PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

DETERMINAÇÃO DE MICRONUTRIENTES EM MULHERES DIABÉTICAS: UMA ANÁLISE SÉRICA DE SETE ELEMENTOS

DETERMINATION OF MICRONUTRIENTS IN DIABETIC WOMEN: A SERUM ANALYSIS OF SEVEN ELEMENTS

تحديد العناصر الغذائية الدقيقة لدى النساء المصابات بالسكري: تحليل المصل لسبعة عناصر

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Received 22 May 2023; received in revised form 14 July 2023; accepted 14 November 2023

RESUMO

Introdução: O corpo humano necessita minerais e micronutrientes. É importante estimar os micronutrientes (Fe^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}, Cu^{+2}, K^{+}, Na^{+}) presentes no soro de pacientes do sexo feminino com diabetes mellitus (T2DM) conforme o necessário para o metabolismo da insulina e da glicose, especialmente zinco, manganês e magnésio, que ativam as áreas receptoras de insulina. **Objetivo**: Estimar as concentrações dos micronutrientes (µg/mL) e determinar seus efeitos na resistência à insulina e diabetes mellitus. **Métodos**: Este estudo foi conduzido no Centro de Endocrinologia e Diabetes da Província de Maysan (Iraque) de janeiro a abril de 2023 incluiu 120 mulheres com idades entre (20 – 65) anos separadas em dois grupos. O grupo controle incluiu 40 mulheres saudáveis, e o grupo de pacientes incluiu 80 mulheres com diabetes mellitus (T2DM). O IBM SPSS Statistics e o teste t foram utilizados para comparar os dois grupos. **Resultados**: Os resultados mostraram significância estatística no nível ($P<0.05$) para a concentração média de Magnésio, Manganês, Zinco, Cobre, Potássio e Sódio, enquanto a concentração de Ferro foi não significativa ($P>0.05$) nos pacientes com (T2DM) em comparações com o grupo controle. A concentração dos micronutrientes nos grupos saudáveis e diabetes mellitus foi $Na^{+} > Cu^{+2} >$ $\mathbf{Zn^{+2}}$ $> Mn^{+2} > Fe^{+2} > K^{+} > Mg^{+2}$ e $Na^{+} > Cu^{+2} >$ $\mathbf{K^{+}}$ $> Mg^{+2} > Fe^{+2} > Zn^{+2} > Mn^{+2}$ respectivamente. No grupo T2DM, o microelemento de maior concentração observada foi o íon Sódio, enquanto o Manganês (Mn^{+2}) teve a menor concentração. Por outro lado, no grupo controle, o Sódio (Na^{+}) mostrou a maior concentração e o Magnésio (Mg^{+2}) a menor. **Discussão**: Os níveis sanguíneos dos micronutrientes Cu, Mg, K, Na, Mn e Zn variaram amplamente. As quantidades de Zinco, Ferro, cobre, Sódio e manganês foram menores no grupo diabetes mellitus. Por outro lado, magnésio e potássio foram maiores no grupo diabetes mellitus. **Conclusões**: As variações das concentrações dos microelementos foram estatisticamente significativas entre os grupos diabetes mellitus e controle, exceto para o Ferro, demonstrando a complexa relação entre os níveis de micronutrientes e distúrbios metabólicos.

Palavras-chave: *Microelementos, Diabetes mellitus, Micronutrientes, Resistência à insulina, metabolismo.*

ABSTRACT

Background: The human body needs minerals and micronutrients. It is important to estimate the micronutrients (Fe^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}, Cu^{+2}, K^{+}, Na^{+}) in the serum of female patients with diabetes mellitus (T2DM) as needed for insulin and glucose metabolism, especially zinc, manganese, and magnesium, which activate insulin receptor sites. **Aim:** Estimate the micronutrient concentrations by (µg/mL) and determine their effects on insulin resistance and diabetes mellitus. **Methods:** The study was conducted at Maysan Province Endocrinology and Diabetes Center from January to April 2023. It included 120 women aged (20 – 65) years separated into two groups. The control group included 40 healthy women, and the patient group included 80 women with diabetic

