

Comparative Evaluation of Micronutrients, Trace Elements, Inflammatory Markers and Glycemic Parameters in Patients With Type 2 Diabetes Mellitus and Healthy Controls

Jyoti Pathak¹, Dr Jaya Jain¹, Dr Ashutosh Jain¹, Dr.Ved Prakash²

¹Research Scholar, Department of Biochemistry, Index Medical College Hospital and Research Centre Indore, Pathakj890@gmail.Com

¹Professor, Department of Biochemistry, Index Medical College Hospital and Research Centre Indore, Jain02jaya@gmail.Com

¹Assistant Professor, Department of Physiology, Index Medical College Hospital and Research Centre Indore, Ashutosh.Jain689gmail.Com

²Assistant Professor, Department of Physiology, MMCMSR ,Sadopur Ambala, Vedjnr23@gmail.Com

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is associated with chronic hyperglycemia, inflammation, and metabolic abnormalities. Altered micronutrient and trace-element status contribute to impaired glycemic control and inflammatory activity. **Objective:** To compare glycemic parameters, micronutrients, trace elements, C-reactive protein (CRP), and adiponectin levels between patients with T2DM and apparently healthy controls. **Methods:** This hospital-based case-control study included 244 participants aged 25-60 years: 122 patients with T2DM and 122 apparently healthy controls. Fasting plasma glucose was estimated using the glucose oxidase-peroxidase method, while HbA1c was measured by immunoturbidimetry. Serum vitamin D, calcium, copper, iron, phosphorus, magnesium, CRP, and adiponectin were estimated using standard laboratory methods. Data were analysed using SPSS version 28.0. Group comparisons were performed using appropriate statistical tests, and $p < 0.05$ was considered statistically significant. **Results:** Patients with T2DM had significantly higher fasting plasma glucose (172.4 ± 36.2 vs. 91.6 ± 10.4 mg/dL), HbA1c ($8.6 \pm 1.5\%$ vs. $5.3 \pm 0.6\%$), CRP (7.2 ± 2.6 vs. 2.4 ± 1.3 mg/L), and copper levels (148 ± 20 vs. 112 ± 16 µg/dL) than controls (all $p < 0.001$). Vitamin D, calcium, magnesium, phosphorus, iron, and adiponectin were significantly lower in the T2DM group ($p < 0.001$). Vitamin D, magnesium, and adiponectin showed inverse correlations with HbA1c and CRP, whereas copper and CRP showed positive correlations with HbA1c. **Conclusion:** T2DM was associated with poor glycemic control, micronutrient imbalance, altered trace-element status, increased inflammation, and reduced adiponectin. Comprehensive biochemical assessment provide useful additional information in the evaluation of patients with T2DM.

Keywords:

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia caused by insulin resistance, progressive pancreatic β -cell dysfunction, and inadequate insulin secretion. It is one of the most important non-communicable diseases worldwide because of its increasing prevalence and association with substantial morbidity, mortality, and economic burden (1). The prevalence of T2DM is increasing rapidly in India due to urbanization, sedentary lifestyles, unhealthy dietary patterns, obesity, genetic predisposition, and population ageing. Although

hyperglycemia is the principal biochemical feature of T2DM, the condition is not restricted to glucose dysregulation. It is a complex metabolic disorder involving oxidative stress, chronic low-grade inflammation, endothelial dysfunction, altered adipokine secretion, and disturbances in micronutrient and trace-element homeostasis (2). According to the International Diabetes Federation (IDF) Diabetes Atlas, 11th edition (2025), approximately 589 million adults aged 20–79 years were living with diabetes worldwide in 2024, corresponding to a global prevalence of 11.1%

(approximately 1 in 9 adults). The global number of adults living with diabetes is projected to increase to approximately 853 million by 2050, while around 81% of adults with diabetes currently live in low- and middle-income countries (3). India represents a substantial proportion of the global diabetes burden, with approximately 90.0 million adults aged 20–79 years living with diabetes in 2024, corresponding to an age-standardised prevalence of 10.5%. India therefore accounts for approximately 1 in 7 adults living with diabetes worldwide, and the number of adults with diabetes in the country is projected to increase to approximately 157 million by 2050, with the age-standardised prevalence projected to reach 12.8%. In comparison, the World Health Organization (WHO) reported that approximately 830 million people worldwide were living with diabetes in 2022, with the prevalence among adults aged ≥ 18 years increasing from 7% in 1990 to 14% in 2022 (4).

Persistent hyperglycemia contributes to diabetic complications through multiple interconnected pathways, including increased formation of advanced glycation end products, activation of the polyol and protein kinase C pathways, mitochondrial dysfunction, and increased generation of reactive oxygen species. These processes lead to oxidative damage of proteins, lipids, DNA, pancreatic β -cells, and vascular tissues. Oxidative stress also activates inflammatory signalling pathways, which can worsen insulin resistance and contribute to the development of microvascular and macrovascular complications such as nephropathy, retinopathy, neuropathy, and cardiovascular disease (5,6). Therefore, assessment of metabolic and inflammatory abnormalities is important for understanding the overall biochemical profile of patients with T2DM.

Fasting plasma glucose and glycated hemoglobin (HbA1c) are widely used markers for evaluating glycemic control. Fasting plasma glucose reflects current glucose status, whereas HbA1c represents the average blood glucose concentration over the preceding two to three months. Elevated HbA1c is associated with a greater risk of diabetes-related complications and is a valuable indicator of long-term glycemic control. Chronic hyperglycemia may itself alter the metabolism and distribution of several vitamins, minerals, and trace elements through increased urinary losses, impaired intestinal absorption, altered renal handling, oxidative stress, and inflammatory responses. Thus, micronutrient abnormalities in T2DM may be both a consequence and a contributor to poor metabolic control (7).

Vitamin D has received considerable attention because of its possible role in pancreatic β -cell function, insulin secretion, insulin sensitivity, calcium regulation, and immunomodulation. Vitamin D receptors are expressed in pancreatic β -cells and peripheral tissues involved in glucose metabolism. Low serum vitamin D

concentrations have frequently been reported among patients with T2DM and have been associated with poor glycemic control, insulin resistance, and increased risk of diabetic complications. However, evidence from supplementation studies remains inconsistent, indicating that vitamin D status may be influenced by obesity, dietary intake, sunlight exposure, physical activity, renal function, and disease duration (8,9). Nevertheless, vitamin D remains an important micronutrient for investigation in patients with T2DM.

