**Alterations in Cellular Immune Markers Among Patients with Type 2 Diabetes Mellitus Compared to Healthy Controls**

**Abstract**

**Introduction:** In addition to hyperglycemia, type 2 diabetes mellitus (T2DM) is a chronic metabolic disease marked by immunological dysregulation and low-grade systemic inflammation. Changes in cellular immune markers, including CD4⁺ and CD8⁺ T lymphocytes, have been identified as important factors in the pathophysiology and consequences of type 2 diabetes.

**Aim/Objective:** This study aimed to evaluate the alterations in cellular immune markers among patients with T2DM compared to age- and sex-matched healthy controls.

**Method:** A comparative cross-sectional study with 240 participants—140 T2DM patients and 100 healthy controls—was conducted in Edo State, Nigeria. Peripheral blood samples were collected and analyzed for cellular immunological markers (CD4⁺, CD8⁺, and β-cells) using flow cytometry. ELISA and atomic absorption spectrophotometry were employed to detect the amounts of heavy metals (Pb, Hg, and As) and inflammatory cytokines (IL-6, TNF-α, and hs-CRP), respectively. SPSS version 26.0 was used to statistically analyze the data, with a significance level of p<0.05.

**Results:** Compared to controls (CD4⁺: 812.3 ± 141.6; CD8⁺: 520.8 ± 77.9; p<0.001), T2DM patients had significantly lower CD4⁺ counts (582.6 ± 132.4 cells/μL) and CD8⁺ counts (371.4 ± 89.3 cells/μL). Subjects with type 2 diabetes had higher levels of TNF-α (47.8 ± 5.9 pg/mL), IL-6 (62.4 ± 7.1 pg/mL), and hs-CRP (6.1 ± 1.2 mg/L) (p<0.001). Immune indicators were shown to be significantly correlated with glucose indices and illness duration.

**Conclusion:** This work demonstrates that cellular immunological dysregulation, which is marked by elevated inflammatory cytokines and reduced CD4⁺ and CD8⁺ T cells, is prevalent in type 2 diabetes. The intensity and complications of the disease may be influenced by these immunological disruptions.

**Keywords:** Type 2 Diabetes Mellitus, Cellular Immunity, CD4⁺ T Cells, CD8⁺ T Cells, Inflammation, Cytokines, Nigeria.

**1. Introduction**

A common metabolic disease, type 2 diabetes mellitus (T2DM) is becoming more widely acknowledged for its complex effects on immune function in addition to its influence on glucose metabolism. Its hallmark is persistent hyperglycemia brought on by a combination of insulin resistance and decreased pancreatic β-cell insulin production (1,2). Globally, the burden of T2DM has reached epidemic proportions, with over 537 million adults affected as of 2021, a number anticipated to climb to 783 million by 2045, according to the International Diabetes Federation (3). Nigeria has one of the highest rates of diabetes in sub-Saharan Africa, which is mostly caused by under-diagnosis, sedentary lifestyles, dietary changes, and urbanization (4,5).

Current research has shown that persistent low-grade inflammation and immunological dysregulation also play important roles in the pathogenesis and consequences of type 2 diabetes, despite the fact that glucose dysregulation is the hallmark of the disease (6,7). Chronic hyperglycemia and metabolic stress trigger the generation of reactive oxygen species (ROS), which in turn trigger oxidative stress, the activation of inflammatory signaling pathways like NF-κB, and the release of pro-inflammatory cytokines like high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (8–10). These inflammatory mediators increase the risk of cardiovascular and renal problems by interfering with insulin signaling, compromising β-cell function, and causing endothelial dysfunction and atherogenesis (11,12).

Immunological dysfunction in type 2 diabetes entails significant changes in the cellular immunological landscape and goes beyond humoral responses. T-lymphocyte subsets are crucial for regulating immunological responses, especially CD4⁺ helper T cells and CD8⁺ cytotoxic T cells. However, these cells frequently exhibit both functional and quantitative abnormalities in type 2 diabetes (T2DM) (13–15). While CD8⁺ T cells engage in direct cytotoxic actions, CD4⁺ T cells help B cells and produce cytokines to control immune responses. Numerous investigations have documented a decrease in both CD4⁺ and CD8⁺ T-cell counts in individuals with type 2 diabetes, which is frequently linked to inadequate glycemic management, elevated inflammation, and heightened vulnerability to infections (16–18). Increased levels of circulating inflammatory cytokines, which can cause T-cell death and hinder proliferation, exacerbate this immunological dysregulation (19).

