sup>7</sup> The burden of diabetes and other chronic noncommunicable diseases in Ethiopia has been grossly overlooked by concerned bodies as a consequence of government's intense focus on communicable disease and malnutrition agendas. The few studies done on diabetes were confined on the issue of quality of care and patients satisfaction in the follow-up clinics. Thus, research on diabetes in this country can only be described as inadequate. To remedy this situation, research on the causation aspect of the disease needs to be conducted. To the best of our knowledge, the relationship between diabetic hyperglycemia and relevant blood chemistry and trace metal elements has not yet been investigated in Ethiopia. The study results may contribute to the development of evidence-based decision and intervention strategies to enhance a healthier lifestyles and better diabetes care. Therefore, this study is aimed to evaluate the status of serum ferritin and some selected trace metal elements, such as zinc ( $Zn^{+2}$ ), magnesium ( $Mg^{+2}$ ), chromium ( $Cr^{+3}$ ), iron ( $Fe^{+3}$ ), copper ( $Cu^{+2}$ ), manganese ( $Mn^{+2}$ ), calcium ( $Ca^{+2}$ ), and phosphorus ( $Po_4^{-3}$ ), among type 2 diabetes mellitus (T2DM) patients at Jimma University Specialized Hospital (JUSH), Ethiopia.

## Subjects and method

### Study subjects and design

Comparative cross-sectional study was used among 214 patients with T2DM and 214 age, sex, and educational level matched nondiabetes individuals who were in the outpatient department at JUSH using consecutive sampling technique. The study was conducted in JUSH from February 15, 2015 to October 30, 2015. JUSH is one of the oldest public hospitals in the country, located about 350 km southwest of the capital, Addis Ababa.

### Inclusion and exclusion criteria

All patients aged 20–79 years with a history of diabetes more than 3 years from their diagnosis at the time of data collection were included. Subjects with a history of 1) pregnancy and lactation, 2) use of any drugs affecting electrolytes at the time of data collection, 3) taking nutritional supplements, such as magnesium-containing laxatives, 4) chronic disorders of the liver, kidney, and cardiovascular system, 5) endocrine disorders, 6) established psychiatric disorder and on antidepressant and/or antipsychotic therapy, 7) HIV/AIDS, 8) malignancy,

and 9) substance abuse were excluded from the study. Study subjects in the nondiabetes group were included and excluded based on the same criteria as stated earlier.

### Ethical consideration

Prior to participation for the study, written informed consent was obtained from the study subjects. The purpose of the study was clearly described to the study subjects including the benefits and risks of the study. Information concerning the study subjects was kept confidential. The specimen collected from the study subjects was only analyzed for the intended purposes. The study was performed in accordance with the ethical principles outlined in the Helsinki Declaration<sup>8</sup> and guidelines for Good Clinical Practice.<sup>9</sup> Ethical approval was obtained from the Jimma University ethical review board. Letter for permission to conduct the study was obtained from JUSH medical director office.

### Data collection

Each study subjects was interviewed and completed the questionnaire in the presence and assistance of trained data collectors (Medical Doctors and Laboratory Technologist). Information concerning age, sex, family history of diabetes, history of hypertension and dyslipidemia, and other information was collected using questionnaire. Anthropometric measures included height and weight; waist and hip circumference; respiratory rate; examination of extremities, eye, skin, and sensory-motor functionality and all routine investigations were measured according to standard protocols. Blood pressure and heart rate were measured three times every 2 minutes after an initial 10-minute rest period.

### Specimen collection and storage

Overnight fasting venous blood (10 mL) was taken from the median cubital vein of subjects using sterile disposable plastic syringe and preserved in ethylenediamine tetraacetic acid (EDTA.K<sub>3</sub>)-containing vacuum tubes (Weihai Hongye, Weihai, People's Republic of China) and plain vacutainer tubes (Shandong Branden Medical Devices Co., Ltd, Beijing, People's Republic of China). Samples in the EDTA.K<sub>3</sub>-containing vacuum tubes were sent to JUSH Laboratory Department for fasting blood glucose (FBG) determination. Samples in the plain vacutainer tubes were immediately brought to the Medical Physiology Laboratory of Jimma University for processing. These samples were centrifuged at 3000 rpm for 10 minutes, and the serum (3.2 mL) was transferred to labeled 3.6 mL Nunc™ cryogenic tubes (NUNC INC) and stored at  $-20^{\circ}C$  until analysis.