DOI: 10.52571/PTQ.v20.n45.2023.05_KHALAF_pgs_38_48.pdf

mellitus (T2DM). The IBM SPSS Statistics and the t-test were used to compare the two groups. **Results:** The results showed a statistically significant difference at the level ($P<0.05$) for the average concentration of Magnesium, Manganese, Zinc, Copper, Potassium, and Sodium, while Iron concentration was a non-significant decrease at ($P>0.05$) in the patients with (T2DM) compared with the control group. The concentration of micronutrients in diabetes mellitus and healthy groups was Na^{+} > Cu^{+2} > **Zn^{+2}** > Mn^{+2} > Fe^{+2} > K^{+} > Mg^{+2} and Na^{+} > Cu^{+2} > **K^{+}** > Mg^{+2}> Fe^{+2} > Zn^{+2} > Mn^{+2} respectively. In the T2DM group, the trace element with the highest observed concentration was the Sodium ion, while Manganese (Mn^{+2}) had the lowest concentration. Conversely, in the control group, Sodium (Na^{+}) showed the highest concentration and Magnesium (Mg^{+2}) the lowest. **Discussion**: Sodium had the greatest micronutrient content in both the control and T2DM groups. Micronutrient differences may affect insulin resistance and diabetes. **Conclusions:** The variation in the micronutrient concentration was statistically significant between the diabetes mellitus and control groups, except for Iron, demonstrating the complex relationship between micronutrient levels and metabolic disorders.

Keywords: *Microelements, Diabetes mellitus, Micronutrients, Insulin resistance, metabolism.*

الملخص

الخلفية: يحتاج جسم الانسان إلى المعادن والمغذيات الدقيقة. من المهم تقدير المغذيات الدقيقة (ايون الحديد، ايون المغنيسيوم، ايون المنغنيز، ايون الزنك، ايون النحاس، ايون البوتاسيوم، وايون الصوديوم) في مصل المرضى الإناث المصابات بداء السكري من النوع الثاني لضرورتها لأستقلاب الأنسولين والجلوكوز، خاصة الزنك والمنغنيز والمغنيسيوم، التي تنشط مواقع مستقبلات الأنسولين. **الهدف**: تقدير تراكيز المغذيات الدقيقة بالميكروغرام/مل وتحديد تأثيرها على مقاومة الانسولين وداء السكري. **طرائق العمل**: أجريت الدراسة في مركز الغدد الصماء والسكري في محافظة ميسان خلال الفترة من كانون الثاني الى نيسان 2023، وقد شملت مائة وعشرون امرأة تتراوح اعمارهن بين (20-65) سنة وتم تقسيمهن الى مجموعتين. ضمت مجموعة السيطرة اربعون امرأة سليمة، ومجموعة المرضى شملت ثمانون امرأة مصابة بداء السكري من النوع الثاني. تم أستخدام IBM SPSS أصدار 26، وأختبار t لمقارنة المجموعتين. **النتائج**: أظهرت النتائج وجود فرق معنوي ذو دلالة إحصائية عند مستوى ($P<0.05$) لمتوسط تركيز المغنسيوم والمنغنيز والزنك والنحاس والبوتاسيوم والصوديوم، بينما سجل تركيز الحديد انخفاضاً غير معنوي عند مستوى ($P>0.05$) لدى المرضى الذين يعانون من مرض السكري من النوع الثاني بالمقارنة مع مجموعة السيطرة. أن تركيز العناصر النزرة في مجموعات مرضى السكري والاصحاء هي ايون الصوديم> ايون النحاس> ايون الزنك> ايون المنغنيز> ايون الحديد> ايون البوتاسيوم> ايون المغنيسيوم و ايون الصوديم> ايون النحاس> ايون البوتاسيوم> ايون المغنيسيوم> ايون الحديد> ايون الزنك> ايون المنغنيز على التوالي. في مجموعة مرضى السكري النوع الثاني، كان العنصر النزر ذو أعلى تركيز ملاحظ هو ايون الصوديوم، بينما كان ايون المنغنيز أدنى تركيز. على العكس من ذلك، في مجموعة السيطرة، أظهر ايون الصوديوم أعلى تركيز وايون المغنيسيوم هو الأدنى. **المناقشة**: كان للصوديوم أكبر محتوى من المغذيات الدقيقة في كل من مجموعتي السيطرة ومرضى السكري من النوع الثاني. قد تؤثر اختلافات المغذيات الدقيقة على مقاومة الأنسولين ومرض السكري. **الاستنتاجات**: ان التباين في تركيز العناصر النزرة ذو دلالة إحصائية بين مجموعات داء السكري والسيطرة، بإستثناء الحديد، مما يدل على العلاقة المعقدة بين مستويات المغذيات الدقيقة والاضطرابات الأيضية.

