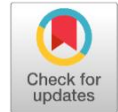




## RESEARCH ARTICLE



## Evaluation of interleukin 6 and interleukin 4 levels in the serum of type 1 diabetic

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## Abstract

Cytokines play critical roles in regulating interactions between pancreatic beta cells and immune cells in the development of type 1 diabetes. It has been shown that pro-inflammatory cytokines released during the disease can exacerbate the disease, while anti-inflammatory cytokines provide protection. This study aimed to evaluate IL-6 and IL-4 levels in the serum of children with type 1 diabetes and compare them to healthy controls. Seventy children with type 1 diabetes and thirty-three healthy children were recruited between September and December 2024. Serum levels of interleukin-6 (IL-6) and interleukin-4 (IL-4) were measured using an ELISA assay. The results showed a significantly higher mean IL-6 concentration in patients ( $12.95 \pm 0.83$ ) compared to healthy controls ( $2.62 \pm 0.33$ ) with a significant difference ( $p=0.001$ ). Although the average IL-4 concentration was higher in healthy controls ( $41.77 \pm 13.34$ ) than in patients ( $18.71 \pm 7.08$ ), this difference was not statistically significant ( $p=0.10$ ). The study also revealed significant correlations between IL-6 and IL-4 concentrations and the duration of the disease. Additionally, a weak positive correlation was observed between IL-6 levels and glycated hemoglobin (HbA1c), body mass index (BMI), and fasting blood sugar (FBS), as well as a weak positive relationship between IL-6 concentrations in male and female diabetic patients. This study demonstrates a significant elevation of pro-inflammatory IL-6 levels in children with type 1 diabetes compared to healthy peers, suggesting a state of chronic inflammation associated with the disease. While IL-4 levels were lower in diabetic patients, the difference did not reach statistical significance.

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## 1. Introduction

Diabetes mellitus (DM) refers to a group of multifactorial metabolic disorders characterised by elevated blood glucose levels (hyperglycemia) resulting from defects in the body's insulin production or impaired insulin action [1]. This leads to a disruption in the metabolic balance of carbohydrates, proteins, and fats. If left untreated, this dysfunction can progress to serious complications affecting multiple organ systems. DM affects millions of people worldwide, regardless of gender or age [2].

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterised by the progressive destruction of pancreatic beta cells. This condition results from a breakdown in immune system regulation, primarily mediated by Type 1 T-helper (Th1) cells, accompanied by the activation and infiltration of immune cells into pancreatic islets. These immune mechanisms collectively lead to beta-cell destruction and subsequent marked hyperglycemia [3]. Approximately 5–10% of patients with diabetes have Type 1 diabetes [4]. In 2021, T1DM affected 8.4 million individuals globally, of whom 18% (1.5 million) were under 20 years of age [5].

Genetic and immunological studies have directly implicated cytokines in the pathogenesis of T1DM. Cytokines are considered the primary mediators of inflammation [6] and have been proposed as triggers of beta-cell damage in humans through the generation of nitric oxide (NO) [7]. Cytokines are small, soluble cellular regulatory proteins with a molecular weight of less than 30 kDa. They govern the initiation and maintenance of immune responses in the body and serve as critical factors in the pathogenesis of autoimmune diseases, including type 1 diabetes mellitus [8]. Cytokines are classified into: Anti-inflammatory cytokines (e.g., IL-10, TGF- $\beta$ , and IL-4), pro-inflammatory cytokines (e.g., IL-1, IL-6, and TNF- $\alpha$ ). These cytokines are primarily secreted by Th1 and Th2 cells [3]. Growing evidence suggests that an imbalance in Th1/Th2 responses and their associated cytokines plays a critical role in the pathogenesis of autoimmune diseases, including T1DM. However, studies remain controversial

whether T1DM is primarily a Th1-mediated autoimmune disease, Th2-mediated, or involves both subsets [4].