Magnesium is another essential mineral involved in glucose metabolism and insulin action. It acts as a cofactor for enzymes required for carbohydrate metabolism, cellular energy production, insulin-receptor phosphorylation, and glucose transport. Hypomagnesemia is commonly observed in patients with T2DM, particularly in those with poor glycemic control, and may occur because of increased renal magnesium loss during hyperglycemia. Low magnesium levels may impair insulin signalling and promote insulin resistance, creating a cycle in which poor glycemic control and magnesium depletion worsen each other. A systematic review and meta-analysis has demonstrated that hypomagnesemia is common in individuals with T2DM, while clinical evidence suggests that magnesium supplementation may improve glycemic parameters in selected patients (10,11). Calcium and phosphorus are also essential for insulin secretion, intracellular signalling, bone metabolism, cellular energy generation, and membrane stability. Calcium is required for normal insulin release from pancreatic β -cells, while phosphorus contributes to adenosine triphosphate production and cellular energy metabolism. Altered calcium and phosphorus homeostasis in T2DM may result from vitamin D deficiency, dietary factors, renal impairment, medication use, and chronic metabolic dysregulation. Therefore, simultaneous measurement of vitamin D, calcium, phosphorus, and magnesium may provide a broader understanding of mineral metabolism in diabetic individuals (12).

Trace elements such as iron and copper have a dual role in T2DM. Iron is essential for oxygen transport, mitochondrial metabolism, and enzymatic reactions; however, excess iron may increase oxidative stress through the generation of reactive free radicals. Studies have reported associations between higher iron stores, particularly ferritin concentrations, and the risk of T2DM, although serum iron alone should be interpreted carefully because it is influenced by inflammation, dietary intake, and iron-binding proteins (13,14). Copper is necessary for several enzymes, including antioxidant enzymes such as superoxide dismutase. However, excessive copper may promote oxidative damage through redox reactions. A meta-analysis of serum and plasma studies has reported altered copper status in diabetes, supporting the need to evaluate copper along with other metabolic markers (15).

Chronic low-grade inflammation is a key feature of T2DM and contributes substantially to insulin resistance, vascular dysfunction, and diabetic complications. C-reactive protein (CRP) is an acute-phase reactant and a widely used marker of systemic inflammation. Elevated CRP concentrations have been associated with obesity, insulin resistance, hyperglycemia, and an increased risk of T2DM. A systematic review and meta-analysis of prospective studies demonstrated a significant association between elevated CRP concentrations and incident T2DM (16). In contrast, adiponectin is an adipose tissue-derived hormone with insulin-sensitizing, anti-inflammatory, and anti-atherogenic properties. Lower adiponectin concentrations are frequently observed in obesity and T2DM and are associated with impaired glucose metabolism. A systematic review and meta-analysis reported that higher adiponectin levels are associated with a lower risk of developing T2DM (17).

The relationship among micronutrients, trace elements, glycemic control, inflammation, and oxidative stress is complex and bidirectional. Deficiencies in vitamin D and magnesium may impair insulin secretion, insulin sensitivity, and antioxidant defence. Alterations in iron and copper may influence oxidative stress and inflammatory activity. In turn, chronic inflammation and

hyperglycemia can disrupt mineral metabolism and accelerate metabolic deterioration. These interactions may ultimately contribute to vascular injury and the development of diabetic complications. However, as micronutrient concentrations may be influenced by diet, body mass index, medication use, renal function, and disease duration, observed associations should not be interpreted as proving causality. Previous studies have often assessed vitamin D, magnesium, iron, copper, CRP, or adiponectin separately. This approach may not fully explain the interconnected biochemical disturbances present in T2DM (18–20). Therefore, the present hospital-based case-control study was designed to compare fasting plasma glucose, HbA1c, vitamin D, calcium, magnesium, phosphorus, iron, copper, CRP, and adiponectin between 122 patients with T2DM and 122 apparently healthy controls aged 25–60 years. In comparison with earlier studies that examined individual micronutrients or single inflammatory markers, the present study evaluates multiple micronutrients, trace elements, glycemic indices, CRP, and adiponectin within the same cohort. This integrated analysis may provide a clearer understanding of the association between nutritional status, inflammation, and glycemic control in T2DM. The study workflow is presented in **Figure 1**

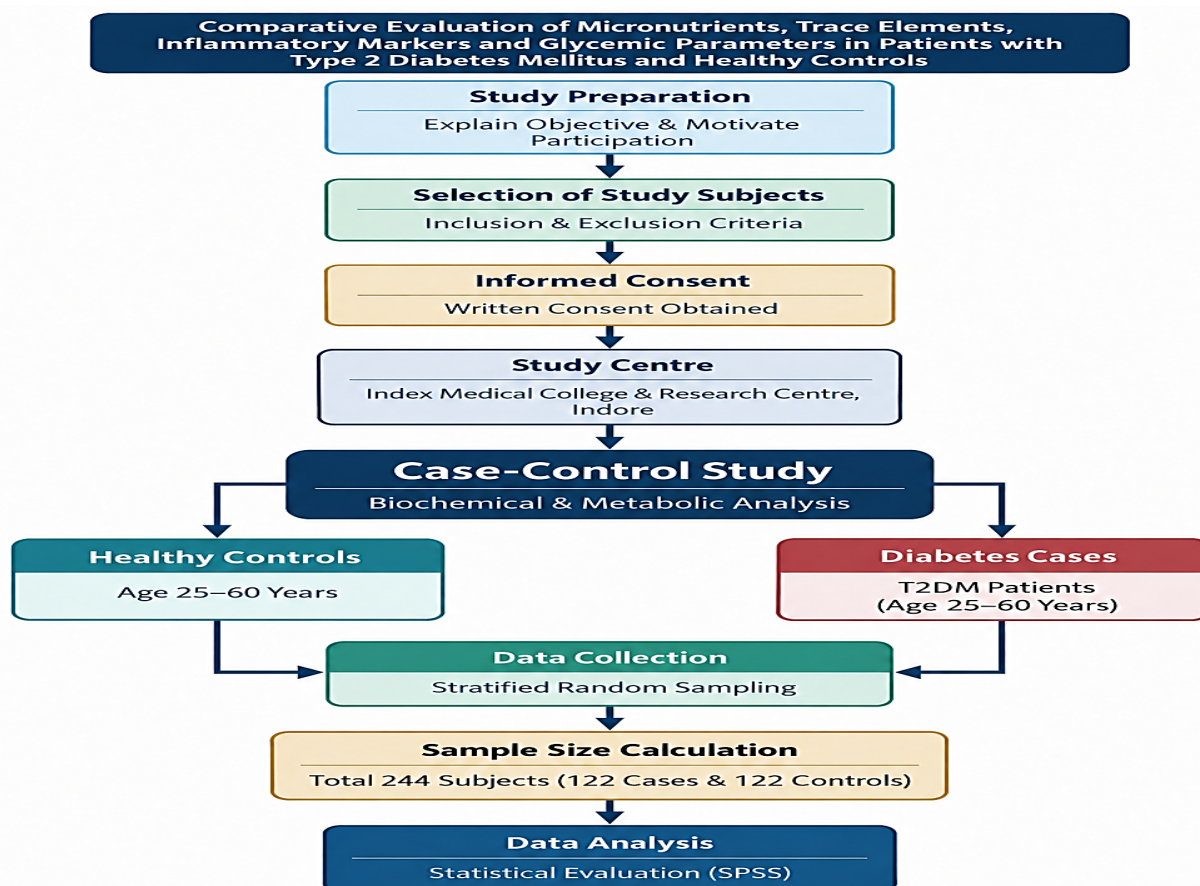


Figure 1. Schematic representation of the methodological workflow employed in the present study, including participant selection, blood sample collection, estimation of glycemic parameters, micronutrients, trace elements, CRP, and adiponectin, followed by comparative statistical analysis between patients with type 2 diabetes mellitus and apparently healthy controls.