الكلمات المفتاحية: *العناصر الدقيقة، داء السكري، المغذيات الدقيقة، مقاومة الأنسولين، التمثيل الغذائي.*

1. INTRODUCTION:

The trace elements (TEs) and micronutrients are present at very low concentrations in natural and perturbed environments and are usually required in low amounts by humans ≤100 mg/day). However, TEs are essential for several physiological processes, mainly involved in the immunity system and metabolism, and serve as cofactors for multiple enzyme system reactions (Dubey *et al*., 2020). Specifically, the imbalance of Zinc (Zn), copper (Cu), iron (Fe) chromium (Cr), vanadium (V), and selenium (Se) seems to be associated with T2DM development and progression as well as T2DM-derived complications (Moreno-Navarrete *et al*., 2014). It has been proposed that both TE deficiencies or overload could relate to oxidative stress, which is closely related to insulin resistance and diabetes (Dubey *et al*., 2020). In addition, Cr, Zn, Cu, Fe, and Se have antioxidant effects and might ultimately enhance insulin action by activating insulin receptor sites or incrementing insulin sensitivity. Zinc is a crucial trace element that directly affects insulin production, storage, and release. It is found in secretory vesicles in the pancreatic cells, where it helps insulin crystallize (Rodríguez-Pérez *et al*., 2021). Zinc deficiency disturbs insulin homeostasis, causing cells to secrete less insulin (Dascalu *et al*., 2022).

In addition, Sodium is an essential element for healthy physiological activities, people with type 2 diabetes (T2DM) may suffer from osmotic diuresis because of hyperglycemia, which raises sodium excretion in the urine (hyponatremia). Hyponatremia is associated with several detrimental clinical symptoms and pathophysiological changes in T2DM patients (Cheng *et al.,* 2022). Poor glucose metabolism may be caused by Mn^{+2} deficiency, it functions as a cofactor in several enzymes, including those responsible for mitochondrial glycoprotein synthesis. These enzyme's activity is decreased by a lack of Mn^{+2} ions (Chen *et al*., 2022). The cellular Mg^{+2} ion is an essential cofactor for numerous carbohydrate-related metabolic pathways. Due to its vital involvement in the Mg-ATP complex, which is a crucial component for all of the glycolysis' rate-limiting enzymes, magnesium specifically regulates the activity of all enzymes involved in phosphorylation processes (Veronese and Barbagallo 2021). Copper is the third most common essential transition metal in humans (Gembillo *et al*., 2023), a necessary metal that functions as a pro-oxidant and an antioxidant as well as a catalytic cofactor of enzymes (Mohammadifard *et al*., 2017).

Additionally, slight copper deficiency may speed up the development of several diseases, including diabetes. To ensure cardiovascular health, Cu^{+2} is crucial. According to some studies, a lack of these ions may increase the chance of developing CVD, especially in patients with T2DM with and without Diabetic Kidney Disease (DKD). Hyperglycemia, serum heavy metal concentrations, and their binding proteins all support oxidative stress (OS) (Gembillo *et al*., 2022). Potassium in fact, almost 98% of the potassium (K^{+1}) of the body is found in intracellular fluid, making it one of the primary intracellular cations in the human body. The K^{+1} is essential for many physiological processes, particularly the neuro-endocrine system and control of blood pressure (BP) (Elia *et al*., 2022), as well as higher morbidity and mortality in diabetics with heart failure (HF) and CKD are clinical issues linked to hypokalemia (Coregliano-ring *et al*., 2022). Finally, Iron Metabolism of glucose is impacted by Iron, impairment of iron uptake may influence glucose metabolism. Insulin sensitivity, vascular resistance, viscosity, and oxidative damage may all be impacted by the serum ferritin content in T2DM patients (Dubey *et al*., 2020). Numerous studies have also shown that a much milder condition of iron overload (IO), caused by an excessive amount of dietary Iron or a variety of other reasons, is also a risk factor for the development of T2DM (Gao *et al*., 2022). This study aimed to determine some trace elements in the blood serum of women who were diabetes mellitus patients and compare them with healthy women in Maysan province southeast of Iraq.

2. MATERIALS AND METHODS:

2.1. Materials

Sodium chloride (NaCl), zinc sulfate ($ZnSO_4$), manganese dioxide (MnO_2), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), copper sulfate pentahydrate ($CuSO_4.5H_2O$), potassium chloride (KCl, ferrous *sulfate* ($FeSO_4$) (Thomas beaker), all the chemicals are analytical grade. Concentrated nitric acid (HNO_3) 69%, hydrochloric acid (HCl) 37%, and perchloric acid ($HClO_4$) 70% (AppliChem).