Both pro-inflammatory and anti-inflammatory cytokines have been well-documented in their roles in T1DM pathogenesis[9]. Anti-inflammatory cytokines such as IL-10, TGF- $\beta$ , IL-5, IL-4, IL-2, IL-15, IL-33, and IL-35 can stimulate regulatory functions, suppress inflammation, restore immune tolerance, prevent beta-cell damage. Conversely, pro-inflammatory cytokines including IL-6, TNF- $\alpha$ , IFN- $\alpha$ , IL-17, and IL-21 exacerbate inflammation by promoting proliferation and activation of diabetogenic immune cells, recruiting pathogenic T-cells (Th1, Th17, CD8+) ,activating natural killer (NK) cells [3] Researchers have hypothesised that Th1-type inflammatory cytokines are excessively secreted in children with Type 1 Diabetes Mellitus (T1DM), leading to cellular damage and disease progression. Conversely, levels of Th2-type anti-inflammatory cytokines were elevated under the influence of homeostatic regulation [10].

Recent advancements in immunometabolism have shifted the perspective on cytokine analysis from being mere inflammatory markers to becoming predictive tools for diabetic complications. Current longitudinal studies have demonstrated that elevated IL-6 levels are not only indicators of current inflammation but also significant predictors of microvascular damage, including early-stage diabetic nephropathy and retinopathy, even before clinical symptoms manifest [11]. Furthermore, the persistent imbalance between pro-inflammatory (IL-6) and anti-inflammatory IL-4 signals is now utilized in Machine Learning (ML) models to stratify patients based on their risk of rapid beta-cell decline and disease progression [12].

Among pro-inflammatory cytokines, Interleukin-6 (IL-6) is a pleiotropic cytokine with diverse effects ranging from inflammation and host defence to tissue injury, immune regulation, and non-immune functions in most cell types and tissues beyond the immune system ADDIN [8]. IL-6 is secreted by numerous cell types, including immune cells, fibroblasts, endothelial cells, skeletal muscle, and adipose tissue. Elevated IL-6 concentrations have been reported in various clinical disorders, where it appears to regulate a wide array of inflammatory responses[13].

IL-6 contributes to the exacerbation of autoimmune diseases by stimulating inflammatory cellular infiltration, a phenomenon clearly observed in the pancreas of NOD mice [3]. Nevertheless, serum IL-6 levels in patients with T1DM remain inconsistent in research findings, despite evidence linking its overexpression in pancreatic beta cells to severe insulinitis characterized by the infiltration of B cells, T cells, and macrophages [14].

Among anti-inflammatory cytokines, Interleukin-4 (IL-4) is an important immune-regulatory cytokine composed of 129 amino acids, with a molecular weight ranging from 12 to 20 kilodaltons [15]. IL-4 plays a role in the pathogenesis of various autoimmune diseases, including diabetes [16] It is mainly produced by Th2 T cells as well as NK cells, mast cells, eosinophils and basophils [17] It has many different functions, as it increases the humoral response [4], acts as a stimulator of T and B cell proliferation, regulates B cell differentiation and promotes Th2 and inhibits Th1 differentiation [18], and has been indicated as an anti-inflammatory in autoimmune diseases based on its protective effects in models of diabetes and rheumatoid arthritis patients[19].

The existing evidence regarding serum levels of cytokines (particularly IL-6 and IL-4) in diabetic patients remains limited and inconsistent. Several studies report elevated serum IL-6 concentrations in diabetic patients [20], Other studies demonstrate reduced IL-6 levels[21], Some studies indicate increased IL-4 concentrations in T1DM patients versus healthy controls[22], Contrarily, other studies show higher IL-4 levels in healthy individuals compared to T1DM patients [23],And the precise relationship between these cytokines levels and the pathogenesis of type 1 diabetes has not been conclusively established across various studies. Given that the precise relationship between cytokine profiles and the pathogenesis of Type 1 Diabetes Mellitus (T1DM) remains a subject of ongoing debate, and since their specific roles in the inflammatory progression of the disease are not yet fully elucidated, this study was designed to achieve the following objectives:

- To Quantify and Compare the circulating serum concentrations of pro-inflammatory (IL-6) and anti-inflammatory (IL-4) cytokines in children with T1DM versus age-matched healthy controls.