MATERIALS AND METHOD

2.1. Study Design and Study Population

A hospital-based case-control study was conducted in the Department of Biochemistry, Central Research Laboratory, and Central Clinical Laboratory of Index Medical College & Research Centre, affiliated with Malwanchal University, Indore, Madhya Pradesh, India. The institution is a tertiary-care teaching hospital located in Index City, NH-59A, Nemawar Road, District Indore, Madhya Pradesh (452016), India. The study was designed to comparatively evaluate glycemic parameters, micronutrients, trace elements, and inflammatory and metabolic markers among patients with type 2 diabetes mellitus (T2DM) and apparently healthy controls. A total of 244 participants aged 25–60 years were enrolled, comprising 122 patients with T2DM (case group) and 122 apparently healthy individuals (control group). Participants were selected according to predefined inclusion and exclusion criteria.

The sample size was determined using the standard formula (21) for estimation of sample size for a proportion:

$$n = \frac{Z^2 P(1 - P)}{d^2}$$

where n represents the required sample size, Z is the standard normal deviate corresponding to a 95% confidence level (1.96), P is the expected prevalence/proportion, and d represents the desired precision.

An expected prevalence of 8.7%, as reported for diabetes in India in the IDF Diabetes Atlas 2021, and a precision of 5% were considered for the calculation (22). The calculated sample size was approximately 122 participants per group. Accordingly, 122 participants were recruited into the T2DM case group and 122 into the health control group, giving a total sample size of 244 participants.

2.2 Clinical Examination, Sampling and Data Collection

All participants underwent clinical assessment according to the study protocol. Relevant demographic characteristics, medical history, diabetes status, and available clinical and laboratory information were recorded using a structured data-collection format. Participants in the case group were identified based on a documented diagnosis of T2DM and relevant clinical and laboratory findings, whereas the control group comprised apparently healthy individuals without a known history of diabetes or relevant comorbidities. Participants were

recruited through the outpatient and clinical services of the study centre after fulfilment of the eligibility criteria.

2.3 Ethical Approval and Informed Consent

The study protocol was reviewed and approved by the Institutional Ethics Committee of Index Medical College & Research Centre, Malwanchal University, Indore, before commencement of the study. The study was conducted in accordance with applicable ethical principles for research involving human participants and the Declaration of Helsinki. The objectives, procedures, and requirements of the study were explained to the participants, and written informed consent was obtained before enrolment and sample collection. Confidentiality of participant information was maintained throughout the study.

2.4 Inclusion and Exclusion Criteria

Participants aged 25–60 years of either sex were eligible for inclusion. The case group consisted of individuals diagnosed with T2DM, while apparently healthy individuals without diabetes or relevant comorbidities were included as controls. Participants were enrolled only after providing written informed consent. Individuals with type 1 diabetes mellitus, pancreatic disease, renal disease, chronic systemic illness, or conditions or treatments likely to substantially influence blood glucose or the biochemical parameters under investigation were excluded. Individuals receiving micronutrient supplements that could affect the measured micronutrient concentrations were also excluded.

2.5 Sample Collection and Processing

Fasting venous blood samples were collected from the participants under aseptic conditions by trained laboratory personnel. Approximately 5 mL of venous blood was collected using appropriate collection tubes according to the requirements of the respective investigations. For serum-based assays, blood collected in plain tubes was allowed to clot at room temperature for approximately 15–20 min, followed by centrifugation at 2,000–3,000 rpm for 10 min to separate the serum. For fasting plasma glucose estimation, blood was collected in an appropriate fluoride-containing tube to inhibit glycolysis, whereas EDTA-anticoagulated whole blood was used for HbA1c estimation. The separated serum/plasma and whole-blood samples were processed and stored according to the requirements of the respective assays until analysis. Hemolyzed samples were avoided wherever applicable.

2.6 Estimation of Fasting Plasma Glucose

Fasting plasma glucose was estimated by the glucose oxidase–peroxidase (GOD–POD) method. The method is based on enzymatic oxidation of glucose-by-glucose oxidase to form gluconic acid and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide reacts with phenol and 4-aminoantipyrine to produce a colored quinoneimine compound. The intensity of the developed color is proportional to the glucose concentration and was measured spectrophotometrically at approximately 505 nm. The assay was performed using the appropriate commercial reagent kit and standard according to the manufacturer's instructions (23).

2.7 Estimation of Glycated Hemoglobin (HbA1c)

HbA1c was estimated using an immunoturbidimetric method. The assay is based on the specific antigen–antibody reaction between HbA1c and antibodies directed against glycated hemoglobin. EDTA-anticoagulated whole-blood samples were processed using the appropriate hemolysing reagent, and the resulting immune reaction produced turbidity proportional to the HbA1c concentration (24). HbA1c was measured using an automated biochemical analyzer, with the result expressed as a percentage of total hemoglobin. The assay was performed according to the manufacturer's recommended protocol.

2.8 Estimation of Serum Vitamin D

Total serum 25-hydroxyvitamin D [25(OH)D] was quantitatively estimated using a fluorescence immunoassay/enzyme-linked fluorescent assay (ELFA) based on a competitive antigen–antibody reaction. Serum 25(OH)D competes with a fluorescently labelled vitamin D analogue for specific antibody-binding sites. The resulting fluorescence signal is inversely related to the concentration of vitamin D in the sample (25). The analysis was performed using the Mini VIDAS immunoassay analyzer or the analyzer used in the laboratory, following the manufacturer's instructions.

2.9 Estimation of Serum Calcium

Serum calcium was estimated using the O-cresolphthalein complexone (OCPC) method. In an alkaline medium, calcium ions react with O-cresolphthalein complexone to form a purple-colored complex. The intensity of the color is directly proportional to the calcium concentration and was measured spectrophotometrically at approximately 570–580 nm (26). Serum calcium was estimated using the appropriate commercial reagent system according to the manufacturer's protocol.

2.10 Estimation of Serum Copper

Serum copper was estimated by a colorimetric method based on the formation of a colored complex between

copper ions and the specific chromogenic reagent. Protein-bound copper is released under acidic conditions, followed by reduction of Cu^{2+} to Cu^{+} and subsequent complex formation with the chromogen. The intensity of the resulting color is proportional to the copper concentration and was measured spectrophotometrically at the wavelength specified by the assay protocol, approximately 540–580 nm (27). The assay was performed using the appropriate commercial reagent system according to the manufacturer's instructions.

2.11 Estimation of Serum Iron

Serum iron was estimated using the ferrozine colorimetric method. In an acidic medium, iron is released from transferrin and ferric ions (Fe^{3+}) are reduced to ferrous ions (Fe^{2+}). The ferrous ions react with ferrozine to form a colored complex, the intensity of which is directly proportional to the concentration of iron in the sample (28). Absorbance was measured at approximately 560–562 nm, and serum iron concentration was calculated against the appropriate standard/calibration curve.

