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# Impact of Heavy Metal Accumulation on Physiological and Immunological Parameters in Type 2 Diabetes Patients

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**Annotation: Background & Aim**: Heavy metal exposure has been associated with metabolic alteration and inflammation, which could promote the progression of type 2 diabetes mellitus (T2DM). Heavy metals along with detection limit results on physiological and immunological parameters were carried out in T2DM patients.

**Materials & Methods**: Case-control study was performed among 110 T2DM patients and 110 healthy subjects at Kirkuk Teaching Hospital (May–September 2025). Fasting Blood Glucose (FBS), HbA1c, liver function enzymes (ALT, AST, ALP), pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and blood levels of Pb, Ni and Cd were evaluated. Relationships of heavy metals to clinical and immunological parameters were examined.

**Results**: FBS (168.4 ± 35.2 vs 92.6 ± 11.8 mg/dL) and HbA1c (8.3 ± 1.2 vs 5.4±0.5%) were significantly higher in T2DM patients compared to the controls ($p < 0.001$). Liver enzymes, TNF-α, IL-6 and IL-1β and blood heavy metals (Pb: 12.3 ± 3.1 µg/dL, Ni: 7.8 ± 2.4 µg/L,Cd: 1.9 ± 0.7 µg/dL) levels were significantly higher among patients compared with controls (all $p < 0.01$). Pb and Cd were strongly correlated with FBS, HbA1c and cytokines levels ($r = 0.39–0.61$, $p < 0.01$), whereas Ni exerted a less pronounced but significant correlation.

**Conclusion**: Chronic heavy metals

exposure is related with unsatisfactory glycemic control and systemic inflammation in T2DM patients, which underscores the significance of environmental toxicants as a modifiable risk factor.

**Keywords:** Heavy metal, T2DM, cytokines, HbA1c, Interleukins.

## Introduction

Type 2 diabetes mellitus (T2DM), a prevalent metabolic disease is featured with insulin resistance and deficiency, resulting in sustained hyperglycemia and various complications (1). Prevalence of T2DM among Iraqi adults has been documented at a rate between 8.5%-13.9%, and up to 19.7% in some sub-samples from regions as the Basrah (2,3). Such a high prevalence highlights the critical need for identifying modifiable environmental and biological risk factors that affect disease progression. Heavy metals, such as lead (Pb), cadmium (Cd), and mercury (Hg) are linked to oxidative stress, inflammation and abnormal glucose metabolism (4–6). Subchronic exposure to these metals can disrupt the pancreatic β-cell function, enhancing insulin resistance and pro-inflammatory cytokines such as IL-6 and TNF-α (7,8). Heavy metal exposure is associated with changes in some physiologic markers, such as fasting blood sugar (FBS), HbA1c, liver enzymes, and renal function tests (9,10). Heavy metals are stable environmental contaminants that come from industrial waste, traffic exhaust, polluted water and food [9]. In urban pockets of Iraq, including Baghdad and Basrah, we have documented the exposure of ground air and soil to Pb and Cd, leading to chronic exposure amongst inhabitants (11). Early Iraqi studies demonstrated in T2DM patients higher blood levels of these metals compared to controls, and their significant association with glucose and kidney function biomarkers. These results may indicate that environmental factors contribute to the severity and complications of T2DM in the Iraqi population (12). Although the impact of heavy metals on metabolic and immune systems are known, there are few studies that have combined both physiological and immunological measures in T2DM. The majority of the studies have addressed specifically biochemical or inflammatory values isolatedly; only few papers considered the multitarget effects of both metals over the immune dysregulation and organ functionality at once. It is important to understand these interactions because chronic long-term low-level exposure to heavy metals may worsen systemic inflammation, oxidative stress and exacerbate disease progression in diabetic patients (9, 11, 13). There are no extensive studies in the literature in Iraq examining the concurrent influence of accumulation heavy metals on physiological and immunological indices among T2DM patients. This reduces our ability to understand the environmental drivers of disease severity, delaying targeted interventions. The purpose of this study was To evaluate whether the levels of heavy metal accumulation have any effects on some symptomatic or immunological indicators in Iraq T2DM patients, possibly shed light on the possible impact of environmental exposure to disease development.

## Materials and Methods

### Study Design and Participants

A hospital-based case–control study was performed in Kirkuk Teaching Hospital during the period from May to September 2025 comprising of 110 T2DM patients and an equal number of healthy controls matched for age and sex. A written informed consent was taken from each subject of the study, and local ethical committee approved the study.

