Serum levels of IL-18 and IL-22 are Associated with Glycemic Control Status in Patients with Insulin-Independent Diabetes Mellitus

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ABSTRACT

Type 2 diabetes (T2D) is a complex, chronic metabolic disease. Novel studies point to the involvement of immunological mechanisms in T2D pathogenesis. Glycated hemoglobin (HbA1C) is a reliable biomarker for verifying glycemic control status. We investigated the pathological role of inflammatory cytokines, IL-18 and IL-22, in T2D development and their correlation with glycemic control status. This is a case–control study. Conducted through January to July/ 2023. The study included patients with T2D who were divided into two groups according to their serum HbA1C levels, the controlled, HbA1C≤ 6.5%, and uncontrolled hyperglycemic groups ≥ 6.5%. Sex- and age-matched non-diabetic volunteers were included as a control group. Serum IL-18 and IL-22 were measured using the enzyme-linked immunosorbent assay technique. Fasting blood sugar and HbA1C values were analyzed as part of the routine testing of patients in the study hospital. Our results showed that serum IL-18 and IL-22 concentrations were significantly higher in patients with T2D compared to healthy controls (P ≤ 0.001). Furthermore, both cytokines exhibited a significant correlation with glycemic status (P ≤ 0.05 in both cases). Only IL-22 showed a significant correlation with disease duration (P ≤ 0.05). IL-18 and IL-22 are critical modulating factors in glycemic control in T2D development. Exploring the role of such inflammatory mediators could aid in the discovery of novel anti-inflammatory cytokine-based therapies for treating or preventing T2D.

Keywords: T2D; IL-18; IL-22; HbA1C; hyperglycemia; proinflammatory cytokines

INTRODUCTION

T2D is a complex chronic metabolic disease of adults. T2D represents a major public health concern as it accounts for roughly 90% of all DM cases. The disease is fundamentally characterized by hyperglycemia. It is recognized that both insulin resistance and inadequate insulin production by pancreatic β-cells represent the major pathologic mechanisms of T2D[1,2].

Although T2D is recognized as a multifactorial disease that arises from the complex interplay between genetic and environmental triggers, a growing body of evidence from around the globe highlights the critical role of chronic low-grade inflammatory responses in the course of T2D development[3]. Pancreatic islet dysfunction is the core pathologic process implicated in T2D development. A growing body of evidence has also revealed that targeted immunotherapy (anti-inflammatory therapy) can reduce insulin resistance and improve pancreatic β-cell function[5].

Accumulating evidence points to the involvement of multiple immune cell types in T2D pathogenicity[4]. The authors of numerous studies have reported a correlation between T2D development and increased serum levels of inflammatory mediators, such as CRP; cytokines, such as IL-1β, IL-6, TNF-α, and IL-18; and chemokines, such as CCL2, CCL5, CX3CL1, and CXCL10[5].

IL-18, a member of the IL-1β family, was first characterized as an IFN-γ-inducing factor. It can be produced by immune cells, such as macrophages and dendritic cells, and non-immune cells, such as keratinocytes, endothelial cells, some epithelial cells, and smooth muscle cells. It is implicated in the development and progression of inflammatory and autoimmune disorders[6]. IL-18/IL-18R gene expression in adipose tissue has been reported to be higher in non-diabetic obese individuals compared with overweight and slim subjects and is associated with insulin resistance[7].

IL-22 is an inflammatory cytokine that belongs to the IL-10 family and plays a critical role in tissue remodeling during inflammatory processes. Upregulated plasma levels of IL-22 have been recorded in numerous inflammatory disorders and autoimmune diseases[11]. It is produced by both innate and adaptive immune cells; NK cells;

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neutrophils, CD4 T helper cells type 22 (Th22) and Th17; and innate lymphoid cells type 3 (ILC-3)[3,8]. CD4 T helper cells contribute to the initiation of inflammation. In addition to Th-1, Th-2, and Th-17, newly discovered subsets of Th-9 and Th-22 have been found to contribute to the pathogenicity of metabolic disorders[9,10].

The results of a meta-analysis conducted in 2021 revealed that extracellular matrix organization and cytokine–cytokine interactions are fundamental factors in T2D progression and that two genes, IL-6 and IL-11, are among the genes most closely associated with T2D[12]. IL-6 and IL-11 upregulation in the blood can induce fibrosis, a process involved in pancreatic β-cell dysfunction[12].