2.12 Estimation of Serum Phosphorus

Serum inorganic phosphorus was estimated using the ammonium molybdate method. In an acidic medium, inorganic phosphate reacts with ammonium molybdate to form a phosphomolybdate complex. The resulting reaction product was measured spectrophotometrically at the wavelength specified by the analytical procedure, and the phosphorus concentration was calculated using the corresponding standard/calibrator. Non-hemolyzed serum samples were used for analysis to minimize interference from intracellular phosphate (29).

2.13 Estimation of Serum Magnesium

Serum magnesium was estimated using the xylidyl blue method. Magnesium ions react with xylidyl blue under alkaline conditions to form a colored magnesium–xylidyl blue complex. EGTA present in the reagent system binds calcium ions and minimizes calcium interference. The intensity of the developed color is proportional to the magnesium concentration and was measured spectrophotometrically at approximately 520–550 nm (30). The concentration was calculated using the appropriate magnesium standard/calibration procedure.

2.14 Estimation of C-Reactive Protein

Serum C-reactive protein (CRP) was estimated using a turbidimetric immunoassay. The method is based on an antigen–antibody agglutination reaction in which CRP in the serum reacts with anti-CRP antibodies associated with latex particles, resulting in the formation of immune complexes and increased turbidity. The change in turbidity was measured at approximately 630 nm, and CRP concentration was determined using the appropriate

calibration system according to the manufacturer's instructions (31).

2.15 Estimation of Adiponectin

Serum adiponectin concentration was determined using a sandwich enzyme-linked immunosorbent assay (ELISA). In this assay, adiponectin present in the sample binds to specific antibodies immobilized on the microplate. An enzyme-linked detection antibody subsequently binds to the captured adiponectin, forming an antibody–antigen–antibody sandwich. Following washing, a chromogenic substrate such as tetramethylbenzidine (TMB) was added, and the developed color was measured at approximately 450 nm. Adiponectin concentration was determined from the standard calibration curve generated using known standards (32).

2.16 Quality Control and Assay Validation

Appropriate quality-control procedures were followed throughout the laboratory investigations. Commercial control materials at appropriate concentration levels were analyzed along with study samples wherever applicable. Calibration of the analytical instruments was performed using the manufacturer's recommended calibrators and standards. Reagents were checked for validity, stored under recommended conditions, and used according to the manufacturer's instructions. Appropriate precautions were taken to minimize interference from hemolysis, lipemia, contamination, improper sample handling, and pipetting errors.

2.17 Statistical Analysis

The data were compiled and analyzed using SPSS version 28.0. Continuous variables were assessed for distribution and expressed as mean $\pm$ standard deviation (SD) for normally distributed data or as median with appropriate measures of dispersion where applicable. The biochemical, micronutrient, trace-element, inflammatory, and metabolic parameters were compared between the T2DM and healthy control groups using appropriate statistical tests. For normally distributed continuous variables, the independent-samples *t*-test was used, whereas the Mann–Whitney U test was applied for non-normally distributed variables. Categorical variables were analyzed using the chi-square test or Fisher's exact test, where appropriate. A two-tailed $p < 0.05$ was considered statistically significant, with a 95% confidence interval.

RESULTS AND DISCUSSION

3.1 Study participants

The study included 244 participants aged 25–60 years, comprising 122 patients with type 2 diabetes mellitus (T2DM) and 122 apparently healthy controls. The age distribution of the two groups was comparable, with most participants belonging to the 46–55-year age group. Male participants constituted 60.7% of the T2DM group and 63.9% of the control group. Thus, the groups were broadly comparable with respect to age and sex distribution. The age-wise distribution of participants in the T2DM and healthy control groups is presented in Figure 2.

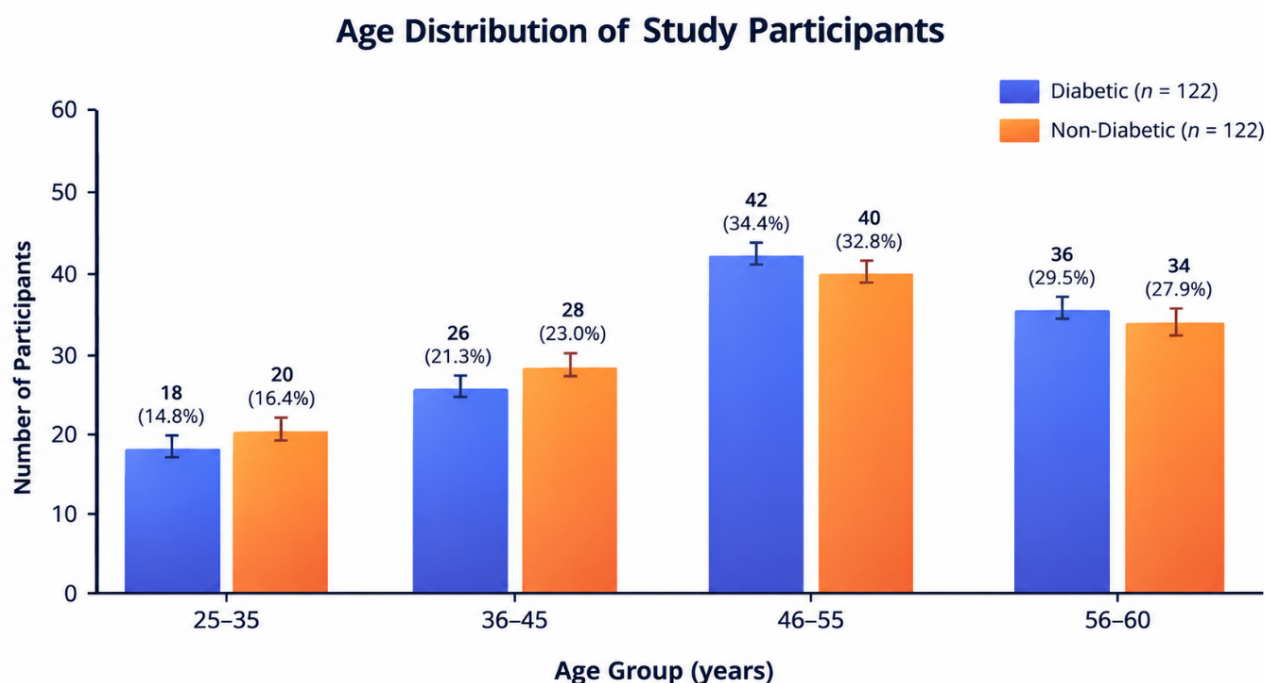


Figure 2. Age-wise distribution of diabetic and non-diabetic study participants. The distribution shows the number and corresponding percentage of participants across the 25–35, 36–45, 46–55, and 56–60-year age groups in the diabetic and non-diabetic study groups (n = 122 in each group).

