INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

2023 March; 29(2): 175-179
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

IL-6 Levels Analysis Controlled in Type 2 Diabetes Mellitus Patients and Uncontrolled

Moonika Todingan, Rachmawati Muhiddin, Liong Boy Kurniawan

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
E-mail: moonika@gmail.com

ABSTRACT

Interleukin-6 (IL-6) is one of the pro-inflammatory cytokines responsible for inducing tissue-specific and/or systemic inflammation, which is a major contributor to the induction of inflammation of pancreatic islet cells. Inflammation of pancreatic cells causes impaired insulin secretion and Type 2 Diabetes Mellitus (T2DM). This study aims to determine the levels of IL-6 in T2DM patients with different levels of severity. A cross-sectional study of 46 subjects was performed with 21 in the controlled T2DM group and 25 in the uncontrolled T2DM group. Interleukin-6 levels were measured using the ELISA method. The statistical tests used were the Mann-Whitney test and the Spearman test. The test results were significant if the p-value <0.05. The level of IL-6 in uncontrolled T2DM was higher (64.00±77.65 pg/mL) than in controlled T2DM (31.25±11.04 pg/mL). Although the levels in both groups were different, the value was not statistically significant (p=0.120). There was no significant correlation found between HbA1c and IL-6 (p=0.125, r =0.230). Several experimental studies have shown that IL-6 inhibits glucose-stimulated insulin secretion from pancreatic islets in experimental animals. However, some of them revealed that acute exposure to IL-6 did not appear to affect pancreatic islet cell function, which is still controversial today. This study found a tendency of increased IL-6 in high-severity T2DM compared to low-severity T2DM although not statistically significant. Further studies with more clinically homogeneous samples are still needed.

Keywords: Type 2 diabetes mellitus, IL-6

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occurs due to defects in insulin secretion, insulin action, or both. Diabetes mellitus is called "the silent killer" because this disease can affect all organs of the body and cause various complaints. Diseases that can be caused include visual impairment, cataracts, heart disease, kidney disease, impotence, difficult healing wounds, gangrene, lung infections, blood vessel disorders, and stroke. Symptoms occurring in people with diabetes mellitus are polydipsia, polyuria, polyphagia, weight loss, and tingling.[1,2]

Epidemiological research shows a tendency for increased incidence and prevalence of Type 2 Diabetes Mellitus (T2DM) in various parts of the world. The World Health Organization (WHO) predicts an increase in the number of people with DM, making it a global health threat. The WHO predicts an increase in the number of people with diabetes in Indonesia, from 8.4 million in 2000 to around 21.3 million in 2030. This report shows an increase in the number of people with diabetes of 2-3 times in 2035. Meanwhile, the International Diabetes Federation (IDF) predicts an increase in the number of people with diabetes in Indonesia from 9.1 million in 2014 to 14.1 million in 2035.[2,3] Insulin resistance in muscle and liver cells, and pancreatic beta cell failure have been recognized as the pathophysiology of central damage in T2DM.

Recent research has shown that beta cell failure occurs earlier and is more severe than previously thought.[4-6] Glycosylated hemoglobin (HbA1c) reflects blood glucose levels corresponding to the life span of erythrocytes, which is about 120 days. High HbA1c levels were found in patients with high fasting blood glucose levels, elevated postprandial blood glucose levels, or both. Of all the glycated proteins, HbA1c is used as the gold standard for controlling blood sugar in patients with T2DM.[7,8]

In recent decades, there has been evidence that cytokines induce an acute phase response (referred to as low-grade inflammation), which is part of the activation of the innate immune system and is strongly associated with the pathogenesis of T2DM and is associated with complications such as dyslipidemia and atherosclerosis. Low-grade

systemic inflammation plays a role in the induction of stress on the endoplasm due to increased metabolic demand for insulin. Type 2 diabetes is characterized by peripheral insulin resistance and decreased insulin production, accompanied by chronic low-grade inflammation in peripheral tissues such as adipose, liver, and muscle. This illustrates the important role of inflammation in the pathogenesis of T2DM, which is considered an immune disorder.[4,9]