Glycated hemoglobin (HbA1C) is widely applied as a proxy for glycemic control in patients with diabetes mellitus[2]. HbA1C is a reliable biomarker (indicator) that reflects the average blood glucose level over the previous 3 months. In this study, we aimed to investigate the possible association between two inflammatory cytokines, IL-18 and IL-22, and T2D, in addition to verifying their association with glycemic status.

MATERIALS AND METHODS

This study was conducted under the ethical approval of the Institutional Review Board of the College of Medicine, Al-Nahrain University, Baghdad, Iraq (Approval No.20240541). Clinical and demographic information was recorded by clinicians through patient–physician interviews after obtaining written informed consent from the participants. We employed a prospective, case–control, single-center design in the present study. Sample collection was conducted at Alemammain Alkadhmani Medical City from January to July 2023. The inclusion criteria were as follows: Adults aged older than 18 years. The patient group included patients of both sexes with a confirmed diagnosis of T2D. Patients with T2D were classified according to HbA1C value into two groups: controlled hyperglycemia with an HbA1C ≤ 6.5% and uncontrolled hyperglycemia with an HbA1C ≥ 6.5%. The control group included adults of both sexes who met the basic eligibility criteria for healthy blood donors at the time of blood collection. Exclusion criteria included patients with type 1 diabetes mellitus (T-1DM), latent autoimmune diabetes (LADA) or hybrid diabetes, pregnant women, and patients with cancer or any autoimmune and/or autoinflammatory diseases.

Venous blood samples were collected in serum separator tubes to enable rapid and efficient serum separation, centrifuged in Eppendorf tubes, and stored at -20 °C until use. IL-18 and IL-22 were measured using the ELISA technique (ELK biotechnology, China), with experiments conducted at the Medical Research Center at Al-Nahrain University. Fasting blood sugar (FBS) and HbA1C were measured as part of routine laboratory tests at Al-Imammain Alkadhemain Medical City.

Statistics: A series of analyses were performed using SPSS-23 software. General demographic characteristics of the participants were examined. Categorical data are described as counts and percentages, and Fisher's exact test was employed to assess the association between the studied markers. Non-normally distributed data are presented as the medians and means ± standard deviations (±SDs), and comparisons were made using the Mann–Whitney U test. A 95% confidence interval was applied, with a P-value less than or equal to 0.05 considered significant.

RESULTS

Forty-two patients with T2D (12 men and 30 women) and 41 controls (20 men and 21 women) were selected for inclusion in the present study. Among the patients with T2D, 15 (35.71%) were found to have elevated HbA1C levels and 27 (64.29%) were found to have an controlled glycemic status. Regarding sex, no significant difference was found between the two groups, with a P-value of 0.083. Furthermore, no significant difference was found regarding the age of patients with T2D versus those in the control group, with medians of 56.5 years and 45 years, respectively, and a P-value of 0.632. Regarding smoking status, 83.3% of T2D patients were smokers; in comparison, all of the controls were non-smokers, with a P-value <0.001. Regarding CRP,

Table 1. Comparison of clinical baseline characteristics of the study population.

Categorical variable		Study groups				P-value
		T2D*		Control		
Sex	Female	30	71.43%	21	51.22%	0.083
	Male	12	28.57%	20	48.78%	
Smoking	No	35	83.30%	41	100.00%	<0.001
	Yes	7	16.70%	0	0.00%	
CRP†	Negative	27	64.30%	41	100.00%	<0.001
	Positive	15	35.70%	0	0.00%	
Retinopathy	No	25	59.50%			
	Yes	17	40.50%			
Nephropathy	No	25	59.50%			
	Yes	17	40.50%			
Neuropathy	No	35	83.30%			
	Yes	7	16.70%			
Stroke	No	40	95.20%			
	Yes	2	4.80%			
Heart Disease	No	37	88.10%			
	Yes	5	11.90%			
PVD‡	No	35	83.30%			
	Yes	7	16.70%			
HbA1C§ status	Controlled	15	35.71%			
	Uncontrolled	27	64.29%			

*T2D: type 2 diabetes; †CRP: C-reactive protein; ‡PVD: peripheral vascular disease; §HbA1C: glycated hemoglobin.

Table 2. Laboratory characteristics of the study population.

Variable	T2D*			Control			P-value
	Median	5%	95%	Median	5%	95%	
Age (years)	56.5	51	59	45	39	53	0.632
Disease duration (years)	6	5	8	.	.	.	
FBS† (mg/dL)	200.5	170	250	86	83	91	<0.001
HbA1C‡ (mmol/L)	7.5	6.7	8.3	5.3	5.3	5.4	<0.001
IL18 §(pg/ml)	531.46	446	575.78	269.2	236.25	345.1	<0.001
IL22†† (pg/ml)	14.48	0	47.94	16.9	0	22.1	<0.001

*T2D: type 2 diabetes; †FBS: fasting blood sugar; ‡HbA1C: glycated hemoglobin; §IL-18: interleukin 18; ††IL-22: interleukin 22.