3.2 Glycemic parameters

Fasting plasma glucose and HbA1c were significantly higher in patients with T2DM than in healthy controls. Mean fasting plasma glucose was 172.4 ± 36.2 mg/dL in the T2DM group compared with 91.6 ± 10.4 mg/dL in the control group ($p < 0.001$). Mean HbA1c was also significantly higher among patients with T2DM than controls ($8.6 \pm 1.5\%$ vs. $5.3 \pm 0.6\%$; $p < 0.001$). These findings confirm the presence of persistent hyperglycemia and poor long-term glycemic control in the T2DM group.

Chronic hyperglycemia can affect insulin signaling, promote inflammation, and alter the metabolism of vitamins, minerals, and trace elements (33). Therefore, the increased fasting plasma glucose and HbA1c levels provide the metabolic background for the biochemical abnormalities observed in the present study. The comparison of fasting plasma glucose and HbA1c levels between patients with T2DM and healthy controls is shown in **Figure 3**. Both glycemic parameters were higher in the T2DM group.

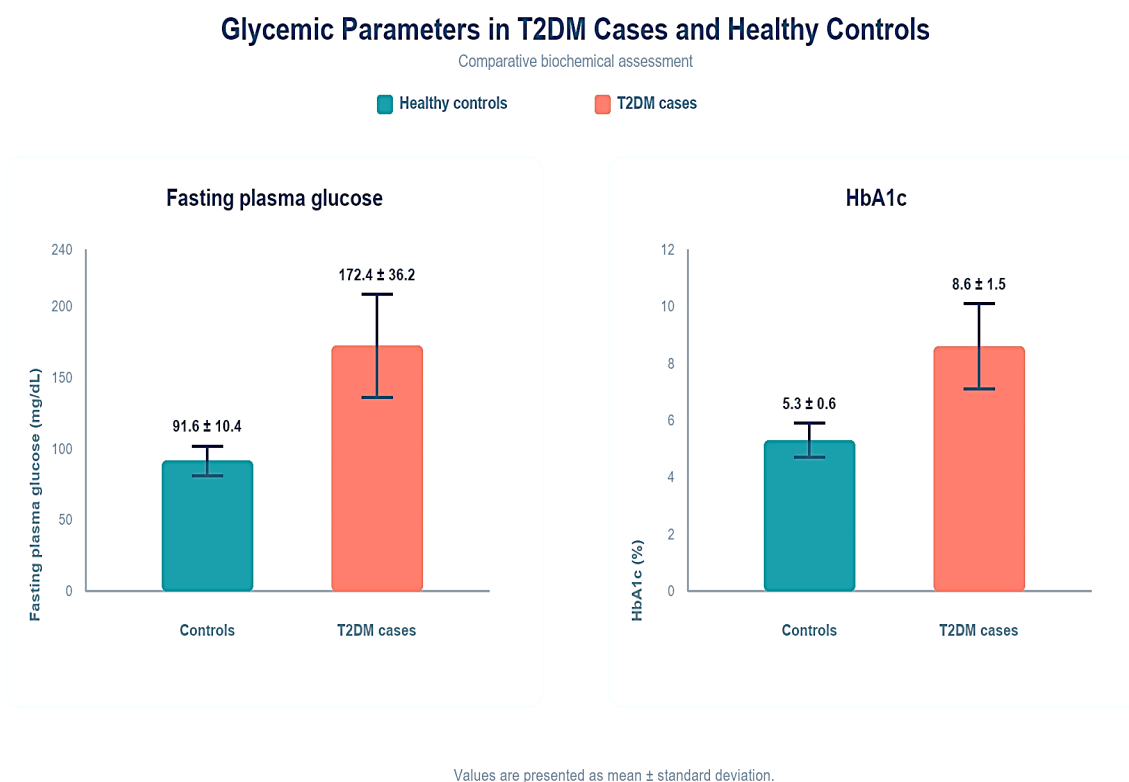


Figure 3. Comparison of fasting plasma glucose and HbA1c levels between patients with type 2 diabetes mellitus and healthy controls. Values are expressed as mean $\pm$ standard deviation.

3.3 Vitamin D and mineral profile

Serum vitamin D levels were significantly lower in patients with T2DM than in controls (19.2 ± 5.8 ng/mL vs. 27.8 ± 6.3 ng/mL; $p < 0.001$). The lower vitamin D concentration in the T2DM group suggests a higher burden of vitamin D deficiency or insufficiency among diabetic participants. Vitamin D may be associated with pancreatic beta-cell function, insulin secretion, insulin sensitivity, and inflammatory regulation (34). Its inverse

correlation with HbA1c ($r = -0.52$, $p < 0.001$) and CRP ($r = -0.54$, $p < 0.001$) indicates that lower vitamin D levels were associated with poorer glycemic control and greater inflammation (35). **Figure 4** presents the comparison of serum vitamin D, calcium, magnesium, and phosphorus levels between patients with T2DM and healthy controls. All measured micronutrient and mineral parameters were lower in the T2DM group.

Serum calcium, magnesium, and phosphorus were also significantly lower in patients with T2DM. Mean calcium concentration was 8.5 ± 0.7 mg/dL in cases and 9.3 ± 0.6 mg/dL in controls. Magnesium was 1.7 ± 0.3 mg/dL in cases compared with 2.2 ± 0.4 mg/dL in controls, while

phosphorus was 3.2 ± 0.5 mg/dL and 3.9 ± 0.6 mg/dL, respectively. All differences were statistically significant ($p < 0.001$).