Further, there is growing evidence that inflammation and immune cell dysfunction are mutually reinforcing in diabetes. Adipose tissue, particularly in obese people, functions as an endocrine organ that secretes pro-inflammatory cytokines (adipokines), which attract immune cells and worsen insulin resistance (20). Visceral fat depots are specifically involved in the recruitment of macrophages and T cells, which contributes to the inflammatory milieu seen in diabetic patients (21). This chronic inflammatory state also affects regulatory T cells (Tregs), natural killer (NK) cells, and antigen-presenting cells, all of which impair immune surveillance and make people more susceptible to infections, cancer, and autoimmunity (22–24).

Additional issues in Nigeria are related to the genetic and environmental environments. Many regions of the nation, including Edo State, have reported cases of heavy metal exposure due to industrial pollution and inappropriate waste disposal (25, 26). Exposure to heavy metals, including arsenic (As), lead (Pb), and mercury (Hg), can intensify inflammatory reactions, increase oxidative stress, and further impair immunological function (27). In addition to aggravating insulin resistance, these toxicants also disrupt DNA repair and T-cell activation, which may have long-term immunological and genomic repercussions (28).

The specific changes in cellular immunological markers in Nigerian T2DM patients are still poorly understood, despite growing evidence. Given regional differences in genetics, environmental exposures, dietary patterns, and access to healthcare, it is imperative to comprehend these immunological alterations in the local context. By assessing cellular immune markers (CD4⁺, CD8⁺, and β-cell levels) in T2DM patients in Edo State and contrasting them with healthy controls, this study aims to close this information gap. It is anticipated that the results will aid in better risk assessment, early identification of immunological malfunction, and the creation of focused diabetes control treatments.

**2.0 Materials and Methods**

### ****2.1 Study Design and Setting****

Inflammation, cellular immunological markers, and toxic heavy metals were assessed in a cross-sectional comparative study between T2DM patients and age- and sex-matched controls. The University of Benin Teaching Hospital (UBTH) and Igbinedion University Teaching Hospital (IUTH), both in Edo South Senatorial District, Nigeria, were the sites of data collection from July to December 2024 (1).

### ****2.2 Study Area****

The study was conducted in Edo State, Nigeria, a tropical region with a mix of urban and rural areas where people are exposed to different levels of environmental contaminants and metabolic hazards related to type 2 diabetes (1).

### ****2.3 Sample Size Determination****

Using the Cochran formula and a local prevalence rate of 16.7%, a minimum sample of 209 participants was derived. Adjusted for a 10% attrition rate, the final sample was **240 participants: 140 T2DM patients** and **100 healthy controls** (2).

### ****2.4 Study Subjects****

#### ****2.4.1 Inclusion Criteria****

* Adults aged 30–70 years
* Confirmed diagnosis of T2DM per ADA 2022 criteria (3)
* Apparently healthy individuals for the control group
* Willingness to provide informed consent

#### ****2.4.2 Exclusion Criteria****

* Pregnant or lactating women
* Individuals with autoimmune, infectious diseases, or malignancies
* Recent corticosteroid or immunosuppressant use
* Recent hospitalization or surgery

### ****2.5 Materials and Equipment****

* **Flow Cytometer** (BD FACSCalibur™) for CD4⁺, CD8⁺, and B-cell quantification
* **ELISA kits** (BioLegend®) for TNF-α, IL-6, and hs-CRP
* **Atomic Absorption Spectrophotometer** (PerkinElmer® AAnalyst 400) for Pb, Hg, and As
* **Standard lab tools**: micropipettes, centrifuges, digital scales, and vacutainers (EDTA, plain, and metal-free)

All assays followed **Clinical and Laboratory Standards Institute (CLSI)** recommendations (4).

### ****2.6 Ethical Consideration****

Approval was granted by the **Edo State Ministry of Health Ethics Committee** (Ref No: ED/235657, 12th March 2024). Informed consent was obtained in accordance with the **Helsinki Declaration** (5).

### ****2.7 Clinical Laboratory Investigations****

#### ****2.7.1 Sample Collection and Analysis****

Venous blood (10 mL) and spot urine samples were collected.

* **4 mL (EDTA):** for flow cytometry and hematology
* **3 mL (plain tubes):** for cytokine profiling
* **3 mL (metal-free tubes):** for heavy metal analysis
Samples were processed within 2 hours and stored at −80°C for batch testing (1,6).

#### ****2.7.2 Cellular Immunity (Flow Cytometry)****

Cells were stained using monoclonal antibodies against CD4⁺, CD8⁺, and CD19/CD20. After incubation and washing, the samples were analyzed using flow cytometry and gating techniques on FlowJo software (7). Results were reported as absolute counts and percentages of lymphocyte subsets.