2.2. Methods

2.2.1. Samples Collection

The current study was carried out during the period from January to April 2023 at the Maysan province Endocrinology and Diabetes Center as well as at a few private clinical laboratories. 120 blood samples were collected from women. Ages 20-65 years were separated into two groups: a control group including 40 healthy women and a diabetic group with type 2 diabetes (T2DM) including 80 women. A diabetes specialist examined the women's blood samples, diagnosed diabetes, and identified some cases of obesity based on their BMI and HbA1c readings. Smokers, pregnant, and chronic disease patients were excluded. Five milliliters of blood were drawn from each woman and collected in gel tubes to complete serum separation from the other blood components so the gel would be as a boundary between the two layers, allowed to stand for twenty minutes to allow blood to coagulate, centrifuged at 4000 rpm for 5 minutes to obtain blood serum, transferred into plain tubes and sent to the laboratory for analysis, all the ethical procedure were followed.

2.2.2. Samples Digestion

The samples were digested by adding (10 mL) of a mixture containing (HNO_3, 69% $HClO_4$, 70%) concentrated acids (4:2 v/v ratio) very strong oxidizing agents, to (0.5 mL) serum, heated for one hour at (120 ℃) in a hot plate, dissolved metal nitrate, water and carbon dioxide produced until the volume became (1mL), to concentrate the trace elements and clear if not, it must be filtered to remove any coagulants make the solution turbid like lipoprotein, cooling to a room temperature, transferred quantitatively to volumetric flask 25 mL and diluted with deionized water up to the mark (Badran *et al*., 2017).

2.2.3. Preparation of stock solutions of Elements (1000 µg/mL)

According to (Komarova *et al*., 2021), all stock solutions of elements were prepared in a volumetric flask (100 mL); Sodium by dissolving (0.2540 g) of NaCl in 10 mL deionized water, and potassium by dissolving (0.1907g) of KCl in 10 mL deionized water, The working standard solutions for Sodium and potassium prepared by serial dilution (1-8 µg/mL). Zinc by dissolving (0.2469 g) of $ZnSO_4$ in 5 mL of 5% HCl, (this is a diluted acid used to prevent any adsorption of the metal ions on the internal surface of the volumetric glass), While manganese by dissolving (0.1582 g) of MnO_2 in 5 mL of 5% HCl, Magnesium by dissolving (1.0143g) of MgSO4.7H20 in 5 mL of 5% HCl. The copper by dissolving (0.3927 g) of $CuSO_4.5H_2O$ in 5 mL of 5% HCl, and Iron by dissolving (1.0143g) of $FeSO_4$ in 5 mL of 5% HCl. The working standard solutions for Zinc, manganese, Magnesium, copper, and Iron are prepared by serial dilution (0.5-2 µg/mL).

2.2.4. Determination of Trace Elements

Standard solutions of the trace elements were used to determine the standard calibration curves for Zn^{+2}, Mg^{+2}, Cu^{+2}, Mn^{+2}, and Fe^{+2} by Flame atomic absorption spectroscopy Aurora (AI-1200), following the user manual instructions as shown in Table 1, the flame contains 99.9% acetylene gas and compressed air, while Na^+ and Li^+ are determined by flame photometer- PFP7 (atomic emission) as shown in Table 2, the flame contains butane gas and compressed air. The limit of detection LOD for each element, the lowest concentration level that can be determined, the signal above the background signal by three times the standard deviation of the blank solution signal as shown in Equation 1. Where: the slope of calibration curve of the element, standard deviation (s), the standard deviation (s) of the data," as per Equation 2.

LOD= 3.3* s/slop (Eq. 1)

$$s = \frac{\sqrt{\sum(xi-\vec{x})^2}}{n-1} \quad \text{(Eq. 2)}$$

Where: xi absorbance or emission values of the blank solution, x¯average of absorbance or emission values of the blanks, n number of measurements.

2.2.5. Statistical analysis

The results are expressed as mean ± Standard Deviation (SD), this analysis was completed using IBM SPSS statistics, version 26 (IBM Co., Armonk, NY, USA), by utilizing a t-test under the significant threshold ($p \leq 0.05$) (Steel et al., 1997).

3. RESULTS AND DISCUSSION:

3.1. Results

The results in Table 3 showed a significant difference at the level ($P<0.05$), for the average concentration of Magnesium, Manganese, Zinc, Copper, Potassium, and Sodium. In contrast, Iron concentration was a non-significant difference at ($P>0.05$) in the patients with (T2DM) by compared with the control group.