## Specimen preparation and analysis

Fasting plasma glucose was determined by glucose hexokinase method (ABX Pentra Glucose hexokinase 13CP kit). Serum C-reactive protein (CRP; ABX Pentra CRP 12CP kit) and ferritin (ABX Pentra ferritin 5CP kit) were determined by radioimmunoassay method. Both clinical methods were performed using automated chemistry analyzer ABX Pentra 400 (HORIBA Medical Diagnostics Instruments & Systems, Montpellier, France). Trace element levels were determined using wet acid digestion method.<sup>10–12</sup> All sample containers were meticulously washed with nonionic detergents solution and rinsed triply with distilled water and dried in the oven at 80°C for 24 hours. Each sample was mixed with 3 mL concentrated nitric acid and hydrogen peroxide [ $\text{HNO}_3\text{--H}_2\text{O}_2$ ] solution (2:1, v/v) in a beaker for 10 minutes and heated at 120°C on a hot plate until complete digestion had taken place. The beaker was loosely covered by 150 mm Pyrex watch glass to avoid contamination from the air. Then, the samples were brought to 5.4 mL and allowed to cool at room temperature while continuously adding triple distilled water to maintain the volume. After cooling down, the digested samples were centrifuged at 10,000 rpm for 30 seconds and the organic supernatant aspirated directly into the flame. Finally, the clear samples were analyzed for trace elements using atomic absorption spectrophotometry (Shimadzu AA-670, Kyoto, Japan).

## Data quality assurance

To ensure the quality of the data, good laboratory practice was followed in all stages of the work. All reagents used were checked for their expiry date and used according to the manufacturer's instructions.

## Data analysis

Data were entered into Epi-info version 3.5.1 and then exported to SPSS version 20 (SPSS Inc., Chicago, IL, USA). Independent *t*-test was performed to determine significant differences between type 2 diabetics and nondiabetics in terms of FBG, weight, body mass index (BMI), waist-to-hip ratio (WHR), blood pressure (systolic and diastolic), ferritin, and trace metal elements. Levene's test for homogeneity of variances was calculated in order to accept or reject the null hypothesis. To determine possible relationships between parameters, Pearson's correlation coefficient (*r*) was used. All variables with *p*-value less than 0.05 were considered as statistically significant.

## Reference ranges

The reference ranges used to determine serum ferritin, trace metal elements, FBG, and CRP were obtained from different

literature<sup>13–15</sup> and presented as follows:  $\text{Zn}^{+2}$  (75–120  $\mu\text{g/dL}$ ),  $\text{Mg}^{+2}$  (1.6–2.6  $\text{mg/dL}$ ),  $\text{Cr}^{+3}$  (0.05–0.5  $\mu\text{g/L}$ ),  $\text{Fe}^{+3}$  for male (65–165  $\mu\text{g/dL}$ ) and for female (50–70  $\mu\text{g/dL}$ ), ferritin for male (20–250  $\mu\text{g/L}$ ) and for female (10–120  $\mu\text{g/L}$ ),  $\text{Cu}^{+2}$  (70–140  $\mu\text{g/dL}$ ),  $\text{Mn}^{+2}$  (0.4–1.1  $\mu\text{g/L}$ ),  $\text{Ca}^{+2}$  (4.64–5.28  $\text{mg/dL}$ ),  $\text{PO}_4^{-3}$  (2.5–4.5  $\text{mg/dL}$ ), FBG ( $\leq 126$   $\text{mg/dL}$ ), and CRP ( $\leq 10$   $\text{mg/dL}$ ).

## Results

It is common scientific knowledge that there is a strong association between type 2 diabetes and increased FBG, BMI, waist circumference, and WHR. The results of the present study are consistent with this fact. As shown in Table 1, FBG, BMI, WHR, and systolic and diastolic blood pressures in type 2 diabetes patients were significantly ( $p < 0.0001$ ) higher than in nondiabetes study subjects.

Study subjects with T2DM had a significant ( $p < 0.0001$ ) lower serum  $\text{Zn}^{+2}$ ,  $\text{Mg}^{+2}$ ,  $\text{Cr}^{+3}$ ,  $\text{Fe}^{+3}$ , and ferritin concentrations than nondiabetics (Table 2). In contrast, the serum  $\text{Cu}^{+2}$  concentration was significantly ( $p < 0.0001$ ) higher in T2DM patients than in nondiabetics. Significant differences were not observed in both study subjects with regard to the serum concentration of  $\text{Mn}^{+2}$ ,  $\text{Ca}^{+2}$ , and  $\text{Po}_4^{-3}$ .