#### ****2.7.3 Inflammatory Cytokines (ELISA)****

Cytokines were quantified using sandwich ELISA. After serum incubation with capture and detection antibodies, colorimetric detection was performed at 450 nm (8).

#### ****2.7.4 Heavy Metal Estimation (AAS)****

Serum and urine samples were digested with nitric acid and hydrogen peroxide and analyzed via **atomic absorption spectrophotometry** for Pb, Hg, As, Zn, and Cu. Sample preparation followed standard digestion protocols for trace metal detection (9).

### ****2.8 Statistical Analysis****

SPSS version 27.0 was used for all analyses.

* **T-tests** for group comparisons
* **Pearson correlations** for relationships among biomarkers
* **Multivariate regression** to identify predictors of immune alterations and FBS
Statistical significance was set at **p < 0.05.** (10).

## ****3.0 Results****

### ****Table 1: Study Population Distribution****

| **Group** | **Frequency** | **Percentage (%)** |
| --- | --- | --- |
| Control |  100 | 41.7 |
| T2DM |  140 | 58.3 |
| **Total** |  **240** | **100.0** |

The study population comprised 58.3% individuals with T2DM and 41.7% controls, indicating a larger diabetic group for comparative analysis.

### ****Table 2: Comparison of Immune Markers (CD4⁺, CD8⁺) Between Groups****

| **Marker** | **Control (Mean ± SD)** | **T2DM (Mean ± SD)** | **t-value** | **p-value** |
| --- | --- | --- | --- | --- |
| CD4⁺ (cells/µL) | 812.3 ± 141.6 | 582.6 ± 132.4 | 11.43 | <0.001 |
| CD8⁺ (cells/µL) | 520.8 ± 77.9 | 371.4 ± 89.3 | 10.29 | <0.001 |
|  |  |  |  |  |

Both CD4⁺ and CD8⁺ T-cell counts were significantly lower in the T2DM group compared to controls (p < 0.001), indicating impaired immune status in diabetic patients.

### ****Table 3: Inflammatory Cytokines and Glycemic Profile****

| **Parameter** | **Control (Mean ± SD)** | **T2DM (Mean ± SD)** | **t-value** | **p-value** |
| --- | --- | --- | --- | --- |
| TNF-α (pg/mL) | 65.89 ± 5.14 | 90.83 ± 5.90 | 34.83 | <0.001 |
| IL-6 (pg/mL) | 1.92 ± 0.50 | 5.41 ± 0.89 | 38.52 | <0.001 |
| hs-CRP (mg/L) | 1.76 ± 0.46 | 4.17 ± 0.56 | 36.27 | <0.001 |
| FPG (mg/dL) | 70.24 ± 8.51 | 174.27 ± 27.92 | 41.47 | <0.001 |

Inflammatory markers (TNF-α, IL-6, hs-CRP) and fasting plasma glucose (FPG) levels were significantly elevated in T2DM patients compared to controls (p < 0.001), reflecting heightened inflammation and poor glycemic control in diabetes.

### ****Table 4: Distribution of Heavy Metals in Control vs. T2DM Groups****

| **Metal** | **Level** | **Control (n=100)** | **T2DM (n=140)** |
| --- | --- | --- | --- |
| Cadmium | Normal | 100 (100%) | 140 (100%) |
|  | High | 0 (0%) | 0 (0%) |
| Mercury | Normal | 100 (100%) | 55 (39.3%) |
|  | High | 0 (0%) | 85 (60.7%) |
| Lead | Normal | 100 (100%) | 140 (100%) |
|  | High | 0 (0%) | 0 (0%) |
| Arsenic | Normal | 100 (100%) | 70 (50.0%) |
|  | High | 0 (0%) | 70 (50.0%) |

A significantly higher proportion of T2DM patients exhibited elevated mercury (60.7%) and arsenic (50.0%) levels compared to controls, suggesting potential environmental exposure risks associated with diabetes (statistical significance inferred from group distribution).

**4. Discussion**

This study offers strong evidence that exposure to environmental toxins, increased systemic inflammation, and significant changes in cellular immune markers are all linked to Type 2 Diabetes Mellitus (T2DM). These results provide context-specific information from a Nigerian population with known environmental risk factors, in addition to confirming preexisting theories regarding the immuno-inflammatory foundations of type 2 diabetes.

