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# The "Trace Mineral Fingerprint" as a Predictor of Homeostatic Model Assessment of Insulin Resistance Progression in Type 2 Diabetics

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## Abstract

**Context:** Trace minerals imbalance has been implicated in the progression of type 2 diabetes mellitus (T2DM).
**Aim:** This study assessed the levels of copper (Cu), zinc (Zn), iron (Fe), superoxide dismutase (SOD), insulin (INS), Glycated haemoglobin (HbA1c) and chromium (Cr) in subjects with T2DM.
**Materials and Methods:** This case-control study enrolled 45 T2DM patients attending diabetes Clinic at the University of Calabar Teaching Hospital and 45 age-matched non-diabetic controls. The fasting plasma glucose (FPG), HbA1c and Super oxide dismutase, SOD were determined by colorimetry and insulin by ELISA method. Serum Cu, Zn, Fe, and Cr were determined by Atomic Absorption Mass Spectrometry. The HOMA-IR, HOMA-IR/HbA1c, Cu/Zn, Fe/Zn and Cu/Fe ratios were computed. Data generated were analyzed using SPSS version 27, (IBM, USA). The ANOVA and Bonferroni post hoc analyses, Receiver Operator Characteristic curve (ROC) was utilized to determine the performance metrics of the indices. The normality of the data was determined by Shapiro-Wilk test. The confidence interval was set to 95% at $\alpha = 0.05$.
**Results:** The FPG, INS, Cu, Fe, Fe/Zn Cu/Zn, HOMA-IR, and HOMA-IR/HbA1c levels were significantly higher while Zn, SOD and Cr were significantly lower in diabetics subjects with HbA1c <7% and ≥ 7% than in controls ($p<0.05$). The HbA1c correlated positively with Fe/Zn ($r=0.582$, $p=0.000$). At potential cutoff of 1.558 Cu/Zn demonstrated AUC of 0.781, with a sensitivity of 71%%, Gini index of 0.562 and K-S metrics of 0.622 in predicting poor glycaemic control.
**Conclusion:** Trace mineral fingerprint may complement HbA1c and HOMA-IR in monitoring glycaemic control and rising insulin resistance in T2DM.

Keywords: Type 2 diabetes mellitus, oxidative stress, trace minerals, glycemic control, insulin resistance.

## Introduction

Type 2 diabetes mellitus (T2DM) continues to rise worldwide and is increasingly recognized as a metabolic disorder influenced not only by insulin resistance and impaired glucose metabolism but also by oxidative stress and trace element imbalance.[1,2] Trace minerals finger print may be useful in predicting progressive insulin resistance and oxidative stress build-up and poor glycemic control in type 2 diabetics.[3] Previous studies have demonstrated that dysregulation of trace minerals such as copper (Cu), zinc (Zn), iron (Fe), and chromium (Cr) contributes significantly to redox imbalance, inflammation, and poor glycaemic control.[4,5,6] The ratios between

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minerals such as Cu:Zn and their impact on insulin sensitivity may reflect the overall level of oxidative stress, rate of degradation of SOD and release of its metal cofactors, and rising insulin resistance well beyond looking at the minerals in isolation.[7,8] This study explores whether a specific "mineral signature" in serum can predict poor glycaemic control and rising insulin resistance in T2DM more accurately than FPG or HbA1c alone, may serve as a "Mineral Homeostasis Index" that complements HbA1c or HOMA-IR in long-term monitoring. This study hypothesized that in individuals with Type 2 Diabetes Mellitus (T2DM), a high serum Cu:Zn ratio combined with Cr deficiency and Fe overload significantly correlates with a decrease in Superoxide Dismutase (SOD) activity, which independently predicts a worsening of HOMA-IR and a subsequent elevation in HbA1c, regardless of the duration of the disease.

Copper and iron are essential trace minerals, but when dysregulated, act as redox-active catalysts, driving the production of highly damaging superoxide and hydroxyl radicals via Fenton and Haber–Weiss reactions., amplifying oxidative injury in pancreatic β-cells and peripheral tissues.[9-11] Conversely, zinc and chromium are recognized for their protective antioxidant and insulin-sensitizing effects.[12,13] Zinc contributes to insulin biosynthesis and stability and modulates antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), while chromium enhances glucose uptake by promoting insulin receptor phosphorylation and downstream PI3K/Akt signaling.[14-16] Dysregulation of these micronutrients impairs antioxidant defense systems, thereby intensifying oxidative stress which is a major factor in the pathogenesis and development of complications of diabetes.[17,18]

Moreover, studies have reported altered serum levels of these trace elements in T2DM patients, often reflected by elevated Cu/Zn and Fe/Zn ratios, which serve as sensitive indicators of oxidative stress severity and metabolic imbalance.[19,4] Excess iron accumulation has been linked with insulin resistance via mitochondrial oxidative damage and lipid peroxidation, while elevated copper promotes chronic low-grade inflammation and endothelial dysfunction.[5,20] Conversely, deficiencies in zinc and chromium are associated with reduced antioxidant enzyme activity, higher fasting glucose, and increased HbA1c, highlighting their role in glycaemic regulation.[21] This study assessed fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), serum insulin levels, trace elements (copper, zinc, iron and chromium), superoxide dismutase (SOD) activity and insulin resistance among subjects with type 2 diabetes mellitus (T2DM), attending clinics in the University of Calabar Teaching Hospital (UCTH), Calabar, Southern Nigeria

## Materials and Methods

***Study design:*** This case-control study was conducted in Calabar Metropolis from June 2024 to February 2025 to determine a "Mineral Homeostasis Index" that complements HbA1c in long-term monitoring of subjects with T2DM. The diabetic subjects were categorized into those with HbA1c ≤7% and >7%, those with Cu/Zn ≤1.7 and >1.7 also those with Zn ≤ 74 µg/dl and >74 µg/dl.