3.2. Discussion

The results showed a significant decrease at ($p<0.05$) of Na^{+1} concentration in DM patients (303.126±73.407) compared with the control group (463.732 ±67.660) µg/mL as shown in Table 3 and Figure 2. The results of sodium ion agree with (Huang *et al*., 2022). These findings point to a potential role for low normal serum sodium levels in T2DM patients' impaired bone turnover. According to our knowledge, no research has specifically examined the relationship between normal blood sodium levels and bone turnover in people with T2DM. Numerous research has focused on the sodium connection between hyponatremia and bone health in people with subarachnoid hemorrhage (Moro *et al*., 2007; Corona *et al*., 2018).. Patients were receiving incorrect antidiuresis syndrome treatment or antiepileptic medication. In T2DM patients, serum sodium levels are related to bone turnover. In this study, blood sodium levels were strongly positively linked with the markers of bone turnover, osteocalcin, and the N-terminal propeptide of type-I procollagen but not with levels of the C-terminal telopeptide CTx. Patients with subarachnoid hemorrhages who experienced transient mild hyponatremia frequently also showed significant decreases in bone production but not in bone resorption. The lack of a link between sodium and CTx levels could be attributed to two variables: low bone turnover in T2DM patients is mostly due to decreased bone production. Furthermore, the mobilization of bone salt stores to maintain circulation sodium concentrations is a key component of the mechanisms through which hyponatremia can accelerate bone resorption; however, when sodium levels are within the usual range, this effect can be less pronounced (Huang *et al*., 2022). The relationship between sodium and potassium can be affected by diabetes (Baqar *et al.,* 2020). According to some research, there may be a link between type 2 diabetes and low levels of potassium. In addition, certain diabetes medications can affect potassium levels, such as Insulin, Diuretics, Potassium-sparing diuretics, non-selective beta-blockers, direct renin inhibitors, Angiotensin-converting enzyme (ACE) inhibitors, and Angiotensin receptor blockers (ARBs). When sodium levels rise, potassium levels tend to decrease, and vice versa. Low concentrations of insulin, such as in diabetic ketoacidosis, cause potassium to leave cells, thus raising potassium in the plasma, sometimes even in the presence of total body potassium deficiency.Therefore, it is important to monitor and manage sodium and potassium levels in patients with diabetes to ensure an adequate balance of these electrolytes.

The zinc results showed a significant decrease at ($p<0.05$) in Zn^{+2} concentration in DM patients (58.475 ±30.561), compared with the control group (154.425 ±12.719) µg/mL as shown in Table 3 and Figure 1. The results of this study for zinc ions agree with Zhang *et al*. (2021) and Dascalu *et al*. (2022). Zinc is the trace metal found in the body in the second-highest concentration. More than 300 enzymes depend on zinc for proper function, and numerous physiological processes, including cell division, tissue repair, bone formation, membrane stability, growth and development, pregnancy, fertility, brain activity, taste, and appetite, depend on it. Different findings about serum zinc in T2DM were found in the investigations that were undertaken. In their studies, many experts discovered that diabetic patients' serum zinc levels were lower than healthy controls. Additionally, they demonstrated that in the diabetic group, there was a negative association between blood zinc levels and both serum glucose levels and basal HbA1c values, demonstrating the value of zinc supplementation for enhancing glycaemic control and lowering HbA1c levels. Serum zinc levels in diabetic and hypertensive patients were shown to be greater in diabetes patients compared to control groups (Zhang *et al*., 2021).

Manganese results showed a significant decrease at ($p<0.05$) in Mn^{+2} ions concentration in DM patients (38.19346.513±) µg/m compared with the control group (149.2 ± 18.069) µg/mL as shown in Table 3 and Figure 1. The results of this study for Manganese ion agree with (Liu *et al*., 2022). The median serum Mn^{+2} levels of the diabetic group were considerably lower than those of the control group. The study's results bolster earlier research indicating lower serum Mn^{+2} concentrations in individuals with T2DM compared to controls. Furthermore, another study identified reduced intra-arterial Mn^{+2} concentrations in pre-DM individuals compared to control, potentially associated with an elevated risk of heart attacks and strokes. The study revealed a significant increase in urine Mn^{+2} concentration in T2DM patients compared to controls. This finding supports assertions by some writers that individuals with T2DM might experience lower blood Mn^{+2} levels due to increased urine Mn^{+2} excretion. The supplementation of Mn^{+2} may potentially decrease the incidence of T2DM-related issues by enhancing the activity of the enzyme manganese superoxide dismutase (Mn-SOD) (Lassen *et al.,* 2018).