- To Analyze the Correlation between these cytokine levels and key clinical parameters, including disease duration, glycemic control (measured by HbA1c), and anthropometric measurements (BMI), to identify potential drivers of chronic inflammation.
- To Investigate Sexual Dimorphism in cytokine expression by comparing IL-6 and IL-4 concentrations between male and female pediatric patients to determine if gender influences the inflammatory response in T1DM.

## **2. Materials and Methods**

### **2.1. Study Design**

This study was conducted on 103 children aged 1-16 years, including 70 children diagnosed with type 1 diabetes (33 males, 37 females) who were regular attendees at the Misrata Specialist Center for Diabetes and Endocrine Regulation and Treatment, along with 33 non-diabetic children (17 males, 16 females). Although the sample sizes were unequal (n=70 for patients and n=33 for healthy controls), this distribution is statistically acceptable in clinical research. The allocation ratio does not significantly compromise the statistical power of the study, To account for this imbalance, robust statistical methods were employed to ensure that the variance heterogeneity does not affect the validity of the results.

The diagnosis of pediatric diabetes was established through clinical follow-up files. Furthermore, pancreatic autoantibodies were screened to confirm the diagnosis of the disease.

Samples were collected between September and December 2024. Demographic data such as sex, age, weight, and height were recorded for all participants. For diabetic patients, additional parameters including HbA1c, fasting blood glucose, and disease duration were documented.

Based on disease duration, the diabetic cohort was divided into two groups:

*Group 1:* Children with a disease duration of 1 year or less (n = 20).

*Group 2:* Children with a disease duration of more than 1 year (n = 50).

### **2.2. Exclusion Criteria**

Participants from both groups (patients and healthy controls) were excluded if they presented with infectious or autoimmune diseases other than diabetes. Additionally, patients diagnosed with T2DM were also excluded from the study.

### **2.3. Ethical Approval**

Written informed consent was obtained from all participants parents (both diabetic and non-diabetic groups) before to initiating any study-related procedures. The research protocol was also reviewed and approved by the ethics committee at the Misrata Specialized Center for the regulation and treatment of diabetes and endocrine patients.

### **2.4. Sample Collection**

Five milliliters of venous blood were collected in the morning after a 9–12 hour fasting period using EDTA tubes. The samples were then centrifuged [3000 rpm for 15 minutes]. The plasma was transferred to cryotubes and stored at -20°C until use.

### **2.5. Quantification of Cytokines (IL-6, IL-4), Glycated Hemoglobin (HbA1c), and Fasting Blood Sugar (FBS) in Type 1 Diabetes**

Serum levels of the cytokines IL-4 and IL-6 were measured in the diabetic children included in the study and compared with those of healthy controls using a sandwich enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (MAGLUMI®, Germany). All cytokine concentrations were expressed in picograms per milliliter (pg/mL).

Glycated haemoglobin (HbA1c) was measured using the Mispa i3 analyser, while fasting blood Sugar levels were determined using the Mispa cxi pron analyser (both from Agappe Diagnostics).

## 2.6. Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16. Quantitative variables were expressed as mean  $\pm$  standard deviation, while qualitative variables were presented as numbers and percentages.

An independent t-test was used to compare two groups, and ANOVA was employed for comparisons among more than two groups to assess statistical differences. The Pearson correlation coefficient was calculated to examine the relationships between cytokines and other parameters. A p-value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Serum Interleukin-6 Concentration Levels

**Table 1.** demonstrates significantly higher serum IL-6 concentrations in diabetic patients compared to healthy controls. The mean IL-6 level was  $12.95 \pm 0.83$  pg/mL in diabetic children versus  $2.62 \pm 0.33$  pg/mL in healthy subjects. Statistical analysis using an independent t-test revealed a highly significant difference ( $P=0.0001$ ) in IL-6 levels between the diabetic and control groups.