Study setting: The study population consisted of type 2 diabetes mellitus participants attending the Diabetes Clinic of the University of Calabar Teaching Hospital (UCTH), Calabar, Southern Nigeria.

***Study population:*** A total of 90 participants comprising of 45 participants with confirmed T2DM, according to the World Health Organization (WHO) criteria and 45 apparently healthy age-matched controls residing within the same geographic location were recruited into this study.

***Inclusion and exclusion criteria:*** The inclusion criteria were adults (35–65 years) diagnosed with T2DM for at least 1 year, while subjects on mineral supplements, those with chronic kidney disease (CKD), chronic liver disease, alcohol consumption, smoking habit, substance abuse, or individuals with inflammatory conditions that might skew ferritin (Fe) or SOD levels were excluded from the study.

***Data Collection:*** Sociodemographic data was collected from the participants using a well-structured interviewer questionnaire.

***Anthropometric indices:*** The weight, in kilograms (kg) and height in meters (m) of the subjects were measured using the bathroom scale and Stadiometer respectively. Body mass index (BMI) was computed as the ratio of weight in kilogram (kg) to height in meters squared ($m^2$). Systolic (SBP) and diastolic blood pressure (DBP) were measured in

millimeter mercury (mmHg), using the sphygmomanometer.

***Study procedure:*** A standard venepuncture method was used to obtain 8mL of fasting venous blood from all participants after an overnight fast of 8–10 hours. Two milliliters (2 mL) of blood was dispensed into fluoride oxalate bottles for fasting plasma glucose. Two milliliters (4 mL) of blood was dispensed into $K_2$EDTA anticoagulated bottles for glycated hemoglobin estimation, while 2 mL was dispensed into plain sample bottles, allowed to clot and centrifuged at 3,000 rpm for 5 minutes at room temperature. The sera were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20°C until analysis.

***Sample size calculation:*** Sample size was determined according to the method of Monti.[22]

$$n = ((Z_\alpha + Z_\beta)^2 \times p(1-p)) / (p0 - p1)^2$$

- Zβ= Beta error at 80% power is 0.84
- Zα= Z value associated with 95% confidence level is 1.96, ie, (Alpha error at 95% level of confidence)
- P0= The anticipated probability of "exposure for people with the disease condition" (98%=0.98)
- P1= is the proportion/anticipated probability of "exposure for people without the disease condition" 86% = 0.86 (estimated from the postulated odds ratio)
- ṗ=mean proportion between cases and control group (p1+p2)/2 =0.98+0.86)/2=0.92

d = difference to be detected
(p0 – P1) = 0.98-0.86=0.12
$n = (1.96+0.84)^2 \times 0.92(0.08) / (0.12)^2 = 40$

A minimum sample of 40 Type two diabetes mellitus subjects is required, with an attrition of 10% added, 45 subjects were recruited.

**Laboratory methods**

**Determination of Fasting Plasma Glucose (FPG)**

Fasting plasma glucose was determined by enzymatic Glucose Oxidase–Peroxidase (GOD-PAP) method according to [23], using commercially available kit obtained from Agappe Diagnostics Ltd.

**Determination of Glycated Hemoglobin (HbA1c)**

Glycated hemoglobin was estimated using the column chromatographic method according to [24] using cation-exchange resin kits from Teco Diagnostics, USA. The percentage glycated hemoglobin was determined by measuring the absorbance at 420 nm of both the glycated hemoglobin fraction and the total hemoglobin fraction. The ratio of the two absorbance values gives the percent glycated hemoglobin.

**Determination of Serum Insulin**

Serum insulin concentration was determined using the ELISA method, obtained from Enzo Life Sciences Inc. Boulevard Farmingdale, NY, USA.

**Determination of Serum Iron, Copper, Zinc and Chromium**

Serum iron, copper, zinc and chromium were determined using Atomic Absorption Spectrophotometry (AAS) according to [25] methods.

**Determination of Superoxide Dismutase (SOD) Activity**

Superoxide dismutase activity was determined by the colorimetric method of [26]

**Computation of Insulin Resistance (HOMA-IR)**

Insulin resistance was calculated using the Homeostasis Model Assessment (HOMA-IR) according to the formula:

HOMA-IR = FPG (mmol/L) × Insulin (μU/mL) / 22.5

**Calculation of the predictive values of the evaluated indices**

Positive predictive value (PPV) = TP × 100 / (TP + FP)

Negative predictive value (NPV) = TN × 100 / (TN + FN)

Where TP = True positive, FP = False positive, TN = True negative and FN = False negative.