### Exclusion Criteria

Those with type 1 diabetes, gestational diabetes, chronic liver or kidney disease, autoimmune

diseases (including RA), recent infection, pregnancy and history of heavy metal exposure were excluded to minimise confounding factors.

### Blood Glucose and HbA1c

Fasting Blood Glucose (FBS) and Glycated Hemoglobin (HbA1c) were analyzed on an Automatic Chemistry Analyzer (Biobase, China). FBS was assessed by an enzymatic colorimetric method based on glucose oxidase-peroxidase system and HbA1c photometrically, to indicate average blood sugar levels over 2–3 months. Values were presented in mg/dL for FBS, and % for HbA1c, where means of determinations were carried out in duplicate.

### Liver Enzymes

The Biobase Automatic Chemistry Analyzer was also used to test the serum ALT, AST and ALP. The activities of ALT and AST were measured on the basis of amino group transferred from 2-oxoglutaric acid to L-alanine with formation of a chromogenic product whereas activity of ALP was determined using hydrolysis p-nitrophenyl phosphate into p-nitrophenol. Results were reported in U/L.

### Immunological Parameters (Cytokines)

The serum levels of TNF-α, IL-6 and IL-1β were detected by ELISA (Sunlong Biotech kit, China) with a Biobase microplate reader. Incubation of anti-hFriedburg in microtiter wells and assay protocol Control samples were added to antibody adsorbed wells together with samples, the HRP-conjugated secondary antibodies. The reaction color substrates Chromogen A/B with HRP and stopped by stop solution. Absorbance was measured at 450 nm and the concentrations were given in pg/mL.

### Heavy Metal Analysis

Blood Lead (Pb), Nickel (Ni) and Cadmium (Cd) levels were tested by atomic absorption spectrophotometer apparatus (Biobis, China). Nitric acid/hydrogen peroxide digests were prepared and analyzed by flame or graphite furnace as determined. The concentrations were expressed in µg/dL for Pb and Cd, and in mg/L for Ni based on calibration curves from certified standards.

### Statistical Analysis

Statistical analysis Study data were queried using SPSS ver. Data with continuous variables were presented as mean ± SD. Between patients and controls comparisons were conducted by means of independent t-tests or Mann–Whitney U tests. Statistical analysis Associations between heavy metals and physiological or immunological variables were analysed by Pearson or Spearman correlation, and $p < 0.05$ was regarded as significant (14,15).

## Results

### Demographic Characteristics

The study included 220 participants, comprising 110 T2DM patients and 110 healthy controls. The mean age of patients was 52.3 ± 9.1 years, and controls 51.7 ± 8.8 years ($p = 0.58$). Gender distribution was similar between groups (patients: 55% male, controls: 53% male, $p = 0.77$). BMI was significantly higher in T2DM patients (29.6 ± 3.5 kg/m²) than in controls (25.2 ± 2.9 kg/m², $p < 0.001$).

**Table 1. Demographic Characteristics of Study Participants**

| Characteristic | Patients (n = 110) | Controls (n = 110) | p-value |
|---|---|---|---|
| Age (years) | 52.3 ± 9.1 | 51.7 ± 8.8 | 0.58 |
| Male (%) | 61 (55%) | 58 (53%) | 0.77 |
| Female (%) | 49 (45%) | 52 (47%) | 0.77 |
| BMI (kg/m²) | 29.6 ± 3.5 | 25.2 ± 2.9 | <0.001 |

### Physiological Parameters

Fasting Blood Glucose (FBS) and HbA1c were significantly elevated in T2DM patients, confirming poor glycemic control. Mean FBS was 168.4 ± 35.2 mg/dL in patients versus 92.6 ± 11.8 mg/dL in controls ($p < 0.001$). HbA1c was 8.3 ± 1.2% in patients compared to 5.4 ± 0.5% in controls ($p < 0.001$). Liver enzymes were moderately higher in patients: ALT 42.1 ± 12.5 U/L versus 28.7 ± 8.2 U/L in controls ($p < 0.001$), AST 38.7 ± 10.9 U/L versus 26.5 ± 7.8 U/L ($p < 0.001$), and ALP 105.3 ± 24.6 U/L versus 88.4 ± 19.3 U/L ($p = 0.002$), suggesting mild hepatic involvement possibly associated with diabetes and/or heavy metal accumulation.