**Table 1.** IL-6 concentration in the blood serum of the studied samples

| Interleukin | Samples      | mean  | max   | mini | SD   | P-value |
|-------------|--------------|-------|-------|------|------|---------|
| IL-6        | Healthy(33)  | 2.62  | 6.98  | 0.09 | 0.33 | 0.0001  |
|             | Patients(70) | 12.95 | 33.76 | 3.08 | 0.83 |         |

#### 3.1.1. Serum IL-6 Concentration in male and female subjects

As shown in **Table 2**, results demonstrated significantly elevated IL-6 levels in Female patients ( $13.15 \pm 1.18$  pg/mL) vs. healthy females ( $2.76 \pm 0.46$  pg/mL;  $p = 0.0001$ ), and Male patients ( $12.73 \pm 1.18$  pg/mL) vs. healthy males ( $2.49 \pm 0.48$  pg/mL;  $p = 0.0001$ ). Pearson correlation analysis revealed a positive relationship ( $r = 0.01980$ ) in IL-6 concentrations between diabetic females and males.

**Table 2.** Comparison between the concentrations of IL-6 in the blood serum of samples from males and females.

| Interleukin | Gender             | Mean  | max   | mini | SD   | P-value |
|-------------|--------------------|-------|-------|------|------|---------|
| IL-6        | Females (healthy)  | 2.76  | 6.67  | 0.09 | 0.46 | 0.0001  |
|             | Females (patients) | 13.15 | 33.76 | 3.08 | 1.18 |         |
|             | Males (healthy)    | 2.49  | 6.98  | 0.09 | 0.48 | 0.0001  |
|             | Males (patients)   | 12.73 | 26.09 | 4.02 | 1.18 |         |

Pearson correlation =0.019804

#### 3.1.2. The relationship between IL-6 concentration and HbA1c, FBS, and BMI in children with type 1 diabetes.

As shown in **Table 3**, the study results revealed—based on Pearson correlation analysis a weak positive correlation between IL-6 concentration and HbA1c 0.09, BMI 0.05 and FBS 0.07.

**Table 3.** The relationship between IL-6 concentration, and HbA1c, FBS, and BMI in children with type 1 diabetes.

| Parameters | Pearson Correlation |
|------------|---------------------|
| IL-6-FBS   | 0.07                |
| IL-6-HbA1c | 0.09                |
| IL-6-BMI   | 0.05                |

### 3.2. The concentration level of interleukin-4 (IL-4) in the serum of the studied samples.

**Table 4.** shows an increase in IL-4 concentration in the serum of healthy subjects compared to the patient group. The concentration in healthy serum was  $41.77 \pm 13.34$  pg/mL, while in the patient group, it was  $18.71 \pm 7.08$  pg/mL. However, statistical analysis using the t-test did not reveal a

significant difference (P-value = 0.10) in IL-4 levels between children with diabetes and the healthy control group.

**Table 4.** IL-4 concentration in the blood serum of the studied samples

| Interleukin | Samples      | mean  | max    | mini | SD    | P-value |
|-------------|--------------|-------|--------|------|-------|---------|
| IL-4        | Healthy(33)  | 41.77 | 426.00 | 4.01 | 13.34 | 0.10    |
|             | Patients(70) | 18.71 | 350.00 | 0.50 | 7.08  |         |

### 3.2.1. IL-4 Concentration in the Serum of Male and Female Samples

As shown in Table 5, the results revealed higher IL-4 levels in healthy females ( $38.71 \pm 10.87$  pg/mL) compared to female patients ( $8.04 \pm 2.10$  pg/mL), with a statistically significant difference ( $P = 0.0002$ ). Increased IL-4 levels in healthy males ( $44.65 \pm 24.20$  pg/mL) compared to male patients ( $30.69 \pm 14.68$  pg/mL), but without statistical significance ( $P = 0.6100$ ). Additionally, Pearson correlation analysis showed a negative correlation ( $-0.06419$ ) in IL-4 concentration between female and male patients.