**Ethical approval**

Ethical approval was obtained from Health Research Ethics Committee, University of Calabar Teaching Hospital, Calabar. Protocol assigned number: UCTH/HREC/33/vol.111/147. Written

informed consent was obtained from all participants prior to inclusion in the study. The study was conducted in accordance with the ethical principles for medical research involving human subjects as outlined in the Helsinki Declaration of 1975 (revised 2000).

### Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD). Analyses of variance (ANOVA) was utilized in comparing group means, and Bonferroni post hoc analyses. Pearson's correlation was used to evaluate associations between the parameters studied. The Receiver Characteristic Operator Curve (ROC) was utilized to determine the performance metrics of the metals and the ratios between the metals in predicting poor outcomes in the test subjects. The Shapiro-Wilk test, Box and Whisker plots were utilized in determining the normality of the data. Gini index and Kolmogorov-Smirnov statistics were used to evaluate the performance of the models. The statistical significance was set at $p < 0.05$.

**TABLE 2: Comparison of Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase Trace minerals, and HOMA-IR in Diabetics with Cu/Zn ≤ 1.7ug/dl, Cu/Zn >1.7 ug/dl and controls.**

| Parameters | Cu/Zn ≤ 1.7 (n=20) | Cu/Zn > 1.7 (n=25) | Controls (n=40) | F | P | Effect Size |
|---|---|---|---|---|---|---|
| SBP (mmHg) | 122.00±3.73 | 120.08±3.34 | 124.87±9.31[a] | 3.895 | 0.024* | 0.082 |
| DBP (mmHg) | 77.00±6.69 | 74.76±4.18 | 75.49±8.85 | 0.528 | 0.591 | 0.012 |
| BMI (kg/m²) | 28.23±0.75 | 28.34±1.03 | 24.19±0.84 | 1.888 | 0.157 | 0.042 |
| FPG (mmol/L) | 6.91±1.08 | 8.30±1.04 | 4.33±0.74[b] | 164.916 | <0.001* | 0.798 |
| HbA1c (%) | 6.98±0.52 | 7.60±0.85 | 3.55±0.50[c] | 422.658 | <0.001* | 0.907 |
| Insulin (µU/ml) | 31.45 ± 2.85 | 31.13±2.98 | 13.36±1.96[d] | 589.163 | <0.001* | 0.931 |
| Zinc (µg/dL) | 107.00±29.82 | 56.44±7.48 | 111.62±22.19[e] | 57.149 | <0.001* | 0.569 |
| SOD (µ/ml) | 128.90±9.93 | 121.80±11.31 | 320.73±84.00[f] | 119.009 | <0.001 | 0.732 |
| Cu (µg/dL) | 121.00±20.55 | 155.28±25.39[g] | 121.24±6.35 | 36.149 | <0.001* | 0.454 |
| Fe (µg/dL) | 144.45±17.95 | 161.64±25.52[h] | 128.55±15.76 | 23.746 | <0.001* | 0.353 |
| Cr (µg/dL) | 0.59 ± 0.14 | 0.50±0.16 | 0.84±0.09[i] | 68.051 | <0.001* | 0.610 |
| Cu/Fe | 0.85±0.15 | 0.97±0.17 | 0.95±0.08[j] | 6.301 | 0.003* | 0.127 |
| Fe/Zn | 1.46±0.51 | 2.94 ± 0.74 | 1.21±0.39[k] | 87.781 | <0.001* | 0.669 |
| HOMA-IR | 9.68±2.00 | 11.53±1.91 | 1.54±0.42[l] | 389.355 | <0.001* | 0.900 |
| HOMA-IR/HbA1c | 1.39±0.26 | 1.53±0.24 | 0.73±0.14[m] | 152.870 | <0.001 | 0.778 |

**Key:** results are expressed as mean ± standard deviation, a=significantly lower in Cu/Zn >1.7 than controls, b, c, d, k, l, m=significantly lower in controls than in Cu/Zn >1.7 &≤1.7, e, f &i=significantly higher in controls than in Cu/Zn >1.7 &≤1.7, g, h, j=significantly lower in Cu/Zn ≤1.7 than in Cu/Zn >1.7 &controls. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FPG = fasting plasma glucose; HbA1c = glycated haemoglobin; Zn = serum zinc; Cu = serum copper; Fe = serum iron; Cr = serum chromium; SOD = superoxide dismutase; Cu/Zn = copper-to-zinc ratio; Cu/Fe = copper-to-iron ratio; Fe/Zn = iron-to-zinc ratio; HOMA-IR = homeostatic model assessment of insulin resistance. $p < 0.05$ was considered statistically significant.

**TABLE 1: Comparison of Age, Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase and trace minerals in Diabetics with HbA1c < 7%, diabetics with HbA1c ≥ 7% and Non-Diabetic Controls**