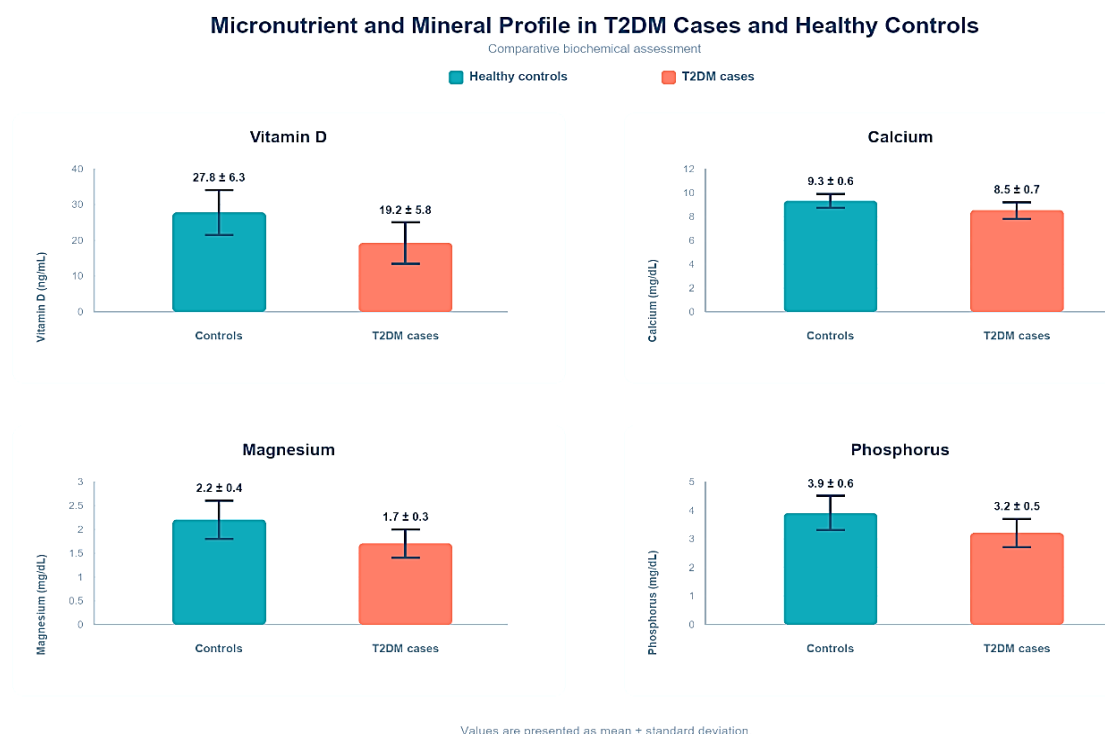


Figure 4. Comparison of serum vitamin D, calcium, magnesium, and phosphorus levels between patients with type 2 diabetes mellitus and healthy controls. Values are expressed as mean ± standard deviation.

Magnesium is particularly important in T2DM because it is involved in glucose metabolism, insulin-receptor activity, and cellular energy production. The negative correlations between magnesium and HbA1c ($r = -0.45$, $p < 0.001$) and CRP ($r = -0.49$, $p < 0.001$) suggest that reduced magnesium concentration was associated with poor glycemic control and increased inflammation. Lower calcium and phosphorus levels may similarly reflect altered mineral homeostasis in T2DM (36). However, these findings should be interpreted as associations because mineral levels may also be affected by dietary intake, vitamin D status, renal handling, and medication use.

3.4. Trace elements

Serum iron concentration was significantly lower in patients with T2DM than healthy controls (64 ± 15 µg/dL vs. 90 ± 18 µg/dL; $p < 0.001$). This finding suggests altered iron metabolism in the diabetic group. Serum iron can be influenced by inflammation, diet, iron stores, and metabolic status; therefore, additional parameters such as

ferritin and transferrin saturation would be needed to fully interpret iron status (37). **Figure 5** illustrates the comparison of serum iron and copper levels between patients with T2DM and healthy controls. Serum iron was lower, whereas serum copper was higher in the T2DM group.

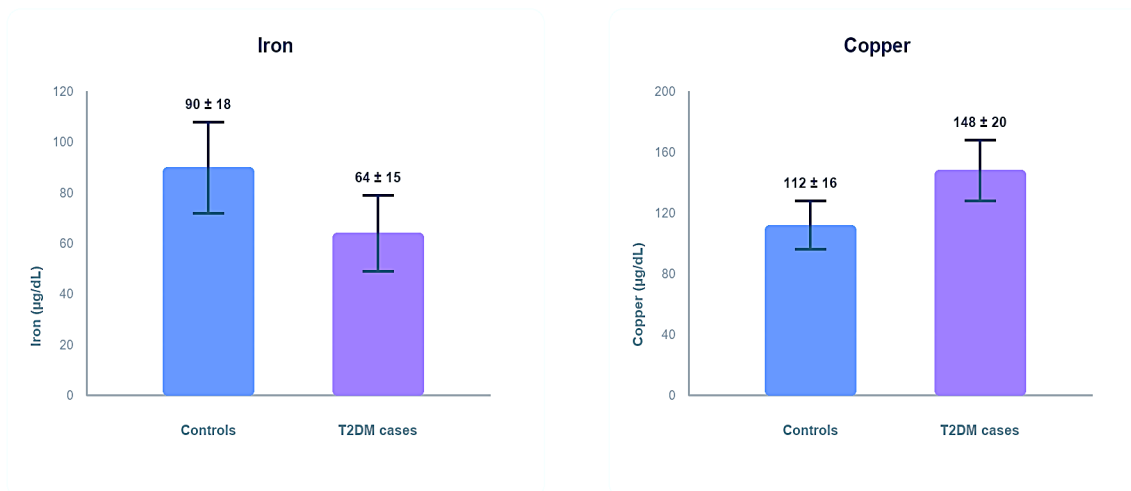
In contrast, serum copper was significantly higher in patients with T2DM than controls (148 ± 20 µg/dL vs. 112 ± 16 µg/dL; $p < 0.001$). Copper showed positive correlations with HbA1c ($r = 0.50$, $p < 0.001$) and CRP ($r = 0.47$, $p < 0.001$). These findings indicate that higher copper concentration was associated with poorer glycemic control and greater inflammatory activity. Copper is essential for normal enzymatic functions, but altered copper status may be associated with metabolic stress and inflammatory processes in T2DM (38).

Figure 5. Comparison of serum iron and copper levels between patients with type 2 diabetes mellitus and healthy controls. Values are expressed as mean ± standard deviation.

Trace Element Profile in T2DM Cases and Healthy Controls

Comparative biochemical assessment

Healthy controls T2DM cases



Values are presented as mean ± standard deviation.

3.5. C-reactive protein and adiponectin

Mean CRP concentration was markedly higher in patients with T2DM than in controls (7.2 ± 2.6 mg/L vs. 2.4 ± 1.3 mg/L; $p < 0.001$). The increased CRP level supports the presence of chronic low-grade inflammation in T2DM. The positive correlation between CRP and HbA1c ($r = 0.58$, $p < 0.001$) further suggests that poor glycemic control was associated with greater inflammatory activity (39). **Figure 6** shows the comparison of serum C-reactive protein and adiponectin levels between patients with T2DM and healthy controls. C-reactive protein was higher, whereas adiponectin was lower in the T2DM group.

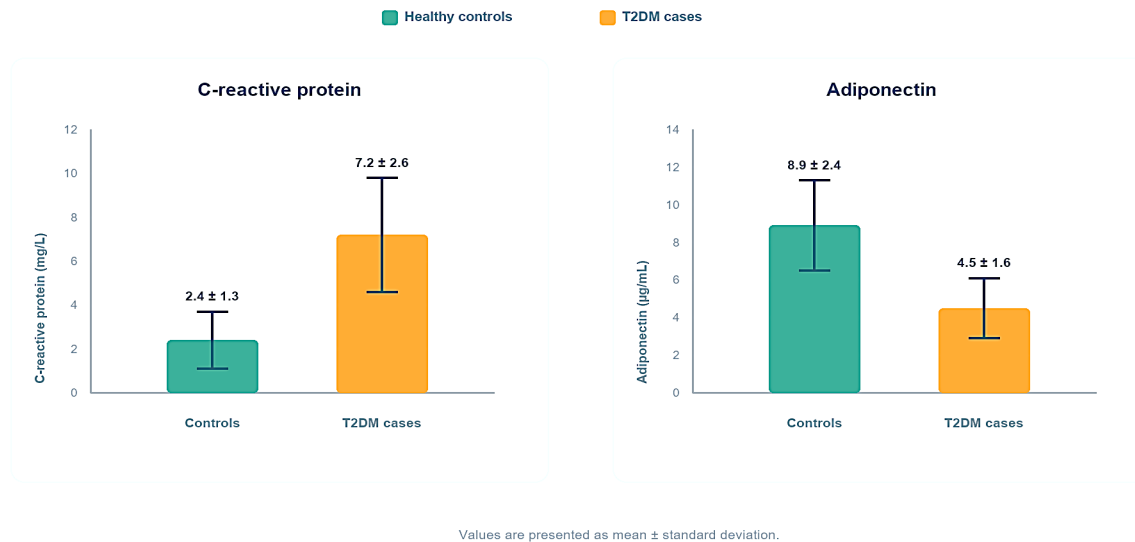
Serum adiponectin was significantly lower in patients with T2DM than in healthy controls (4.5 ± 1.6 µg/mL vs.