**Table 5.** Comparison of IL-4 concentrations in the serum of male and female samples

| Interleukin          | Gender             | mean     | max    | mini | SD    | P-value |
|----------------------|--------------------|----------|--------|------|-------|---------|
| IL-4                 | Females (healthy)  | 38.71    | 147.00 | 4.84 | 10.87 | 0.0002  |
|                      | Females (patients) | 8.04     | 58.00  | 0.50 | 2.10  |         |
|                      | Males (healthy)    | 44.65    | 426.00 | 4.01 | 24.20 | 0.6100  |
|                      | Males (patients)   | 30.69    | 350.00 | 0.50 | 14.68 |         |
| Pearson correlation= |                    | -0.06419 |        |      |       |         |

### 3.2.2. The Relationship Between IL-4 Concentration and HbA1c, FBS, and BMI in Children with Type 1 Diabetes

The study results, as shown in Table 6 and analyzed using Pearson correlation, revealed a negative correlation between IL-4 concentration and HbA1c  $-0.095$ , FBS  $-0.051$ , A positive correlation between IL-4 concentration and BMI  $0.084$ .

**Table 6.** shows the relationship between IL-4 concentration and HbA1c, FBS, and BMI in children with type 1 diabetes.

| Parameters | Pearson Correlation |
|------------|---------------------|
| IL-4-FBS   | -0.051              |
| IL-4-HbA1c | -0.095              |
| IL-4-BMI   | 0.084               |

### 3.3. Relationship Between Disease Duration and IL-6/IL-4 Concentrations in Children with Type 1 Diabetes

This study examined the relationship between the duration of Type 1 diabetes in children and the concentrations of IL-6 and IL-4. The results, as shown in Table 7, revealed significant differences in IL-6 concentrations among children with Type 1 diabetes based on disease duration. The mean IL-6 concentration in the group with a disease duration of 1 year and less was  $5.90$  pg/mL, while the mean concentration in the group with a disease duration of more than one year was  $34.06$  pg/mL.

The results also showed significant differences in the concentration of IL-4 in children with diabetes between the duration of infection of one year or less and the duration of infection of more than one year. The mean IL-4 concentration in the group with a disease duration of one year and less was  $12.88$  pg/mL, whereas the mean concentration in the group with a disease duration of more than 1 year was  $14.19$  pg/mL.

**Table 7.** Relationship between disease duration and IL-6/IL-4 concentrations in children with type 1 diabetes.

| Interleukin | Duration of diabetes/number | mean  | max    | mini | SD    | P-value |
|-------------|-----------------------------|-------|--------|------|-------|---------|
| IL-6        | One year and less /20       | 5.90  | 13.10  | 0.92 | 0.68  | 0.0002  |
|             | Over one year /50           | 34.06 | 350.00 | 0.50 | 16.63 |         |
| IL-4        | One year and less /20       | 12.88 | 24.06  | 5.09 | 1.43  | 0.0001  |
|             | Over one year /50           | 14.19 | 33.76  | 4.82 | 2.81  |         |

#### 4. Discussion

Deregulation in cytokine production, which leads to a dominance of the Th1 cell response over the Th2 cell response, plays a critical role in the pathogenesis of type 1 diabetes [24]. Metabolic issues in type 1 diabetes are often associated with chronic inflammation in the pancreatic islets, resulting in the destruction of insulin-secreting beta cells by auto reactive CD8+ and CD4+ T cells. These immune cells repeatedly release cytokines throughout the progression of the disease [25]. While the potential role of IL-6 in the pathogenesis of diabetes remains a subject of debate [26], some recent studies suggest a strong relationship between IL-6 and the development of type 1 diabetes [27]. In this study, our results showed a significantly higher mean concentration of IL-6 in patients ( $12.95 \pm 0.83$ ) compared to healthy controls ( $22.62 \pm 0.33$ ), with a statistically significant difference ( $p=0.001$ ). Since the serum IL-6 concentration in children with diabetes far exceeded that of healthy children, these findings highlight the importance of IL-6 and other pro-inflammatory factors as potential early biomarkers or risk factors for the disease.