| Parameters | HbA1c < 7% (n = 19) | HbA1c ≥ 7% (n = 26) | Controls (n = 30) | F | P Val. |
|---|---|---|---|---|---|
| Age (years) | 47.73 ± 10.64 | 48.05 ±7.46 | 49.44 ± 7.63 | 0.732 | 0.484 |
| SBP (mmHg) | 121.32 ± 3.97 | 120.65±3.36 | 124.87±9.31[a] | 3.504 | 0.034 |
| DBP (mmHg) | 76.42 ± 6.34 | 75.27±4.85 | 75.4 ± 8.85 | 0.148 | 0.863 |
| BMI (kg/m²) | 28.43 ± 0.96 | 28.18 ±0.87 | 24.19 ± 0.84 | 1.928 | 0.152 |
| FPG (mmol/L) | 7.15 ± 1.00 | 8.07±1.30 | 4.33 ± 0.74[b] | 134.712 | <0.001 |
| INS (µU/mL) | 31.55± 1.62 | 31.11±3.56 | 13.36 ± 1.96[c] | 591.432 | <0.001 |
| Zn (µg/dL) | 92.37 ± 37.63 | 69.07±24.65 | 111.62 ± 22.19[d] | 20.949 | <0.001 |
| SOD (u/mL) | 124.53 ± 8.45 | 125.27±12.98 | 320.73 ± 84.00[e] | 118.724 | <0.001 |
| Cu (µg/dL) | 140.57 ±34.34 | 139.65±24.76 | 121.24 ± 6.35[f] | 9.036 | <0.001 |
| Fe (µg/dL) | 152.58±20.01 | 155.04±26.66 | 128.55 ± 15.76[g] | 17.723 | <0.001 |
| Cr (µg/dL) | 0.57 ± 0.14 | 0.52±0.17 | 0.84 ± 0.09[h] | 64.528 | <0.001 |
| Cu/Zn | 1.85 ± 1.00 | 2.28±0.88 | 1.13 ± 0.25[i] | 24.831 | <0.001 |
| Cu/Fe | 0.93 ± 0.22 | 0.91±0.13 | 0.95 ± 0.08 | 0.796 | 0.455 |
| Fe/Zn | 1.96 ± 0.85 | 2.52±1.01 | 1.21 ± 0.39[j] | 28.176 | <0.001 |
| HOMA-IR | 10.06 ±1.68 | 11.18±2.34 | 2.54 ± 0.42[k] | 335.731 | <0.001 |
| HOMA-IR/HbA1c | 1.49±0.24 | 1.44±0.27 | 0.73±0.14[l] | 143.020 | <0.001 |

**Key:** Results are expressed as mean ± standard deviation, a=significantly higher in controls than in diabetics HbA1c >7.0%, b, c, f, g, i, j, k, & l=significantly lower in controls than in diabetics, d, e, &h=significantly higher in controls than in the diabetics. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FPG = fasting plasma glucose; HbA1c = glycated haemoglobin; Zn = serum zinc; Cu = serum copper; Fe = serum iron; Cr = serum chromium; SOD = superoxide dismutase; Cu/Zn = copper-to-zinc ratio; Cu/Fe = copper-to-iron ratio; Fe/Zn = iron-to-zinc ratio; HOMA-IR = homeostatic model assessment of insulin resistance. $p < 0.05$ was considered statistically significant.

### Results

Comparison of Age, Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase and trace minerals in Diabetics with HbA1c < 7%, diabetics with HbA1c ≥ 7% and Non-Diabetic Controls is shown in Table 1. The Systolic blood pressure, FPG, insulin, Cu, Fe, Cu/Zn, Fe/Zn, Cr, Zn, SOD, HOMA-IR and HOMA-IR/HbA1c ratio varied significantly among the groups ($p<0.05$) while age, DBP, BMI and Cu/Fe did not vary significantly across the groups ($p>0.05$). SBP was significantly higher in controls than in diabetics with HbA1c>7.0% ($p<0.05$). The FPG, insulin, Cu, Fe, Fe/Zn Cu/Zn, HOMA-IR, and HOMA-IR/HbA1c were significantly lower in controls than in diabetics ($p<0.05$), while Zn, SOD and Cr were significantly higher in controls than in the diabetics ($p<0.05$).

Comparison of Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase Trace minerals, and HOMA-IR among

**TABLE 3: Comparison of Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase and Trace Minerals, and indices of insulin resistance in Diabetics with zinc ≤ 74ug/dl, Zinc >74ug/dl and controls**

| Parameters | Zinc <74 µg/dl (n=32) | Zinc >74 µg/dl (n=13) | Controls n=45 | F | P | Effect size |
|---|---|---|---|---|---|---|
| SBP (mmHg) | 120.03±3.04 | 123.15±4.04 | 124.86±9.31a | 4.438 | 0.015* | 0.095 |
| DBP (mmHg) | 74.53±3.89 | 78.77±7.60 | 75.49±8.85 | 1.581 | 0.212 | 0.035 |
| BMI (kg/m²) | 28.34±0.92 | 28.15±0.89 | 27.19±3.64 | 1.904 | 0.155 | 0.042 |
| FPG (mmol/L) | 8.06±1.13b | 6.77±1.12 | 4.33±0.74 | 148.308 | <0.001* | 0.773 |
| HbA1c (%) | 7.54±0.81 | 6.81±0.37 | 3.55±0.50 | 431.123 | <0.001* | 0.908 |
| Insulin (µU/ml) | 30.72±2.93 | 32.71±2.30 | 13.36±1.95 | 635.335 | <0.001* | 0.936 |
| SOD (u/ml) | 123.41±11.94 | 128.77±8.27 | 320.73±84.00 | 118.858 | <0001* | 0.732 |
| Cu (µg/dL) | 146.19±28.69 | 124.92±23.91 | 121.24±6.36 | 15..451 | <0.001* | 0.262 |
| Fe (µg/dL) | 156.53±26.31 | 147.77±15.49 | 128.56±15.76 | 18.840 | <0.001* | 0.302 |
| Cr (µg/dL) | 0.52 ± 0.16 | 0.59 ± 0.16 | 0.84±0.09 | 65.230 | <0.001* | 0.600 |
| Cu/Zn | 2.55 ± 0.73 | 0.98 ± 0.17 | 1.13±0.25 | 97.354 | <0.001* | 0.691 |
| Cu/Fe | 0.95 ± 0.18 | 0.85 ± 0.14 | 0.95±0.08 | 3.342 | <0.001* | 0.071 |
| HOMA-IR/HbA1c | 1.47±0.24 | 1.46±0.29 | 0.73±0.14 | 141.846 | <0.001 | 0.765 |