Supplemental Mn^{+2} may lower the incidence of T2DM issues by boosting the activity of the enzyme manganese superoxide dismutase (Mn-SOD). Understanding that Mn exposure should be kept to a minimum and should not exceed what is commonly found in food is essential (Liu *et al*., 2022).

Magnesium results showed a significant increase at ($p<0.05$) in Mg^{+2} concentration in DM patients (89.05197.686±)compared with the control group (38.43391.543±) µg/mL as shown in Table 3 and Figure 2. The results of this study for Magnesium ion do not agree with Veronese *et al*. (2021). Through several routes, magnesium may enhance insulin sensitivity and glucose metabolism. First, chronic Mg^{+2} deficiency has been linked to reduced post-preceptorial activity, which in turn causes cells to use less glucose, this is known from experimental models. Additionally, Mg^{+2} may enhance pancreatic beta-cells ability to secrete insulin. Improvements in HOMA-IR, especially in those at high risk of diabetes, indicate that the major effect of magnesium appears to be a reduction of insulin resistance, suggesting that magnesium functions more effectively when there is an insulin deposit. Additionally, other experimental data showing that Mg can reduce oxidative stress and inflammatory parameters, two major causes of insulin resistance, confirm the findings about the improvement of insulin sensitivity (Veronese *et al*., 2021).

Copper results showed a significant decrease at ($p<0.05$) in Cu^{+2} concentration in DM patients (126.595 72.825±) compared with the control group (340.170 ± 29.260) µg/mL as shown in Table 3 and Figure 1. The results of this study for Copper ion do not agree with (Khedr *et al*., 2021). Serum levels of Cu were elevated among T2DM patients with poor glycemic control compared to normoglycemic T2DM patients and normal individuals. Increased Cu level among T2DM patients reported previously has been linked with the development of diabetes. Copper is a pro-oxidant, and high levels of Cu induce increased hydrogen peroxide production, resulting in β cell degeneration and the development of T2DM.

On the other hand, zinc is bestowed with antioxidant and anti-inflammatory properties through its ability to down-regulate inflammatory cytokine production. In diabetes mellitus, low Zn levels and high Cu levels tip the scales in favor of an inflammatory environment, diabetes raises free Cu levels, which are toxic and encourage pro-oxidant activity. This is due to elevated Cu levels, decreased ceruloplasmin levels, and decreased Cu binding activity. Protein glycosylation is induced by hyperglycemia, and Cu has a greater affinity for these glycosylated proteins. As a result, this increased oxidative stress and free radical generation predispose T2DM patients to disease-related problems (Khedr *et al*., 2021).

Potassium results showed a significant increase at ($p<0.05$) in concentration in DM patients (98.965±26.774) compared with the control group (84.917±19.798) µg/mL, as shown in Table 3 and Figure 2. The results of potassium ion agree with (Collins *et al*., 2017) but do not agree with (Cheng *et al*., 2022). Potassium chloride (KCl), potassium bicarbonate, potassium citrate, and potassium phosphate are the four primary categories of potassium-containing preparations. When hypophosphatemia is present, potassium phosphate solutions are especially helpful, citrate or bicarbonate solutions when acidosis is present, but potassium chloride is typically the best solution to use. The gastrointestinal tract's mucosa may get irritated by oral KCl tablets, which typically contain 8 mmol K^+, this irritation may potentially result in ulcerations or bleeding, which necessitates consuming a lot of drinks along with the medication.

When using non-steroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors (ACEi), or angiotensin receptor blockers (ARBs) in diabetic patients with reduced glomerular filtration rate (GFR), potassium-sparing diuretics can help prevent the onset of hyperkalemia. Encouragement of the consumption of foods high in potassium, including bananas, tomatoes, lentils, almonds, fish, and meat., while always bearing in mind the glycemic load of each food, is an intriguing strategy for diabetes patients who are prone to hypokalemia (Bandak *et al*., 2017). The major adverse cardiovascular events (MACE) results presented here add to a small but growing body of evidence suggesting that serum potassium (sK^{+1}) variability may be a risk factor for unfavorable clinical outcomes, this increased risk may be concealed by analyses that only take mean sK^{+1} over time or by the evaluation of competing biomarkers. For instance, it is possible to draw comparisons with the more well-established significance of glucose variability as a risk factor and potential surrogate marker for cardiovascular and microvascular problems in diabetes patients (James *et al*., 2021).