The current results are consistent with those reported by El-mohamady et al. and Khaja et al., who demonstrated elevated levels of IL-6 in the serum of patients with type 1 diabetes [7,25]. These findings also align with the results obtained from Siewko et al. and Zorena et al. [28,29]. Additionally, Hamed, Alexandraki, Nuhia, and their colleagues observed increased IL-6 secretion in patients with type 1 diabetes [30,31,8].

In contrast, the results reported by other groups are entirely divergent. For instance, Rydén and Faresjö did not observe elevated IL-6 concentrations in children with type 1 diabetes [32]. Other studies reported no difference or even decreased IL-6 levels in patients with type 1 diabetes [21,33]. Despite these discrepancies, dysregulated IL-6 production and receptor signalling are common features in type 1 diabetes and are frequently associated with pancreatic islet inflammation and beta-cell damage [3].

The significant rise in IL-6 concentrations from 5.90 pg/mL in newly diagnosed patients to 34.06 pg/mL in those with advanced disease duration suggests that IL-6 acts as a critical driver of progressive pancreatic pathology. This chronic elevation facilitates beta-cell depletion by inducing endoplasmic reticulum (ER) stress and activating STAT3-mediated apoptotic pathways [29]. Furthermore, sustained exposure to high IL-6 levels has been shown to impair glucose-stimulated insulin secretion (GSIS) by downregulating essential insulin transcription factors, such as Ins1 and Ins2, thereby diminishing the functional capacity of the remaining beta-cell mass. These molecular mechanisms, characterized by a transition from acute inflammation to chronic destructive signaling, explain the progressive exhaustion of insulin-secreting cells observed as the disease duration increases [34]. These findings align with those reported by Geerlings et al. and Dogan et al., who observed significantly lower IL-6 levels in newly diagnosed cases compared to patients with longer disease duration [19,32].

This contrasts with the findings of Erbağcı et al., who detected higher IL-6 levels in newly diagnosed children with type 1 diabetes compared to those with long-term diabetic complications [35]. Similarly, El-mohamady et al. demonstrated that IL-6 concentrations were significantly lower ( $p < 0.01$ ) in children with long-standing diabetes than in recently diagnosed children. They attributed this to the activation of systemic inflammatory processes during the early stages of type 1 diabetes, which may promote sustained beta-cell destruction [7]. Furthermore, IL-6 concentrations were significantly increased in newly diagnosed diabetic patients in a study by Siewko et al. [28], these differences were attributed to the effects of diabetes treatment regimens and metabolic control [29]. The difference in interleukin-6 (IL-6) levels may reflect a shift from acute autoimmune destruction at diagnosis to

chronic systemic inflammation after one year, driven by oxidative stress and the accumulation of advanced glycation end products (AGEs). This elevation is exacerbated by the end of the partial remission period and increased glycemic variability, making IL-6 a key indicator of long-term metabolic burden, rather than simply a primary immune response.

The elevated concentrations of certain cytokines in children with type 1 diabetes are associated, over time, with an increased risk of developing chronic complications. As the disease duration progresses, these effects become more severe, as high levels of these cytokines are linked to tissue damage in the eyes (retinopathy) and kidneys (nephropathy), particularly in patients with a disease duration exceeding ten years [36].