**Key:** Results are presented as mean ± standard deviation (SD). a=significantly higher in controls than in Zn ≤74ug/dL, b, & c =significantly higher in Zn ≤74ug/dL than in Zn ≥74ug/dL, d, =significantly higher in Zn ≥74ug/dL than in Zn ≤74ug/dL, e &h, = significantly lower in the test than in the controls, g=significantly higher in test than controls, f, I, k, & L, significantly higher in Zn ≤74ug/dL, than in Zn≥74ug/dl and controls, m=significantly lower in controls that in the test groups. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FPG = fasting plasma glucose; HbA1c = glycated haemoglobin; Insulin = serum insulin concentration; SOD = superoxide dismutase; Cu = serum copper; Fe = serum iron; Chr = serum chromium; Cu/Zn = copper-to-zinc ratio; Cu/Fe = copper-to-iron ratio; Fe/Zn = iron-to-zinc ratio; HOMA-IR = homeostatic model assessment of insulin resistance. p < 0.05 considered statistically significant.

**Table 4: Correlation between variables studied**

| Variables | r | p | 95% CI |
|---|---|---|---|
| HbA1c – FE/Zn | 0.582 | 0.000 | 0.342 – 0.745 |
| HbA1c – HOMA-IR | 0.475 | 0.001 | 0.206 – 0.672 |
| INSULIN – Cr | 0.326 | 0.029 | 0.032 – 0.563 |
| Zn – Cu | -0.383 | 0.009 | -0.605 – -0.096 |
| Zn – HOMA-IR | -0.406 | 0.006 | -0.623 – -0.124 |
| SOD – Cu/Zn | -0.324 | 0.030 | -0.561 – -0.030 |
| SOD – FE/Zn | -0.294 | 0.050 | -0.538 – 0.003 |
| SOD HOMA-IR | -0.342 | 0.022 | -0.575 -0.049 |
| Cu – FE | 0.473 | 0.001 | 0.203 – 0.670 |
| Cu – FE/Zn | 0.497 | 0.001 | 0.233 – 0.687 |
| FE Cr | -0.383 | 0.009 | -0.605 -0.097 |
| FE – Cu/Zn | 0.481 | 0.001 | 0.213 – 0.676 |
| Cr – Cu/Zn | -0.315 | 0.035 | -0.554 – -0.020 |
| Cr – FE/Zn | -0.445 | 0.002 | -0.650 – -0.169 |
| Cu/Zn – HOMA-IR | 0.426 | 0.004 | 0.147 – 0.637 |
| FE/Zn – HOMA-IR | 0.415 | 0.005 | 0.134 – 0.629 |
| SOD-HOMA-IR/HbA1c | -0.396 | 0.009 | -0.608 1.00 |
| Cr- HOMA-IR/HbA1c | 0.357 | 0.016 | 0.069-0.586 |

Diabetics with Cu/Zn ≤ 1.7ug/dl, Cu/Zn >1.7 ug/dl and controls is presented in Table 2. The SBP, FPG, HbA1c, insulin, Fe/Zn, HOMA-IR, HOMA-IR/HbA1c, Zn, SOD and Cr varied significantly among the groups (p<0.05). The SBP was significantly lower in diabetics with Cu/Zn >1.7 than controls (p<0.05), FPG, HbA1c, insulin, Fe/Zn, HOMA-IR, and HOMA-IR/HbA1c were significantly lower in controls than in diabetics with Cu/Zn >1.7 and ≤1.7 (p<0.05). The levels of Zn, SOD and Cr were significantly higher in controls than in diabetics with Cu/Zn >1.7 & ≤1.7, Cu, Fe and Cu/Fe were significantly lower in diabetics with Cu/Zn ≤1.7 than in diabetics with Cu/Zn >1.7 and the controls.