Tables 3 and 4 show the mean differences in age, BMI, WHR, systolic and diastolic blood pressures, fasting plasma glucose, ferritin, and trace metal elements among T2DM study subjects who had been taking hypoglycemic agents and insulin as treatment options.

WHR was significantly higher ( $p < 0.0001$ ) among oral hypoglycemic agents taking T2DM study subjects than

**Table 1** Clinical characterization of the diabetes and nondiabetes study subjects

Tested parameters	Study subjects		t-test	p-value
	Diabetes (n=214)	Nondiabetes (n=214)		
Age (years)	43.18±12.68	43.18±12.68	-0.37	0.712
Duration with diabetes (years)	5.83±3.11	-	-	-
Fasting blood glucose (mg/dL)	162.16±29.53	78.56±10.73	38.92	<0.0001*
Body mass index (kg/m <sup>2</sup> )	26.15±4.53	23.94±4.68	4.93	<0.0001*
Waist and hip circumference (cm)	1.43±0.56	1.04±0.37	8.52	<0.0001*
Systolic blood pressure (mmHg)	134.2±22.62	122.04±18.76	6.05	<0.0001*
Diastolic blood pressure (mmHg)	88.36±13.23	84.14±13.92	3.21	<0.0001*

**Notes:** Data were presented as mean (standard deviation). \* $p < 0.05$  indicates a significant difference. Independent *t*-test.

**Table 2** Levels of ferritin and metal elements in the serum of diabetes and nondiabetes study subjects

Tested parameters	Study subjects		t-test	p-value
	Diabetes (n=214)	Nondiabetes (n=214)		
Zn <sup>+2</sup> (µg/dL)	46.26±21.03	72.41±21.47	-12.72	<0.0001*
Mg <sup>+2</sup> (mg/dL)	0.86±0.88	1.82±0.76	-11.95	<0.0001*
Cr <sup>+3</sup> (µg/L)	0.82±1.42	0.35±0.43	4.57	<0.0001*
Fe <sup>+3</sup> (µg/dL)	55.95±20.99	71.19±25.72	-6.70	<0.0001*
Ferritin (µg/L)	24.96±18.60	34.99±18.53	-5.58	<0.0001*
Cu <sup>+2</sup> (µg/dL)	79.61±27.38	71.67±22.38	3.28	<0.0001*
Mn <sup>+2</sup> (µg/L)	0.65±0.91	0.66±0.45	-0.18	0.853
Ca <sup>+2</sup> (mg/dL)	4.28±1.19	4.36±0.56	-0.83	0.407
Po <sub>4</sub> <sup>-3</sup> (mg/dL)	2.16±0.98	2.27±0.56	-1.40	0.162

**Notes:** Data were presented as mean (standard deviation). \*p<0.05 indicates a significant difference. Independent t-test.

**Table 3** Clinical characteristics among oral hypoglycemic agent and injectable insulin users of diabetes study subjects

Tested parameters	Diabetes treatment options		t-test	p-value
	Oral hypoglycemic agents (n=161)	Injectable insulin (n=53)		
Age (years)	42.57±13.4	44.11±9.9	-0.894	0.373
Fasting blood glucose (mg/dL)	159.88±27.4	169.07±34.5	-1.761	0.82
Body mass index (kg/m <sup>2</sup> )	26.3±4.86	25.65±3.35	1.084	0.281
Waist and hip circumference (cm)	1.5±0.59	1.22±0.43	3.778	<0.0001*
Systolic blood pressure (mmHg)	134.3±24.1	133.9±17.5	0.105	0.928
Diastolic blood pressure (mmHg)	87.2±13.2	91.8±12.8	-2.255	0.025

**Notes:** Data were presented as mean (standard deviation). \*p<0.05 indicates a significant difference. Independent t-test.