Table 3. The association between IL-18 and IL-22 and HbA1C and FBS.

		IL22 (pg/ml)	IL18 (pg/ml)
Disease duration (years)	r	0.344*	0.182
	p	0.026	0.25
FBS*	r	0.133	-0.211
	p	0.401	0.18
HbA1C†	r	0.281*	0.383*
	p	0.009	0.012
IL22‡ (pg/ml)	r	1	-0.01
	p	.	0.948
IL18§ (pg/ml)	r	-0.01	1
	p	0.948	.

T2D: type 2 diabetes;* FBS: fasting blood sugar; †HbA1C: glycated hemoglobin; ‡IL-22: interleukin 22; § IL-18: interleukin 18;.

a qualitative measurement was performed. Our results showed that 35.7% of patients with T2D produced a positive result, whereas none of the healthy controls produced a positive result. A significant difference was detected between the two groups, with a P-value <0.001 (Table-1).

Regarding diabetes-related complications, 3 (7.14%) patients suffered from no complications, 28 (66.6%) patients were noted as suffering from one complication, and 11 (26.19%) patients suffered from multiple complications. Furthermore, our data revealed that both retinopathy and nephropathy were the most prevalent complications, with each condition recorded in 40.5% of patients. Conversely, neuropathy and peripheral vascular disease each affected 16.7% of the patients. Heart disease and stroke represented the lowest percentages among the studied complications, 11.9% and 4.8%, respectively (Table 1).

The median FBS of patients with T2D was 200.5 mg/dL versus 86 mg/dL in the control group. The median values of glycated hemoglobin were 7.5 mmol/L and 5.3 mmol/L in the T2D patients and controls, respectively. Regarding the studied inflammatory cytokines, the median value for serum IL-18 was 531.46 pg/ml in the T2D group versus 269.2 pg/ml in the healthy control group, and the IL-22 serum level in T2D patients was 14.48 pg/ml versus 16.9 pg/ml in the healthy control group. A highly significant difference, P-value <0.001, was found for the four mentioned parameters between the T2D group and the healthy control group (Table 2).

The associations between IL-18 and IL-22 and disease duration, FBS, and glycemia status were assessed. Regarding disease duration, only IL-22 showed a positive association, with a P-value of 0.026. Neither of the cytokines showed any association with FBS, with P-values of 0.18 and 0.401, respectively. However, they were found to have a positive association with HbA1C, with P-values of 0.012 and 0.009, respectively (Table 3).

DISCUSSION

Diabetes ranks as one of the highest priority health issues worldwide, primarily due to its serious complications. Diabetes can considerably shorten life expectancy[13]. Innovative studies have highlighted the involvement of immunological mechanisms in T2D pathogenesis[4]. In the present work, we aimed to analyze the association between peripheral IL-18 and IL-22 levels and diabetes control status, which is represented by chronic glycemic control. The most important finding of the present study is the fact that serum levels of IL-18 and IL-22 are significantly higher among patients with T2D compared to healthy controls. Furthermore, the results showed that both cytokines were positively associated with HbA1C levels. In contrast, however, only IL-22 demonstrated a significant association with disease duration.

The present findings are in accordance with accumulating evidence that supports the hypothesis that there is a complex interplay between chronic inflammatory status and T2D development[5,13]. Islet inflammation is associated with serious lesions characterized by increasing numbers of infiltrated macrophages, which produce high levels of proinflammatory cytokines, namely IFN-γ, TNF-a, and IL-1b, resulting in β-cell dysfunction and, consequently, T2D[15]. Published data from previous studies indicate that increased blood levels of the acute phase protein CRP and proinflammatory cytokines such as IL-1β, IL-6, IL-18, and TNF-α have been identified as risk factors contributing to T2D progression[5]. IL-1 beta and IL-1Ra play potential roles in maintaining the energetic balance of pancreatic β-cell function and, consequently, glycemic control in T2D development[14].