The Comparison of Blood Pressure, Indices of Glycemic Control, Superoxide Dismutase and Trace Minerals, and indices of insulin resistance in Diabetics with zinc ≤ 74 ug/dl, Zinc >74 ug/dl and controls is shown in Table 3. The SBP was significantly higher in controls than in diabetics with Zn ≤74ug/dL(p<0.05). The levels of FPG and HbA1c were significantly higher in diabetics with Zn ≤74 ug/dL than in diabetics with Zn >74 ug/dL (p<0.05), insulin level was significantly higher in diabetics with Zn >74 ug/dL than in diabetics with Zn ≤74 ug/dL (p<0.05), SOD and Cr levels were significantly lower in the diabetics than in the controls (p<0.05), Fe was significantly higher in diabetics than controls, Cu, Cu/Zn, Fe/Zn, and

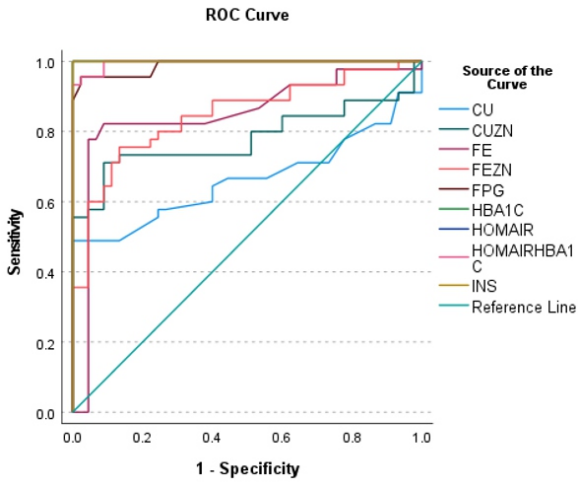


**Figure 1: Receiver operator curve of Copper-Zinc ratio, Fasting plasma glucose, Glycated haemoglobin, Homeostatic Model Assessment of Insulin Resistance, Insulin and Superoxide Dismutase in subject with type 2 diabetes mellitus.**

**Table 5: The performance metrics of the variable studied**

| Variables | Area | S.E | P | 95% C.I | Gini index | Max K-S Metric | Pot. Cutoff | Sen. (%) | 1-spec | Spec. (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FPG (mmol/l) | 0.989 | 0.008 | 0.000 | 0.973 1.005 | 0.977 | 0.933 | 5.750 | 95.6 | 0.022 | 97.8 | 97.8 | 95.7 |
| HbA1c (%) | 1.000 | 0.000 | 0.000 | 1.000 1.000 | 1.000 | 1.000 | 5.700 | 100.0 | 0.000 | 100.0 | 100.0 | 100.0 |
| INS (µU/mL) | 1.000 | 0.000 | 0.000 | 1.000 – 1.000 | 1.000 | 1.000 | 20.800 | 100.0 | 0.000 | 100.0 | 100.0 | 100.0 |
| Cu (µg/dL) | 0.663 | 0.061 | 0.008 | 0.543 – 0.783 | 0.326 | 0.489 | 132.000 | 48.9 | 0.000 | 100.0 | 100.0 | 65.7 |
| Fe (µg/dL) | 0.847 | 0.046 | 0.000 | 0.757 – 0.938 | 0.695 | 0.733 | 137.500 | 77.8 | 0.044 | 95.6 | 95.0 | 80.8 |
| Cu/Zn | 0.781 | 0.053 | 0.000 | 0.676 – 0.886 | 0.562 | 0.622 | 1.558 | 71.1 | 0.089 | 91.1 | 89.2 | 74.1 |
| Fe/Zn | 0.847 | 0.042 | 0.000 | 0.765 0.929 | 0.694 | 0.622 | 1.408 | 75.6 | 0.133 | 86.7 | 85.7 | 77.6 |
| HOMA-IR | 1.000 | 0.000 | 0.000 | 1.000 – 1.000 | 1.000 | 1.000 | 4.847 | 100.0 | 0.000 | 100.0 | 100.0 | 100.0 |
| HOMA-IR/HbA1c | 0.996 | 0.004 | 0.000 | 0.998- 1.003 | 0.991 | 0.933 | 1.146 | 93.3% | 0.000 | 100.0 | 100.0 | 93.7% |

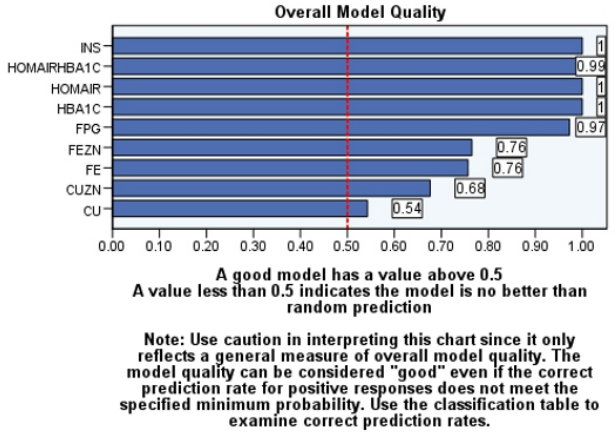


**Figure 2: The overall performance summary of the variables in diagnoses and prediction of insulin resistance and diabetic outcomes**

HOMA-IR, were significantly higher in Zn ≤74ug/dL, than in Zn≥74ug/dl and controls, HOMA-IR/HbA1c was significantly higher Zn ≤74ug/dL and Zn >74ug/dl than in controls ($p<0.05$).

The HbA1c correlated positively and significantly with Fe/Zn ($r=0.582$, $p=0.000$) and HOMA-IR ($r=0.475$, $p=0.001$). The Zn correlated negatively and significantly with Cu and HOMA-IR ($r=-0.383$, $p=0.009$ and $r=-0.406$, $p=0.006$ respectively. The SOD correlated negatively and significantly with Cu/Zn ($r=-0.324$, $p=0.030$) and HOMA-IR ($r=-0.342$, $p=0.022$). The Cu correlated positively and significantly with Fe ($r=0.473$, $p=0.001$) and Fe/Zn ($r=0.497$, $p=0.001$). Fe correlated negatively and significantly with chromium ($r=-0.383$, $p=0.009$) but positively and significantly with Cu/Zn ($r=0.481$, $p=0.001$). Cu/Zn and Fe/Zn correlated positively and significantly with HOMA-IR ($p<0.05$).