**Table 4** Levels of serum ferritin and metal elements among oral hypoglycemic agent and injectable insulin users of diabetes study subjects

Tested parameters	Diabetes treatment options		t-test	p-value
	Oral hypoglycemic agents (n=161)	Injectable insulin (n=53)		
Zn <sup>+2</sup> (µg/dL)	45.8±20.84	47.6±21.76	-0.550	0.583
Mg <sup>+2</sup> (mg/dL)	0.85±0.89	0.89±0.89	-0.216	0.829
Cr <sup>+3</sup> (µg/L)	0.83±1.45	0.78±1.34	0.229	0.819
Fe <sup>+3</sup> (µg/dL)	57.79±20.49	50.37±21.71	2.252	<0.025*
Ferritin (µg/L)	26.72±19.70	19.62±13.58	2.439	<0.016*
Cu <sup>+2</sup> (µg/dL)	81.28±29.06	74.52±20.88	1.56	1.119
Mn <sup>+2</sup> (µg/L)	0.75±0.96	0.35±0.61	3.52	0.001*
Ca <sup>+2</sup> (mg/dL)	4.28±1.28	4.3±0.86	-0.156	0.877
Po <sub>4</sub> <sup>-3</sup> (mg/dL)	2.11±1.07	2.34±0.65	-1.88	0.062

**Notes:** Data were presented as mean (standard deviation). \*p<0.05 indicates a significant difference. Independent t-test.

insulin. Whereas diastolic blood pressure was significantly higher ( $p<0.05$ ) among insulin taking T2DM study subjects than oral hypoglycemic agents (Table 3). Serum iron, ferritin, and manganese levels were significantly higher ( $p<0.05$ ) among T2DM study subjects, who had been taking oral hypoglycemic agent as a treatment option than insulin (Table 4).

Pearson bivariate correlation analysis was applied to investigate the interrelationships of ferritin and trace metal elements and with clinically useful parameters, such as BMI, WHR, and FBG in the serum of T2DM study subjects. Thus, significant positive correlation was detected between serum Zn<sup>+2</sup> with serum Mg<sup>+2</sup> ( $r=0.738$ ), Cr<sup>+3</sup> ( $r=0.233$ ), Ca<sup>+2</sup> ( $r=0.238$ ), and Po<sub>4</sub><sup>-3</sup> ( $r=0.222$ ); serum Mg<sup>+2</sup> with Zn<sup>+2</sup> ( $r=0.738$ ), Cr<sup>+3</sup> ( $r=0.358$ ), and Po<sub>4</sub><sup>-3</sup> ( $r=0.209$ ; Table 5). Serum Zn<sup>+2</sup> also significantly and negatively correlated with BMI ( $r=-0.331$ ), WHR ( $r=-0.340$ ), and FBG ( $r=-0.186$ ). Likewise, serum Mg<sup>+2</sup> and Po<sub>4</sub><sup>-3</sup> are significantly and negatively correlated with BMI, WHR, and FBG (Table 6).

## Discussion

Trace metal elements are very important elements for cellular metabolic reactions, and their deviation from the normal physiologic range may put adverse effect on biological process.<sup>16,17</sup> In our finding, the serum concentration of zinc, magnesium, and chromium in patients with T2DM was significantly lower than the nondiabetes study subjects. The finding is in harmony with previous studies.<sup>18-25</sup> However, some studies showed insignificant difference of serum zinc and magnesium among T2DM patients and nondiabetes study subjects,<sup>26-28</sup> which are inconsistent with the current finding. Zinc and magnesium are essential trace metal elements crucial for the function of more than 300 enzymes.<sup>29-33</sup> The trivalent chromium is often required for the maintenance of normal glucose<sup>34</sup> and fat metabolism.<sup>35</sup> Chromium stimulates insulin signaling pathway and increases glucose transporter protein translocation in muscle cells.<sup>1</sup> However, zinc, magnesium, and chromium deficiency often leads to insulin resistance.<sup>29-37</sup> The exact physiologic mechanism of zinc, magnesium, and chromium deficiency among T2DM patients is unknown, but high urinary loss has been observed as a result of the osmotic effect of glycosuria.<sup>20,25,33</sup> Increased serum iron and ferritin concentrations have been reported to be associated with insulin resistance.<sup>38-45</sup> Our findings on serum iron and ferritin concentration were inconsistent with the previous findings.<sup>38-45</sup> Iron plays an essential role as a cofactor for fuel oxidation and electron transport, but it also has the potential to cause oxidative damage if not