Furthermore, the results of recent investigations have revealed that the utilization of anti-inflammatory cytokines may considerably ameliorate insulin resistance and improve pancreatic β-cell function. Th-1 inflammatory cytokines such as IFN-γ play a decisive role in glucose intolerance in vivo and induce a time-dependent decrement in insulin-induced glucose uptake. It is noted that TNF-α knockout may ameliorate insulin resistance[16]. Chemokines have also been implicated in T2D initiation. The results of a meta-analysis published in 2021 revealed that serum levels of certain peripheral chemokines, CC (CCL1, CCL2, CCL4 CCL5, and CCL11) and CXC (CXCL 8, CXCL10, and CXCL1), are significantly higher in T2D patients; however, this same phenomenon is not observed in patients with prediabetes compared with healthy controls[5]. Our results demonstrating that IL-18 is positively associated with T2D development and glycemia status are in accordance with other scholarly studies. The association between IL-18 and T2D has been noted by the authors of previous studies, with this cytokine being recorded at higher levels in patients with obesity (weight status-related) and those with metabolic disorders such as T2D[17].

He Zhuang and colleagues conducted a Mendelian randomization and genome-wide association study and established, with a high level of

confidence, the causal effect of IL-18 and T2D initiation by evolving instrumental variables according to single-nucleotide polymorphisms (SNP)s of IL-18[14].

Although adipocytes produce IL-18, they are not the main producers of this cytokine; rather, IL-18 is preponderantly produced by infiltrated activated macrophages, thus confirming its immunopathologic role[17]. In addition to infiltrated macrophages, pancreatic α-cells are primary producers of IL-18. This cytokine utilizes the IL-18 receptor (IL-18r), located on pancreatic acinar cells, to control the inflammation process and macrophage infiltration. In addition, IL-18 utilizes the Na-Cl co-transporter (NCC) and glucagon-like peptide-1 receptor (GLP-1r) present on pancreatic β-cells to control their proliferation and insulin production. It has been noted in previous studies that NCC deficiency in β-cells inhibits their proliferation and provokes insulin resistance and hyperglycemic conditions. Furthermore, deficiency in IL-18r on pancreatic acinar cells promotes macrophage infiltration and negatively affects β-cells' function and size, inducing insulin resistance and hyperglycemia[18]. It is documented that NCC regulates β-cell function through the "PI3K activated serine-threonine kinase" (AKT) and "signal transducer and activator of transcription 3" (STAT 3) pathways to control transcription activator pancreatic duodenal homeobox-1 (PDX-1) gene expression, which, in turn, is implicated in β-cell proliferation and activation and insulin secretion[18,19].

Our finding that IL-22 is positively associated with T2D development is also in accordance with the results of other studies. In previous studies, increases in serum levels of IL-22 have been identified in individuals with metabolic disorders, such as T2D and coronary artery disease, compared with healthy individuals[9,4].

The follistatin-mediated mechanism of IL-22 in T2D development has been speculated. IL-22 induces the production of hepatokine named IL-11; this action in turn induces the production of follistatin, which is associated with an increased risk of T2D initiation due to the induction of insulin resistance in adipose tissues[20].

Furthermore, in a study conducted in 2022, researchers found that fasting serum follistatin levels are strongly correlated with fasting hyperinsulinemia in T2D patients[21]. In an in vivo study conducted in 2021, researchers found that glucotoxicity is a crucial trigger for elevated IL-6 and IL-11 levels in T2D patients, with both being correlated with the overproduction of extracellular matrix collagen in pancreatic islets. Consequently, glucotoxicity-induced IL-6 and IL-11 induce fibrosis, subsequently leading to islet dysfunction[12].

Limitations of the study

In the present work, we only analyzed the target parameters, IL-18 and IL-22, in the patients' serum. One improvement in future studies would be to investigate these parameters, at the protein level, in pancreatic tissues using immunohistochemical techniques and to further analyze their gene expression using the real-time polymerase chain reaction (RT–PCR) technique.

CONCLUSION

The results presented herein demonstrate that increased IL-18 and IL-22 levels in the peripheral blood are associated with T2D and glycemic control status represented by HbA1C. Such findings may strengthen previously published data that indicate that inflammation is a potent risk factor for T2D development. Such understanding may pave the way to identifying potential targeted immunotherapy regimes, primarily cytokine-dependent therapy. Such efforts would ameliorate conditions characterized by low-grade chronic inflammation and prevent disease progression from insulin resistance and glucose intolerance to T2D. The clinical value of the application of such therapy requires further verification through more advanced, in-depth studies.

Authorship Contribution: Dr. Ghassaq Alubaidi conceived the original concept, interpreted the results, and wrote the manuscript. Dalyia A. Hamoodi performed the data collection and participated in manuscript preparation. Dr. Haider F Ghazi analyzed the data and was responsible for the statistics. All authors contributed to the final version of the manuscript.

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