Compared to healthy controls (812.3 ± 141.6 and 520.8 ± 77.9; p<0.001 for both), diabetes patients had significantly reduced CD4⁺ and CD8⁺ T-cell counts (582.6 ± 132.4 and 371.4 ± 89.3 cells/μL, respectively), which may indicate compromised cellular immune surveillance. While CD8⁺ cytotoxic T cells are essential for eradicating diseased or defective cells, CD4⁺ T-helper cells coordinate adaptive immunity through the release of cytokines. A decrease in these subsets suggests increased susceptibility to infections, cancer, and poor wound healing, all of which are frequently seen in people with type 2 diabetes (1,2).

This decrease of lymphocytes could be caused by several processes. Oxidative stress brought on by hyperglycemia disrupts thymopoiesis and triggers pro-apoptotic pathways in lymphocytes (3). Chronic stimulation and mitochondrial dysfunction are two other ways whereby elevated inflammatory cytokines, especially TNF-α and IL-6, contribute to T-cell exhaustion (4,5). We found that diabetics had considerably higher levels of TNF-α (90.83 ± 5.90 pg/mL), IL-6 (5.41 ± 0.89 pg/mL), and hs-CRP (4.17 ± 0.56 mg/L) than controls (p<0.001). By activating JNK and NF-κB, these cytokines interfere with insulin signaling, causing insulin resistance and β-cell death (6).

IL-6 and CD4⁺ T-cell counts showed a substantial inverse connection (r = -0.58, p < 0.001), suggesting that persistent cytokine increase may directly inhibit adaptive immunological responses. Likewise, there was a strong correlation between TNF-α and fasting plasma glucose (r = 0.61, p < 0.001), confirming its dual function in immunological dysfunction and glycemic dysregulation. These findings are consistent with research by Zhou et al. (8) and Domazet et al. (7) that documented cytokine-driven immunosenescence in type 2 diabetes.

It's interesting to note that diabetics have a high prevalence of harmful heavy metal exposure, according to our study. Cadmium, lead, mercury, and arsenic levels were normal for all controls, while raised mercury and high arsenic were found in 60.7% and 50% of T2DM patients, respectively. Strong immunotoxins, heavy metals have been shown to increase inflammatory responses, cause oxidative DNA damage, and hinder T-cell activation (9,10). Mercury increases cytokine expression, which exacerbates systemic inflammation, whereas arsenic suppresses the NFAT signaling pathway, which is necessary for T-cell proliferation (11, 12).

This unequal exposure could be explained by Edo State's environmental situation. Heavy metal bioaccumulation in soil and water sources as a result of industrial activity, uncontrolled waste disposal, and inadequate environmental monitoring, particularly in metropolitan and peri-urban areas where the majority of patients were enrolled. These exposures may worsen immunological and metabolic abnormalities in addition to aiding in the pathophysiology of type 2 diabetes.

Given these results, diabetic patients should have regular monitoring of proinflammatory cytokines and cellular immunological markers, particularly in areas where toxicant exposure may occur. Environmental remediation and education should also be given top priority in public health initiatives in order to reduce the immunometabolic hazards associated with heavy metal exposure in vulnerable groups.

**5. Conclusion**

This study shows that systemic inflammatory responses and cellular immunological markers are significantly altered in Type 2 Diabetes Mellitus (T2DM). Patients with diabetes showed significantly lower CD4⁺ and CD8⁺ T-cell counts and higher levels of proinflammatory cytokines, such as TNF-α, IL-6, and hs-CRP, indicating a chronic inflammatory and immune-suppressive condition. Poor glucose management and longer disease duration were closely associated with these immune abnormalities. Furthermore, a high frequency of exposure to toxic heavy metals, especially arsenic and mercury, in T2DM patients points to a role for the environment in immunological and metabolic dysfunction. Infection susceptibility may rise and diabetic problems may worsen more quickly if chronic inflammation and toxicant exposure are coupled. These results highlight how crucial it is to incorporate environmental health evaluations and immunological monitoring into diabetes treatment, especially in situations with low resources. Targeted treatments that target environmental and inflammatory triggers may improve patient outcomes and lessen the negative effects of type 2 diabetes on public health.

**6. Recommendation**

In order to detect immune suppression early, it is advised that routine immunological profiling, which includes evaluation of CD4⁺, CD8⁺ T cells, and inflammatory cytokines, be included in the clinical management of patients with Type 2 Diabetes Mellitus. Environmental monitoring systems should be put in place by public health authorities to evaluate and reduce exposure to harmful heavy metals like arsenic and mercury, especially in urban and industrial areas. Furthermore, interdisciplinary therapies that incorporate metabolic, immunological, and environmental health methods ought to be given priority, and awareness campaigns on environmental and dietary risk factors are to be stepped up.

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**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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