Pro-inflammatory cytokines released during illness can intensify the disease, while anti-inflammatory cytokines provide protection [23]. The anti-inflammatory cytokine IL-4 plays an important role in type 1 diabetes mellitus; however, its role is not yet fully understood, and published findings remain inconsistent. In this study, the mean concentration of IL-4 was higher in healthy individuals than in patients, but without a statistically significant difference ( $p=0.10$ ). This finding aligns with studies by Pérez et al., Nuhair et al., and Abed Nasser, which observed no differences in serum IL-4 levels between diabetic patients and the control group [24,8,36]. Similarly, Jasem's results showed no significant differences in IL-4 concentration levels, with healthy individuals exhibiting higher levels 10 patients[23].

In contrast, Khalaf et al. and Shaker et al. found significantly higher levels of IL-4 in children with diabetes compared to non-diabetic children [12,37]. Similarly, the results of Berwary et al. and Kikodze et al. showed statistically significant differences in IL-4 concentration, revealing that patients had higher IL-4 levels than healthy individuals[38,22]. This increase may be attributed to hyperglycaemia, as well as the reduced response of Th2 helper cells and the increased activity of Th1 effector cells [36]. Additionally, studies have demonstrated that the presence of IL-4 in the serum of diabetic patients plays a regulatory role, preventing the progression of type 1 diabetes. This occurs through CD1d-mediated suppression of NK cells and effector T cells targeting pancreatic beta cells in the islets of Langerhans [39].

The results also showed statistically significant differences in IL-4 concentration among diabetic children when comparing disease duration ( $\leq 1$  year vs.  $>1$  year) ( $P$ -value = 0.0001). Consistent with our findings, IL-4 levels decreased in newly diagnosed type 1 diabetic patients in NOD mice in studies by Kukreja et al. and Kent et al [40,41], and this did not agree with the study by Abed Nasser, who found no significant differences in IL-4 concentration in children with diabetes between the duration of disease (one year or less) and the duration of disease (more than one year[36] ).

Furthermore, the study results revealed a positive correlation between IL-6 concentrations and HbA1c, BMI, and FBS, while a negative correlation was observed between IL-4 concentrations and both HbA1c and FBS. In line with these findings, Hamed et al. reported a strong positive association between serum IL-6 levels in children with type 1 diabetes and both HbA1c and BMI [30]. This relationship may be attributed to hyperglycaemia-induced monocyte activation, which stimulates increased IL-6 secretion through the upregulation of Protein Kinase C (PKC), p38 Mitogen-Activated Protein Kinase (p38 MAPK), and Nuclear Factor-kappa B (NF- $\kappa$ B) activity. These pathways collectively enhance IL-6 transcription and release [42]. Additionally, hyperglycemia-induced mitochondrial oxidative stress generates reactive oxygen species (ROS) [43], which further amplify these inflammatory pathways. Consequently, the elevated IL-6 concentrations recorded in this study likely reflect a state of glucose-driven immune activation, where poorly controlled metabolic parameters exacerbate the inflammatory profile in children with type 1 diabetes.

The positive correlations between BMI and IL-6 concentrations are also consistent with the findings of Park et al., who reported that plasma IL-6 levels were positively associated with body mass index [44]. This association can be attributed to the fact that adipose tissue serves as a significant source of pro-inflammatory cytokines, particularly IL-6, which stimulates lipolysis in human adipocytes [45].

The current study also revealed significantly elevated serum concentrations of IL-6 in both male and female patients compared to their respective healthy control groups. The observed elevation in patients reflects a generalized state of immune activation. This elevation is consistent with

the established role of IL-6 as a key pleiotropic cytokine that orchestrates the systemic inflammatory response and induces the synthesis of acute-phase proteins in response to pathological stimuli [46]. Notably, the higher baseline and reactive levels of IL-6 observed in female patients may be attributed to inherent physiological dimorphism. Adipose tissue, which typically differs in distribution and percentage between genders, is known to contribute up to 30% of circulating IL-6 [47]. This suggests that female body composition may predispose them to a more pronounced inflammatory profile under diseased conditions.