The area under the curve (AUC) receiver operator characteristic curve of fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), insulin, copper (Cu), iron (Fe), copper-to-zinc ratio (Cu/Zn), iron-to-zinc ratio (Fe/Zn) and homeostatic model assessment of insulin resistance (HOMA-IR) among subjects with type 2 diabetes mellitus is represented in Figure 1. HOMA-IR (AUC=1.000, SE=0.000, P=0.000, CI=1.000–1.000), HbA1c (AUC=1.000, SE=0.000, P=0.000, CI=1.000–1.000) and insulin (AUC=1.000, SE=0.000, P=0.000, CI=1.000–1.000) were observed. FPG (AUC=0.989, SE=0.008, P=0.000, CI=0.973–1.005), Fe (AUC=0.847, SE=0.046, P=0.000, CI=0.757–0.938), Fe/Zn (AUC=0.847, SE=0.042, P=0.000, CI=0.765–0.929), Cu/Zn (AUC=0.781, SE=0.053, P=0.000, CI=0.676–0.886) and Cu (AUC=0.663, SE=0.061, P=0.008, CI=0.543–0.783) were also observed. The positive and negative predictive values of HOMA-IR, HbA1c and insulin were PPV=100 % and NPV=100 % respectively. FPG had PPV=97.8 % and NPV=95.7 %. Iron had PPV=95.0 % and NPV=80.8 %, while Fe/Zn had PPV=85.7 % and NPV=77.6 %. Cu/Zn showed PPV=89.2 % and NPV=74.1 %, whereas Cu had PPV=100 % and NPV=65.7 %. The overall test efficiency for HOMA-IR, HbA1c and insulin was 100 %, while FPG, Fe, Fe/Zn, Cu/Zn and Cu were 96.7 %, 86.7 %, 81.7 %, 80.0 % and 73.3 % respectively as presented in Figure 2.

## Discussion

Type 2 diabetes mellitus (T2DM) remains one of the most important global metabolic disorders despite advances in pharmacotherapy and lifestyle interventions.27 Exploring the intersection of trace minerals and glycemic control may offer insight beyond the impact of simple deficiency models toward understanding the role of trace minerals in complex signaling pathways that mediate progressive insulin resistance. This study

investigated serum copper (Cu), zinc (Zn), iron (Fe), chromium (Cr), superoxide dismutase (SOD), and derived ratios such as Cu/Zn, Cu/Fe and Fe/Zn, and evaluated their predictive accuracy for insulin resistance and glycaemic imbalance in patients with T2DM.

The significantly higher FPG, insulin, HOMA-IR, and HOMA-IR/HbA1c observed among diabetics with HbA1c>7.0% suggests poor glycaemic control and marked insulin resistance. Persistent hyperglycaemia increases mitochondrial production of reactive oxygen species, impairs insulin receptor signaling and accelerates β-cell dysfunction.[28] These processes promote systemic oxidative stress and chronic inflammation. This observation is consistent with previous reports demonstrating that oxidative stress plays a central role in insulin resistance and progression of T2DM.[19,27-29]

The elevated levels of Cu, Fe, Fe/Zn, Cu/Zn, and reduced SOD, Cr and Zn in diabetics with HbA1c>7.0% demonstrate the roles of these trace minerals in insulin sensitivity, synthesis and signaling. Elevated Cu and Fe levels act as antioxidant pro-oxidant contribute to oxidative stress impaired glycemic control leading to increased insulin resistance and protein glycation.[30-32] Decrease in the levels of Zn and Cr may be due to increased urinary loss (hyperzinccuria) of zinc and poor nutrition as well as the associated obesity and chronic nature of T2DM. This observation is in line with previous studies,[33-36] which demonstrated that zinc is required for normal insulin secretion and zinc deficiency in pancreatic β cells is involved in abnormal insulin secretion. Similarly, a previous study,[37] had demonstrated that chromium enhances insulin receptor phosphorylation and improves glucose uptake via PI3K/Akt signaling pathways as well as altered gene expression essential for glucose uptake. Reduced chromium levels may therefore aggravate insulin resistance. These findings agree with previous studies which reported that deficiencies of zinc and chromium are strongly associated with impaired glycaemic control and metabolic instability in T2DM patients.[38,39] Thus, reduced cellular glucose uptake as a result of the combination of low insulin production (due to low Zn) and reduced insulin receptor sensitivity (due to low Cr) suggestively lowers the body's ability to move glucose into cells. Also, the deficiencies of both Cr and Zn are linked to increased oxidative stress and inflammation, which are central in the pathogenesis of type 2 diabetes. The balance between 'bad' trace minerals: Cu, Fe and the 'good' trace minerals: Cr and Zn can serve as a 'trace mineral fingerprint' for assessing glycemic control and insulin resistance in T2DM.[40]