Finally, iron results show a non-significant decrease at ($p>0.05$) in (Iron) concentration in patients with DM (83.947±76.631) compared with the control group (105.020±38.074) µg/ml as shown in Table 3 and Figure 1. The results of this study for iron ions agree with Basaki *et al*. (2012), though the difference in Fe^{+2} concentration was not significant. There is general agreement in the literature that homeostasis of trace elements can be disrupted by diabetes mellitus. On the other hand, alterations in the status of trace elements and increased oxidative stress in diabetes mellitus may be linked to the development of insulin resistance and diabetic complications. Reactive oxygen species (ROS) can be produced by free Fe^{+2}, a very pro-oxidant molecule, therefore, it is crucial to maintain the homeostasis of free Fe^{+2} because large levels of it may be detrimental. It has been shown that patients with diabetic retinopathy have greater Fe^{+2} concentrations than healthy individuals. Therefore, it would appear that the pathophysiology of diabetic retinopathy involves oxidative damage brought on by a high concentration of free Fe^{+2}(Basaki *et al*., 2012).

4. CONCLUSIONS:

The findings of the study reveal a compelling link between trace element concentrations and the incidence of Type 2 diabetes mellitus (T2DM), highlighting the intricate interplay between micronutrient levels and metabolic disorders. The determination of trace elements was statistically significant between the diabetes mellitus group and the control group at ($p<0.05$) except Iron at ($p>0.05$). There was a decrease in the level of trace elements (Zn, Mg, Cu, Na) and an increase in K and Mg among patients with T2DM. The highest and lowest trace element concentrations were Na^{+}, Mn^{+2} and Na^{+}, Mg^{+2} in T2DM and control groups.

5. DECLARATIONS

5.1. Study Limitations

The limitations of the study are tied to the sample size used and the methodologies implemented during the research period.

5.2. Acknowledgements

The authors extend sincere gratitude to the staff and technicians of the Chemistry Department at the University of Misan. Their unwavering support, expertise, and dedication were instrumental in successfully completing this study.

5.3. Funding source

The authors funded this research.

5.4. Competing Interests

No conflict of interest exists in this publication.

6. HUMAN AND ANIMAL-RELATED STUDIES

6.1. Ethical Approval

The ethical approval authority was obtained from the Training and Human Development Center at the Misan Health Directorate, Ministry of Health (Approval No: 11, Date: 08-01-2023).

6.2. Informed Consent

The consent form sought participants' authorization to publish data from their involvement in our present research study. This publication aims to disseminate our discoveries within the scientific community. We ensured participants that their individual data would be anonymized to safeguard their privacy. We emphasized that their involvement was entirely voluntary, with the freedom to withdraw consent at any stage. Participants were clearly informed that their signature on the form would signify their agreement to utilize their data in our publication.

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Table 1 *The analytical conditions for the determination of metal ions by FAA*

Ser.	Ion	(λ_{max}) nm	LOD* (µg. mL)	Working rang (µg. mL)	Silt width (nm)	R^2	Equation
1	Zn^{2+}	213.9	0.015	0.5-2.0	0.2	0.9955	y = 37.869 x – 0.0519
2	Mg^{2+}	285.2	0.010	0.5-2.0	0.2	0.9987	y= 9.6259 x – 0.3146
3	Cu^{2+}	324.7	0.004	0.5-2.0	0.2	0.9997	y= 74.38 x + 0.20087
4	Mn^{2+}	279.5	0.002	0.5-2.0	0.2	0.9970	y= 32.7389 x – 0.0514
5	Fe^{2+}	248.3	0.014	2.0-8.0	0.2	0.9977	y = 83.191 x – 0.4170

*LOD means limit of detection

Table 2 *The analytical conditions for determination of metal ions by Flame photometer*

Ser.	Ion	(λ_{max}) nm	LOD (µg. mL)	Working rang (µg. mL)	Silt width (nm)	R^2	Equation
1	Na^+	598.0	0.017	1.0-8.0	0.2	0.9977	Y = 1.1508 x– 0.4284
2	K^+	766.5	0.012	1.0-8.0	0.2	0.9993	Y = 0.512 x– 0.0093

*LOD means limit of detection

Table 3 Descriptive and Inferential statistics of trace element concentrations in DM patients and control groups