## 5. Conclusion

This study demonstrated significantly elevated IL-6 levels and relatively reduced IL-4 levels in children with type 1 diabetes compared to healthy controls. These findings support the presence of a pro-inflammatory state associated with disease duration. However, further longitudinal and mechanistic studies are required to determine the clinical utility of these cytokines as biomarkers.

## Recommendations

- Conduct further studies in other Libyan populations to validate these findings.
- Investigate additional pro- and anti-inflammatory cytokines to better understand their roles in type 1 diabetes.
- Explore the relationship between autoantibodies and pro-/anti-inflammatory cytokines.

## Disclaimer

The paper has never been offered for presentation or publication, including during submission for some other conference.

## Conflicts of Interest

No financial, personal or professional interests' conflict of interest to declare.

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#### تقييم مستويات الإنترلوكين 6 والإنترلوكين 4 في مصل مرضى السكري من النوع الأول

تلعب السيتوكينات أدوارًا حاسمة في تنظيم التفاعلات بين خلايا بيتا البنكرياسية والخلايا المناعية في تطور داء السكري من النوع الأول. وقد ثبت أن السيتوكينات المحفزة للالتهاب التي تُفرز أثناء المرض قد تُفاقمه، بينما توفر السيتوكينات المضادة للالتهاب الحماية. هدفت هذه الدراسة إلى تقييم مستويات الإنترلوكين-6 (IL-6) والإنترلوكين-4 (IL-4) في مصل الأطفال المصابين بداء السكري من النوع الأول ومقارنتها بمستويات الأطفال الأصحاء. تم تجنيد سبعين طفلاً مصاباً بداء السكري من النوع الأول وثلاثة وثلاثين طفلاً سليماً بين شهري سبتمبر وديسمبر من عام 2024. قُيسَت مستويات الإنترلوكين-6 (IL-6) والإنترلوكين-4 (IL-4) في المصل باستخدام اختبار ELISA. أظهرت النتائج ارتفاعاً ملحوظاً في متوسط تركيز الإنترلوكين-6 (IL-6) لدى المرضى ( $0.83 \pm 12.95$ ) مقارنةً بالأصحاء ( $0.33 \pm 2.62$ )، مع وجود فرق دال إحصائياً ( $p=0.001$ ). على الرغم من أن متوسط تركيز الإنترلوكين-4 كان أعلى لدى الأفراد الأصحاء ( $13.34 \pm 41.77$ ) مقارنةً بالمرضى ( $7.08 \pm 18.71$ )، إلا أن هذا الفرق لم يكن ذا دلالة إحصائية ( $p=0.10$ ). كما كشفت الدراسة عن وجود ارتباطات دالة إحصائية بين تركيزي الإنترلوكين-6 والإنترلوكين-4 ومدة المرض. بالإضافة إلى ذلك، لوحظ ارتباط إيجابي ضعيف بين مستويات الإنترلوكين-6 والهيموجلوبين السكري (HbA1c) ومؤشر كتلة الجسم (BMI) وسكر الدم الصائم (FBS)، وكذلك ارتباط إيجابي ضعيف بين تركيزات الإنترلوكين-6 لدى مرضى السكري من الذكور والإناث. تُظهر هذه الدراسة ارتفاعاً ملحوظاً في مستويات الإنترلوكين-6 المحفز للالتهاب لدى الأطفال المصابين بداء السكري من النوع الأول مقارنةً بأقرانهم الأصحاء، مما يشير إلى حالة التهاب مزمنة مرتبطة بالمرض. في حين كانت مستويات الإنترلوكين-4 أقل لدى مرضى السكري، إلا أن هذا الفرق لم يصل إلى مستوى الدلالة الإحصائية.

**الكلمات المفتاحية:** إنترلوكين 6، إنترلوكين 4، مرضى السكري