The significantly elevated Cu/Zn and Fe/Zn ratios observed in diabetic subjects with HbA1c >7%, may indicate a shift toward a pro-oxidant internal environment. Dysregulation in Cu and Fe serve as redox-active metals capable of catalyzing reactive oxygen species generation through Fenton and Haber–Weiss reactions. When present in excess relative to zinc, these metals may amplify oxidative stress and endothelial dysfunction.[41] The positive correlations between Fe/Zn ratio and HbA1c, between Cu/Zn ratio and HOMA-IR, and the negative association between SOD and HOMA-IR/HbA1c, SOD and Cu/Zn, SOD and Fe/Zn suggest that elevated levels iron and copper and reduced zinc levels, may be responsible for the increased oxidative stress (lower SOD), associated with poor glycemic control (higher HbA1c) and increased insulin resistance (higher HOMA-IR) and HOMA-IR/HbA1c in T2DM. That is, the Cu:Zn and Fe/Zn ratios correlate directly with the degradation of SOD activity, and these ratios also can serve as 'trace mineral fingerprint' for rising insulin resistance. These findings suggest that a significant imbalance in trace elements and reduced antioxidant defense mechanisms are linked to the pathogenesis and progression of T2DM. The balance in these trace elements levels may improve insulin synthesis, stability, insulin receptor expression and function as well as SOD activity in T2DM. These findings are similar to those of previous studies,[42,43] that described trace element imbalance as a contributing factor to oxidative damage in diabetes. Superoxide dismutase activity was significantly reduced in the diabetic subjects, suggesting altered antioxidant defense in T2DM. The SOD is a key enzymatic antioxidant responsible for scavenging superoxide radicals.

The significantly higher levels of FPG, HbA1c, insulin, Fe/Zn, HOMA-IR, and HOMA-IR/HbA1c in T2DM with Cu/Zn >1.7 and Cu/Zn ≤1.7 when compared with the controls suggest that increased

derangement in glycemic control (HbA1c) and insulin resistance (HOMA-IR or HOMA-IR: HbA1c), is associated with elevation in the unbound serum Cu and Fe as well as decreases in the antagonistic Zn may be responsible for secondary oxidative stress (Fenton reactions) that worsens insulin resistance. A Cu/Zn level ≥ 22.13 and an Fe/Zn of ≥2.0 is predictive of rising insulin resistance and may complement HbA1c, HOMA-IR and HOMA-IR/HbA1c for long-term monitoring of glycaemic control and insulin resistance. The reduced levels of Zn, SOD and Cr in diabetics with Cu/Zn >1.7 and Cu/Zn ≤1.7 than in the controls suggest that increased hyperglycaemia and insulin resistance (hyperinsulinaemia) may also cause glycation and degradation of the SOD protein structure with the release of its metal cofactors (Cu, Fe). Also, Cr may serve as a "buffer" that prevents the downregulation of insulin receptors during periods of hyperinsulinemia (high serum insulin). The levels of Cr correlated positively with HOMA-IR/HbA1c ratio, suggesting that Cr-repleted T2DM subjects may maintain better glucose control despite high levels of insulin resistance, hence lower Cr levels affect insulin receptor function and insulin sensitivity. This observation is similar to that of a previous study,[44] which reported alteration in trace minerals homeostasis in T2DM patients to vary with glycemic control.

The Receiver Operating Characteristic (ROC) curve analysis showed that HOMA-IR, FPG, insulin, HbA1c, HOMA-IR/HbA1c had the highest diagnostic accuracy, as expected. However, Cu/Zn and Fe/Zn ratios also demonstrated good predictive performance. A favorable diagnostic model should possess high sensitivity and specificity, ideally with an AUROC approaching 0.900. Although glycaemic indices remain superior diagnostic tools, the appreciable AUROC values observed for Cu/Zn and Fe/Zn ratios suggest that these indices may serve as useful adjunct markers. The performance of FPG, HbA1c, insulin, HOMA-IR, HOMA-IR/HbA1c, Cu, Fe, Cu/Zn and Fe/Zn in the diagnosis and prediction of good glycaemic control as demonstrated by the binary classifier models such as Gini index and Kolmogorov-Smirnov statistics with values approaching 1, shows that the models have good performances and ability to distinguish between good and bad glycemic control, thereby effectively predicting outcomes. This "trace mineral fingerprints" may complement HbA1c and HOMA-IR in monitoring glycaemic control and rising insulin resistance in subjects with T2DM.

**Limitations:** This study has certain limitations. The relatively small sample size may limit generalizability of the findings. Dietary intake, inflammatory markers and long-term supplementation history were not comprehensively assessed. Larger longitudinal studies are needed to establish causality and therapeutic implications.

**Conclusion:** The findings of this study suggest that imbalance in the levels of copper, zinc, iron and chromium is associated with oxidative stress, poor glycaemic control and increased insulin resistance in T2DM. Elevated levels of copper, iron, Cu/Zn, and Fe/Zn ratios and decreased levels of chromium, superoxide dismutase and zinc are associated with oxidative stress, poor glycaemic control and rising insulin resistance. This 'trace mineral fingerprint' may complement HbA1c in monitoring glycaemic control and in the prediction of rising insulin resistance. The management of T2DM should include a "Mineral Panel" to identify patients at risk of rapid progression, despite a temporarily stable fasting plasma glucose level.

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