**Table 5** Pearson bivariate correlation analysis on serum ferritin and metal elements among diabetes study subjects

Tested parameters	Zn <sup>+2</sup> (µg/dL)	Mg <sup>+2</sup> (mg/dL)	Cr <sup>+3</sup> (µg/L)	Fe <sup>+3</sup> (µg/dL)	Ferritin (µg/L)	Cu <sup>+2</sup> (µg/dL)	Mn <sup>+2</sup> (µg/L)	Ca <sup>+2</sup> (mg/dL)	Po <sub>4</sub> <sup>+3</sup> (mg/dL)
<b>Zn<sup>+2</sup> (µg/dL)</b>									
<i>r</i>	1	0.738	0.233	0.062	-0.032	-0.296	0.039	0.238	0.222
<i>p</i> -Value	-	<0.0001**	<0.001**	0.367	0.645	<0.0001**	0.572	<0.0001**	<0.001**
<b>Mg<sup>+2</sup> (mg/dL)</b>									
<i>r</i>	0.738	1	0.358	-0.103	-0.042	-0.171	0.079	0.157	0.209
<i>p</i> -Value	<0.0001**	-	<0.0001**	0.134	0.544	0.012**	0.250	0.022	0.002**
<b>Cr<sup>+3</sup> (µg/L)</b>									
<i>r</i>	0.233	0.358	1	0.01	0.031	-0.03	0.068	0.211	0.298
<i>p</i> -Value	<0.001**	<0.0001**	-	0.885	0.653	0.661	0.321	0.002*	<0.0001**
<b>Fe<sup>+3</sup> (µg/dL)</b>									
<i>r</i>	0.062	-0.103	0.010	1	0.665	0.189	0.065	0.020	-0.058
<i>p</i> -Value	0.367	0.134	0.885	-	<0.0001**	0.0001**	0.343	0.772	0.402
<b>Ferritin (µg/L)</b>									
<i>r</i>	-0.032	-0.042	0.031	0.665	1	0.327	0.085	0.024	0.006
<i>p</i> -Value	0.645	0.544	0.653	<0.0001**	-	<0.0001**	0.218	0.732	0.927
<b>Cu<sup>+2</sup> (µg/dL)</b>									
<i>r</i>	-0.296	-0.171	-0.03	0.189	0.327	1	0.275	-0.138	-0.087
<i>p</i> -Value	<0.0001**	0.012**	0.661	<0.0001**	<0.0001**	-	<0.0001**	0.044	0.205
<b>Mn<sup>+2</sup> (µg/L)</b>									
<i>r</i>	0.039	0.079	0.068	0.065	0.085	0.275	1	-0.144	-0.090
<i>p</i> -Value	0.572	0.250	0.321	0.343	0.218	<0.0001**	-	0.036	0.192
<b>Ca<sup>+2</sup> (mg/dL)</b>									
<i>r</i>	0.238	0.157	0.211	0.020	0.024	-0.138	-0.144	1	0.527
<i>p</i> -Value	<0.0001**	0.022	0.002**	0.772	0.732	0.044	0.036	-	<0.0001**
<b>Po<sub>4</sub><sup>+3</sup> (mg/dL)</b>									
<i>r</i>	0.222	0.209	0.298	-0.058	0.006	-0.087	-0.090	0.527	1
<i>p</i> -Value	<0.001**	0.002**	<0.0001**	0.402	0.927	0.205	0.192	<0.0001**	-

Note: \*\*Pearson correlation is significant at the 0.01 level (two tailed).

**Table 6** Pearson bivariate correlation analysis on serum ferritin and metal elements with body mass index, waist-to-hip ratio, and fasting blood glucose among diabetes study subjects

Tested parameters	Body mass index (kg/m <sup>2</sup> )		Waist-to-hip ratio (cm)		Fasting blood glucose (mg/dL)	
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Zn <sup>+2</sup> (µg/dL)	-0.331	<0.0001**	-0.340	<0.0001**	-0.186	0.006**
Mg <sup>+2</sup> (mg/dL)	-0.254	<0.0001**	-0.199	0.003*	-0.136	<0.0001**
Cr <sup>+3</sup> (µg/L)	-0.121	0.077	0.002	0.974	-0.023	0.734
Fe <sup>+3</sup> (µg/dL)	-0.099	0.148	-0.029	0.672	-0.111	0.106
Ferritin (µg/L)	-0.058	0.396	0.053	0.443	-0.100	0.144
Cu <sup>+2</sup> (µg/dL)	0.140	0.04	0.327	<0.0001**	0.057	0.404
Mn <sup>+2</sup> (µg/L)	0.021	0.755	0.282	<0.0001**	-0.008	0.912
Ca <sup>+2</sup> (mg/dL)	-0.335	<0.0001**	-0.372	<0.0001**	-0.141	0.040
Po <sub>4</sub> <sup>-3</sup> (mg/dL)	-0.380	<0.0001**	-0.309	<0.0001**	-0.159	<0.0001**