8.9 ± 2.4 µg/mL; $p < 0.001$). Adiponectin is an insulin-sensitizing and anti-inflammatory adipokine. Its lower concentration in patients with T2DM may be associated with impaired insulin sensitivity and increased inflammatory status. Adiponectin showed significant inverse correlations with HbA1c ($r = -0.63$, $p < 0.001$) and CRP ($r = -0.61$, $p < 0.001$), indicating that lower adiponectin levels were associated with poorer glycemic control and higher inflammation (40).

Figure 6. Comparison of serum C-reactive protein and adiponectin levels between patients with type 2 diabetes mellitus and healthy controls. Values are expressed as mean ± standard deviation.

Inflammatory and Adipokine Profile in T2DM Cases and Healthy Controls

Comparative biochemical assessment



CONCLUSION

This hospital-based case-control study compared glycemic parameters, micronutrients, trace elements, inflammatory markers, and adiponectin levels among 122 patients with type 2 diabetes mellitus and 122 apparently healthy controls. The findings show that T2DM is associated not only with impaired glycemic control but also with important alterations in nutritional, inflammatory, and metabolic status. Patients with T2DM had significantly higher fasting plasma glucose, HbA1c, C-reactive protein, and serum copper levels than healthy controls. In contrast, serum vitamin D, calcium, magnesium, phosphorus, iron, and adiponectin concentrations were significantly lower in the T2DM group. These findings suggest that chronic hyperglycemia in T2DM may be accompanied by micronutrient imbalance, altered trace-element status, increased inflammatory activity, and reduced insulin-sensitizing adipokine levels. The correlation analysis provided additional support for the relationship between these factors. Lower vitamin D, magnesium, and adiponectin levels were associated with higher HbA1c and CRP concentrations. Conversely, higher copper and CRP levels were associated with poorer glycemic control. These relationships indicate that poor glycemic control, inflammation, altered micronutrient status, and reduced adiponectin may occur together in patients with T2DM.

The study highlights the importance of evaluating T2DM as a complex metabolic disorder rather than focusing only on blood glucose levels. Assessment of vitamin D, magnesium, calcium, phosphorus, iron,

copper, CRP, and adiponectin may provide useful additional information regarding the metabolic and inflammatory status of patients with T2DM. However, as this was a hospital-based case-control study, the observed associations cannot establish causation. Factors such as dietary intake, duration of diabetes, medication use, obesity, renal function, and sunlight exposure may influence the measured biochemical parameters. Larger multicentre and prospective studies are needed to confirm these findings and determine whether correcting micronutrient deficiencies or reducing inflammation can improve glycemic control and clinical outcomes in patients with T2DM.

ACKNOWLEDGEMENT

Authors contribution

REFERENCES

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci.* 2020 Aug;21(17).
2. Singh UK, Singh SK, Hemaliya C, Kumar R, Kumar N, Bhatnagar R, et al. Prevalence and Determinants of Type 2 Diabetes Mellitus in a North Indian Population: a Community-Based Cross-Sectional Study. *Maedica (Buchar).* 2026 Mar;21(1):48–56.
3. Genitsaridi I, Salpea P, Salim A, Sajjadi SF, Tomic D, James S, et al. 11th edition of the IDF Diabetes Atlas: global, regional, and national diabetes prevalence estimates for 2024 and

- projections for 2050. *lancet Diabetes Endocrinol*. 2026 Feb;14(2):149–56.
4. Rana NS, Vishvakarma NK, Sonkar SC, Beg MMA. Diabetes: A comprehensive review of the Indian landscape in contrast with global trends. *World J Diabetes*. 2026 Feb;17(2):110701.
5. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid Med Cell Longev* [Internet]. 2020 Jan 1;2020(1):8609213. Available from: <https://doi.org/10.1155/2020/8609213>
6. Burgos-Morón E, Abad-Jiménez Z, Marañón AM de, Iannantuoni F, Escribano-López I, López-Domènech S, et al. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *J Clin Med*. 2019 Sep;8(9).
7. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c Test in Diagnosis and Prognosis of Diabetic Patients. *Biomark Insights*. 2016;11:95–104.
8. Wu C, Qiu S, Zhu X, Li L. Vitamin D supplementation and glycemic control in type 2 diabetes patients: A systematic review and meta-analysis. *Metabolism* [Internet]. 2017;73:67–76. Available from: <https://www.sciencedirect.com/science/article/pii/S0026049517301476>
9. George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* [Internet]. 2012 Aug 1;29(8):e142–50. Available from: <https://doi.org/10.1111/j.1464-5491.2012.03672.x>
10. Pitliya A, Vasudevan SS, Batra V, Patel MB, Desai A, Nethagani S, et al. Global prevalence of hypomagnesemia in type 2 diabetes mellitus - a comprehensive systematic review and meta-analysis of observational studies. *Endocrine*. 2024 Jun;84(3):842–51.
11. Asbaghi O, Moradi S, Kashkooli S, Zobeiri M, Nezamoleslami S, Hojjati Kermani MA, et al. The effects of oral magnesium supplementation on glycaemic control in patients with type 2 diabetes: a systematic review and dose-response meta-analysis of controlled clinical trials. *Br J Nutr*. 2022 Dec;128(12):2363–72.
12. De la Cruz-Concepción B, Flores-Cortez YA, Barragán-Bonilla MI, Mendoza-Bello JM, Espinoza-Rojas M. Insulin: A connection between pancreatic β cells and the hypothalamus. *World J Diabetes*. 2023 Feb;14(2):76–91.
13. Liu J, Li Q, Yang Y, Ma L. Iron metabolism and type 2 diabetes mellitus: A meta-analysis and systematic review. *J Diabetes Investig* [Internet]. 2020 Jul 1;11(4):946–55. Available from: <https://doi.org/10.1111/jdi.13216>
14. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* [Internet]. 2014 Jul 1;30(5):372–94. Available from: <https://doi.org/10.1002/dmrr.2506>
15. Qiu Q, Zhang F, Zhu W, Wu J, Liang M. Copper in Diabetes Mellitus: a Meta-Analysis and Systematic Review of Plasma and Serum Studies. *Biol Trace Elem Res*. 2017 May;177(1):53–63.
16. Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care*. 2013 Jun;36(6):1789–96.
17. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2009 Jul;302(2):179–88.
18. Dubey P, Thakur V, Chattopadhyay M. Role of Minerals and Trace Elements in Diabetes and Insulin Resistance. *Nutrients*. 2020 Jun;12(6).
19. Ibrahim R, Mohamed AA, Abbas ES, Sultan E, Selim MMS, Samy LA, et al. Trace elements in relation to glycemic control and disease complications in children living with type 1 diabetes mellitus. *J Clin Biochem Nutr*. 2026 Jul;79(1):86–96.
20. Berger MM, Amrein K, Barazzoni R, Bindels L, Bretón I, Calder PC, et al. The science of micronutrients in clinical practice – Report on the ESPEN symposium. *Clin Nutr* [Internet]. 2024;43(1):268–83. Available from: <https://www.sciencedirect.com/science/article/pii/S0261561423004284>
21. Ahmed SK. How to choose a sampling technique and determine sample size for research: A simplified guide for researchers. *Oral Oncol Reports* [Internet]. 2024;12:100662. Available from: <https://www.sciencedirect.com/science/article/pii/S2772906024005089>
22. Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. *Indian J Ophthalmol*. 2021 Nov;69(11):2932–8.
23. Basak A. Development of a rapid and inexpensive plasma glucose estimation by two-point kinetic method based on glucose oxidase-peroxidase enzymes. *Indian J Clin Biochem*. 2007 Mar;22(1):156–60.