**Table 2. Physiological Parameters in T2DM Patients and Controls**

| Parameter | Patients (Mean ± SD) | Controls (Mean ± SD) | p-value |
|---|---|---|---|
| FBS (mg/dL) | 168.4 ± 35.2 | 92.6 ± 11.8 | <0.001 |
| HbA1c (%) | 8.3 ± 1.2 | 5.4 ± 0.5 | <0.001 |
| ALT (U/L) | 42.1 ± 12.5 | 28.7 ± 8.2 | <0.001 |
| AST (U/L) | 38.7 ± 10.9 | 26.5 ± 7.8 | <0.001 |
| ALP (U/L) | 105.3 ± 24.6 | 88.4 ± 19.3 | 0.002 |

### Immunological Parameters (Cytokines)

Pro-inflammatory cytokines were significantly elevated in T2DM patients: TNF-α 28.6 ± 7.4 pg/mL versus 12.5 ± 3.8 pg/mL in controls ($p < 0.001$), IL-6 16.9 ± 4.3 pg/mL versus 5.8 ± 2.1 pg/mL ($p < 0.001$), and IL-1β 14.7 ± 3.9 pg/mL versus 6.2 ± 2.0 pg/mL ($p < 0.001$), indicating systemic inflammation associated with T2DM.

**Table 3. Plasma Cytokine Levels in T2DM Patients and Controls**

| Cytokine | Patients (pg/mL) | Controls (pg/mL) | p-value |
|---|---|---|---|
| TNF-α | 28.6 ± 7.4 | 12.5 ± 3.8 | <0.001 |
| IL-6 | 16.9 ± 4.3 | 5.8 ± 2.1 | <0.001 |
| IL-1β | 14.7 ± 3.9 | 6.2 ± 2.0 | <0.001 |

### Heavy Metal Concentrations

Blood levels of heavy metals were significantly higher in T2DM patients, suggesting chronic exposure. Pb was 12.3 ± 3.1 µg/dL in patients versus 5.6 ± 1.8 µg/dL in controls ($p < 0.001$), Ni 7.8 ± 2.4 µg/L versus 3.5 ± 1.2 µg/L ($p < 0.001$), and Cd 1.9 ± 0.7 µg/dL versus 0.8 ± 0.3 µg/dL ($p < 0.001$).

**Table 4. Blood Heavy Metal Levels in T2DM Patients and Controls**

| Metal | Patients | Controls | p-value |
|---|---|---|---|
| Pb (µg/dL) | 12.3 ± 3.1 | 5.6 ± 1.8 | <0.001 |
| Ni (µg/L) | 7.8 ± 2.4 | 3.5 ± 1.2 | <0.001 |
| Cd (µg/dL) | 1.9 ± 0.7 | 0.8 ± 0.3 | <0.001 |

### Correlation Analysis

Pearson correlation analysis revealed strong positive correlations between blood heavy metals and both glycemic and inflammatory markers. Pb was significantly correlated with FBS ($r = 0.61$, $p < 0.001$), HbA1c ($r = 0.58$, $p < 0.001$), TNF-α ($r = 0.53$, $p < 0.001$), IL-6 ($r = 0.57$, $p < 0.001$), and IL-1β ($r = 0.49$, $p < 0.01$). Cd also showed significant correlations with FBS ($r = 0.49$, $p < 0.01$), HbA1c ($r = 0.46$, $p < 0.01$), TNF-α ($r = 0.45$, $p < 0.01$), IL-6 ($r = 0.42$, $p < 0.01$), and IL-1β ($r = 0.39$, $p < 0.01$). Ni displayed weaker but significant correlations with glycemic and cytokine markers ($r = 0.22–0.32$, $p < 0.05$).

**Table 5. Pearson Correlation Coefficients Between Heavy Metals and Glycemic/Immunological Parameters in T2DM Patients**

| Parameter | Pb (r) | Ni (r) | Cd (r) | p-value |
|---|---|---|---|---|
| FBS (mg/dL) | 0.61 | 0.32 | 0.49 | <0.001 |
| HbA1c (%) | 0.58 | 0.28 | 0.46 | <0.001 |
| TNF-α (pg/mL) | 0.53 | 0.25 | 0.45 | <0.001 |
| IL-6 (pg/mL) | 0.57 | 0.27 | 0.42 | <0.001 |
| IL-1β (pg/mL) | 0.49 | 0.22 | 0.39 | <0.01 |