Notes: Data were presented as mean (standard deviation). \*\*Pearson correlation is significant at the 0.01 level (two tailed).

carefully regulated and found in excess in the tissue.<sup>46</sup> Ferritin is a ubiquitous intracellular protein commonly utilized as a marker of body iron stores.<sup>41,43,44</sup> Ferritin can release iron if the blood has a low iron concentration and store excess iron if the blood and tissues have a high iron concentration.<sup>47,48</sup> The imbalance of blood iron and ferritin in diabetes is mysterious.<sup>49</sup> But, inadequate dietary intake, asymptomatic illness,

and poor absorption rate often associated with the decrease of iron and ferritin. Different studies have been showing the increase of serum copper in T2DM patients, while urinary excretion of copper is high.<sup>16,18,21,23,26,27,50,51</sup> Our study has shown significant elevation of serum copper concentration in patients with type 2 diabetes than nondiabetes. Again our finding on serum copper disagree with some other findings

as well.<sup>52</sup> Copper is considered as a powerful enzyme catalyst and its deficiency results in glucose intolerance, hypercholesterolemia, and atherosclerosis.<sup>53</sup> Inconsistent with others findings,<sup>54–57</sup> significant difference was not observed in both T2DM patients and nondiabetes regarding serum manganese. Manganese is an important constituent of some enzymes, such as pyruvate carboxylase and arginase.<sup>1</sup> Normal levels of manganese are involved in the synthesis and secretion of insulin.<sup>54–57</sup> Generally, studies are not adequate on calcium and phosphorus in the blood sample of both diabetes and nondiabetes study subjects. But, data from animal models of diabetes and from studying patients with diabetes reveal increased intracellular calcium level in most tissues.<sup>58,59</sup> However, in the current study, significant difference was not observed in serum calcium and phosphorus in both type 2 diabetes and nondiabetes study subjects. In the current study, we have also compared some clinical parameters, such as BMI, WHR, systolic and diastolic blood pressures, FBG, serum trace metal elements, and ferritin level among oral hypoglycemic agent and injectable insulin users of T2DM study subjects. Thus, WHR, diastolic blood pressure, serum iron, ferritin, and manganese level were significantly higher among oral hypoglycemic agent users of T2DM study subjects than injectable insulin users. Although we do not know the exact mechanism for such differences, usually injectable insulin is much more effective to achieve near normal glycaemic control as early as possible in the course of the disease.<sup>60</sup>

## Limitation of the study

We did not measure tissue and urinary trace metal elements, glycated hemoglobin, and postprandial insulin level of the study subjects. Moreover, the study was cross-sectional and parameters were measured once. Thus, relationship between the measured parameters and the T2DM may not be truly associated. Thus, we would like to recommend the researchers to study more about the association of ferritin, trace metal elements, and T2DM in a cohort study with larger number of study subjects.

## Conclusion

In general, the current study shows decrease of serum zinc, magnesium, chromium, iron, ferritin and increase of serum copper concentration in T2DM study subjects than in nondiabetes mellitus study subjects. But, serum manganese, calcium, and phosphorus did not show significant differences in both the study subjects. Additionally, WHR, serum iron, ferritin, and manganese were significantly higher among oral hypoglycemic agent users of T2DM study subjects.

Moreover, significant positive and negative correlation has been seen between serum zinc with magnesium, chromium, copper, calcium, and phosphorus and magnesium with chromium, copper, calcium, and phosphorus among T2DM study subjects (details are described in Table 5). Zinc, magnesium, and phosphorus also negatively correlated with BMI, WHR, and FBG level (details are described in Table 6). We believe that poor glycaemic control, poor adherences toward insulin and oral hypoglycemic agents, high urinary clearance rate, asymptomatic disease, oxidative stress, and other health and health-related factors could be contributing factors for the existing disturbed trace metal elements among T2DM patients. Thus, following the possible preventable strategies (lifestyle modification and good adherences to medication) may reduce the complication of diabetes and trace metal elements in T2DM patients.

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## Disclosure

The authors report no conflicts of interest in this work.

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