Ion	Min-Max DM* µg/mL	Min-Max C** µg/mL	DM* (µg/mL) n=80 Mean± SD	C** (µg/mL) n=40 Mean± SD	P value
Fe^{+2}	23.16 -183.48	11.17 - 273.37	83.947±76.631	105.020±38.074	0.200
Mg^{+2}	1.79-353.19	1.007- 423.19	89.05197.686±	38.43391.543±	0.034
Mn^{+2}	101.94 -181.37	1.61- 144.15	38.19346.513±	149.26218.069±	000
Zn^{+2}	134.52-179.47	18.405-149.04	58.47530.561±	154.425±12.719	000
Cu^{+2}	259.53-377.90	40.19 - 339.87	126.59572.825±	340.170± 29.260	000
K^{+1}	55 - 125	58.75-128	98.96526.774±	84.91719.798±	0.023
Na^{+1}	180 -577.5	188.75 -314	303.12673.407±	463.73267.660±	000

*DM diabetes mellitus, **C Control

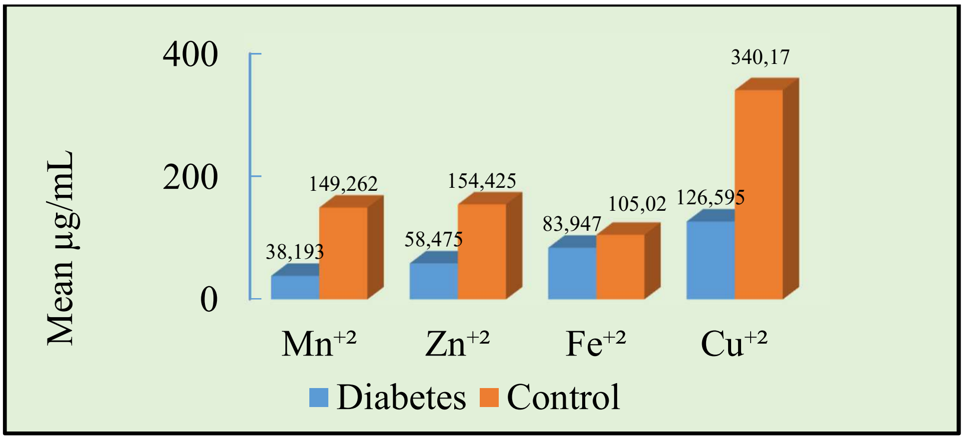

Figure 1 *The mean concentration of (Mn^{+2}, Zn^{+2}, Fe^{+2}, Cu^{+2}) in the blood serum of DM patients and healthy groups*

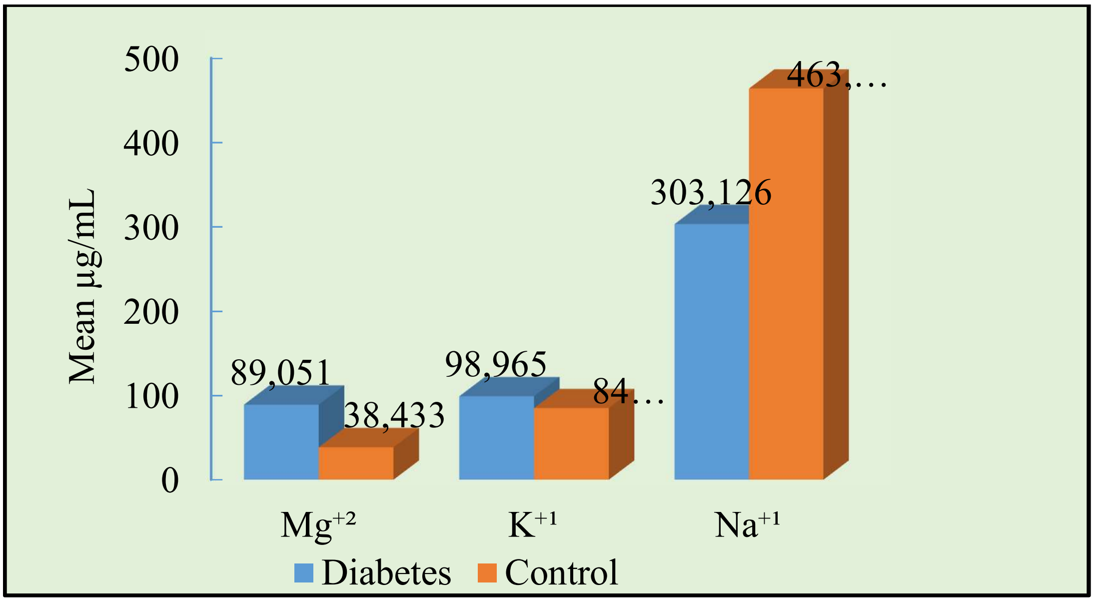

Figure 2 *The mean concentration of (Mg^{+2}, K^{+1}, and Na^{+1}) in the blood serum of DM patients and healthy groups*