24. 24. Metus P, Ruzzante N, Bonvicini P, Meneghetti M, Zaninotto M, Plebani M. Immunoturbidimetric assay of glycated hemoglobin. *J Clin Lab Anal.* 1999;13(1):5–8.
25. 25. Moreau E, Bächer S, Mery S, Le Goff C, Piga N, Vogeser M, et al. Performance characteristics of the VIDAS® 25-OH Vitamin D Total assay - comparison with four immunoassays and two liquid chromatography-tandem mass spectrometry methods in a multicentric study. *Clin Chem Lab Med.* 2016 Jan;54(1):45–53.
26. 26. Stern J, Lewis WHP. The colorimetric estimation of calcium in serum with o-cresolphthalein complexone. *Clin Chim Acta [Internet].* 1957;2(6):576–80. Available from: <https://www.sciencedirect.com/science/article/pii/S0009898157900633>
27. 27. Kraus FB, Mischereit M, Eller C, Ludwig-Kraus B. Characterization and Validation of the LT-SYS Copper Assay on a Roche Cobas 8000 c502 Analyzer. *Lab Med [Internet].* 2017 Feb 1;48(1):10–7. Available from: <https://doi.org/10.1093/labmed/lmw049>
28. 28. Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Anal Biochem [Internet].* 1971;40(2):450–8. Available from: <https://www.sciencedirect.com/science/article/pii/S0003269771904052>
29. 29. Sonnenblick M, Eylath U, Brisk R, Eldad C, Hershko C. Paraprotein interference with colorimetry of phosphate in serum of some patients with multiple myeloma. *Clin Chem.* 1986 Aug;32(8):1537–9.
30. 30. Ratge D, Kohse KP, Wisser H. Measurement of magnesium in serum and urine with a random access analyzer by use of a modified xylydyl blue-1 procedure. *Clin Chim Acta.* 1986 Sep;159(2):197–203.
31. 31. Liu Y, Li M, Zhang H, Gao L, Liu J, Zhu X, et al. Preparation and Characterization of C-Reactive Protein Dual-Particle Latex-Enhanced Immunoturbidimetric Reagents. *BME Front.* 2024;5:85.
32. 32. Zaharia A-L, Oprea VD, Coadă CA, Tănase CE, Ionescu A-M, Chirila SI, et al. Serum Adiponectin Levels Increase in Acute Ischemic Stroke and Correlate with Patients' Outcomes: A Pilot Study. *Biomedicines.* 2024 Aug;12(8).
33. 33. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radic Biol Med [Internet].* 2022;184:114–34. Available from: <https://www.sciencedirect.com/science/article/pii/S0891584922001228>
34. 34. Al-Qahtani FS, Alshaikh AA, Alfaifi SH. The Association Between Vitamin D Deficiency and the Level of Fasting C Peptide Among Patients With Uncontrolled Type 2 Diabetes Mellitus: A Retrospective Cohort Study. *Cureus.* 2024 Apr;16(4):e58133.
35. 35. Lim S, Kim MJ, Lim S, Kim MJ, Choi SH, Shin CS, et al. Association of vitamin D deficiency with incidence of type 2 diabetes in high-risk Asian subjects1234. *Am J Clin Nutr [Internet].* 2013;97(3):524–30. Available from: <https://www.sciencedirect.com/science/article/pii/S0002916523054424>
36. 36. Association Between Serum Magnesium Levels And Glycemic Control (HbA1c) In Obese Patients With Type 2 Diabetes Mellitus: A Narrative Review. *Rev Diabet Stud [Internet].* :730–44. Available from: <https://doi.org/10.70082/ngba3853>
37. 37. Bayih A, Dedefo G, Kinde S, Alem M, Negesso AE, Baye A, et al. Serum ferritin level and associated factors among uncontrolled adult type II diabetic follow-up patients: comparative based cross-sectional study. *BMC Endocr Disord.* 2024 Aug;24(1):144.
38. 38. Li X, Yang L, Mu D, Wang Y. Association between serum copper concentration and type 2 diabetes mellitus in elderly people in Northeast China: a cross-sectional study. *BMC Geriatr.* 2025 Nov;26(1):156.
39. 39. Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, et al. Role of C-Reactive Protein in Diabetic Inflammation. *Mediators Inflamm.* 2022;2022:3706508.
40. 40. Eltahir HB, Ali EM, Mohamed AO. Association of serum adiponectin level with glycemic control and atherogenic lipid profile in Sudanese patients with type 2 diabetes mellitus. *BMC Endocr Disord.* 2025 Oct;25(1):235.

LIST OF ABBREVIATIONS

- **25(OH)D** = 25-Hydroxyvitamin D
- **CRP** = C-reactive protein
- **DNA** = Deoxyribonucleic acid

- **EDTA** = Ethylenediaminetetraacetic acid
- **ELFA** = Enzyme-linked fluorescent assay
- **ELISA** = Enzyme-linked immunosorbent assay
- **GOD-POD** = Glucose oxidase–peroxidase
- **HbA1c** = Glycated hemoglobin
- **IDF** = International Diabetes Federation
- **OCPC** = O-cresolphthalein complexone
- **SD** = Standard deviation
- **SPSS** = Statistical Package for the Social Sciences
- **T2DM** = Type 2 diabetes mellitus
- **TMB** = Tetramethylbenzidine
- **WHO** = World Health Organization