## Discussion

According to our study, mean levels of both FBS and HbA1c in the T2DM group (168.4 ± 35.2 mg /dL and 8.3 ± 1.2% respectively) were significantly higher than the control (92.6 ±11 mg/dL and 5.4±0/5%, p <.001). This is also in agreement with the findings of Ghaidan and Al-Dhamin (16) who had shown higher Pb levels (12.1 ± 3.2 µg/dL) associated with FBS 165 ± 30 mg/dL and HbA1c 8.1 ±1.3% in T2DM patients compared to healthy controls in Baghdad, indicating a possible role for heavy metal exposure and poor glycemic control. Likewise, (17) reported elevation in blood Pb (11.8 ± 3.0 µg/dL) and Cd (1.8 ± 0.6 µg/dL) among diabetic subjects with FBG ≈ 160–170 mg/dL and HbA1c around ~ 8.0% suggesting that environmental heavy metal may be involved in hyperglycemia among the Iraqi population. Our results that pro-inflammatory cytokines (TNF-α: 28.6 ± 7.4 pg/mL, IL-6: 16.9 ± 4.3 pg/mL, IL-1β: 14.7 ± 3.9 pg/mL) were significantly upregulated are similar to those of Al-Musawi et al. (18) showed among patients with diabetes and retinopathy that TNF-α was highest at 27.5 ± 6.8 pg/mL and IL-6 was the highest (16.0 ± 4.1 pg/mL) in a comparison between metal Formaldehyde is listed as an IARC Group 1 human carcinogen exposure may play a role in systemic inflammation. These findings are also consistent to that of Ji et al. Metals and its association with oxidative stress adn inflammation This finding agrees with Lopez-Alarcon et al. (19) who found that multi-metal exposure (Pb, Cd, Hg) is associated with high levels of inflammation markers and IR sugggesting that metals induce T2DM via their mediated oxidative stress and inflammation. Liver enzyme elevations in our data (ALT: 42.1 ± 12.5 U/L, AST: 38.7 ± 10.9 U/L, ALP: 105.3 ±24.6 U/L) are similar to those of El-Sharawy et al. (20), who also reported ALT values of 43.0 ± 13.2 U/L and AST of 37.5 ± 11.0 U/L in urban populations chronically exposed to metal. This indicates that Pb and Cd might cause mild hepatic stress in T2DM patients, thus leading to the aggravation of glucose metabolism. Other types of liver enzyme elevation were observed in Kirkuk workers (21) concomitantly with Pb (11.9 ± 3.0 µg/dL) and Cd (1.7 ± 0.5 µg/dL), confirming the hepatotoxic effect of chronic metal exposure. In our patients, Ni levels (7.8 ± 2.4 µg/L) moderately correlated with the FBS and cytokine levels. This result is compatible with the Al-Harbi et al. (22); they found that environmental Ni exposure in Arabian Gulf inhabitants was associated with increased (5.9 ± 0.6 mmol/L) fasting glucose and markers of metabolic syndrome, showing that even trace metals have a potential effect on glucose homeostasis. Our results extend known correlations by demonstrating strong associations of Pb/Cd in relation to inflammatory cytokines (Pb vs TNF-α: r = 0.53; Cd vs IL-6: r = 0.42, p < 0.01). These findings are consistent with Zhang et al. (23), who observed correlation coefficients of Pb and IL-6 0.55, and Cd and TNF-α 0.47 in adults exposed to several metals, indicating that heavy metals can directly modulate immune responses in T2DM patients. Our results are consistent with those of reports from Erbil and Kirkuk (24), in which blood Pb (11–12 µg/dL) and Cd (1.5–1.9 µg/dL) levels were correlated with increased HbA1c (7.9–8.2%) and inflammatory markers, further suggesting that the environmental heavy metals could be modifiable risk factors for T2DM development. Globally, Ji et al. (19) and Zhang et al. (23) described additive or synergistic actions of Pb, Cd and other heavy metals in relation to diabetes risk and inflammatory activation.

## Conclusions

Long-term exposure to the heavy metals, such as Pb and Cd, is considerably correlated with under controlled glycemia and increased levels of pro-inflammatory cytokines in T2DM patients. Ni also showed moderate associations. These results indicate that ambient exposure to heavy metals might be involved in the development of metabolic and inflammatory complications related to diabetes. Monitoring and mitigating HM exposure may be an effective method to ameliorate clinical events in T2DM subjects.

## Limitations

This was a cross-sectional study; thus, whether heavy metals exposure led to complications of T2DM could not be inferred. Heavy metal exposure assessment was based on blood levels without environmental measurements. This was a single-center study and the generalization of this analysis is impacted. Only three heavy metals (Pb, Ni, Cd) were examined; other possible toxicants were not considered.

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