Several studies have found that type 2 diabetes patients have higher levels of inflammatory markers such as Interleukin-6 (IL-6), C-Reactive Protein (CRP), Plasminogen Activator Inhibitor-1 (PAI-1), Tumor Necrosis Factor- (TNF-), Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1).[3,10] Insulin secretion is mainly affected by the formation of oxidative stress and the induction of inflammation in pancreatic cells. IL-6, which is also a major contributor to the induction of inflammation of pancreatic islet cells, which ultimately leads to impaired insulin secretion and type 2 diabetes, is one of the factors involved in the formation of oxidative stress and inflammation in pancreatic cells. Glucose stimulated insulin secretion from the pancreatic islets in experimental animals, but several studies have also found that acute exposure to IL-6 does not appear to affect pancreatic islet cell function, so this is controversial. Despite this controversy, chronic exposure to IL-6 is responsible for inducing tissue-specific and/or systemic inflammation, which is one of the main causative factors in decreased insulin secretion from pancreatic islet cells. IL-6 is associated with malignancy, cardiovascular disease, renal insufficiency, acute infection, and obesity. A recent study by Nguyen and colleagues identified IL-6 as an important marker for microvascular complications in diabetes and identified it as a mediator that may play a role in the development of cardiovascular disease. Yasa *et al.* found that mean plasma IL-6 levels in DM patients with retinopathy were higher than those without retinopathy.[6,11,12]

Several studies on IL-6 levels in T2DM patients have been carried out in several journals, but to the author's knowledge, no one has reported an analysis of IL-6 levels in DM patients with different severity in Makassar. The purpose of this study was to determine differences in IL-6 levels in T2DM patients with different levels of severity.

METHODS

This research was a cross-sectional study and was carried out at the Clinical Pathology Laboratory Installation at Hasanuddin University Hospital and Dr. Wahidin Sudirohusodo for sampling and research at the Hasanuddin University Medical Faculty Research Unit/Hasanuddin University Hospital. The research was conducted in September-October 2021.

The study population was all patients diagnosed with T2DM by clinicians. The research sample consisted of patients with T2DM who met the research criteria (inclusion criteria) during September-October 2021. The inclusion criteria in question were all patients diagnosed with T2DM by clinicians and who had HbA1c levels checked. Patients with malignancy, acute infection, anemia, a history of cardiovascular disease, and renal insufficiency were excluded from the study. The classification of controlled and uncontrolled type 2 diabetes was based on HbA1c levels. Research subjects with HbA1c levels of ≤ 7% were classified into the controlled T2DM group. Subjects with HbA1c levels of >7% were classified as uncontrolled T2DM groups. Interleukin-6 levels were measured by an Enzyme-Linked Immunosorbent Assay (ELISA) with a double antibody sandwich on streptavidin-biotin base, using the Human IL-6 Platinum ELISA, which was expressed in units of picograms per milliliter (pg/mL).[4]

Data analysis was performed using SPSS. The normality of the data was tested using Kolmogorov-Smirnov. The statistical tests used were the Mann-Whitney test and the Spearman test. The test results were significant if the p-value was 0.05.

This research was conducted after obtaining ethical clearance by considering respect for subject, beneficence, non-maleficence, and justice from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanudin University-RSPTN UH-RSUP Dr. Wahidin Sudirohusodo Makassar with number 609/UN4.6.4.5.31/PP36/2021.

RESULTS AND DISCUSSIONS

A total of 46 patients with a diagnosis of T2DM from the age of 24-80 years, with most aged in the range of 41-60 years, were included. Based on gender, most of the subjects studied were males in the uncontrolled or controlled type 2 diabetes groups (Table 1).

The cross-sectional study involved 46 research subjects who met the inclusion and exclusion criteria. The sample characteristics in Table 1 show that the number of males with T2DM was higher than that of females. Based on the results of the study in Table 1,

Table 1. Characteristics of T2DM patients

Characteristics	Uncontrolled T2DM (n=25)	Controlled T2DM (n=21)
Gender		
Male	13 (52%)	11 (52.4%)
Female	12 (48%)	10 (47.6%)
Age		
21-40 years	1 (4%)	1 (4.8%)
41-60 years	16 (64%)	13 (61.9%)
60-80 years	8 (32%)	7 (33.33%)

Source: Primary data

it was also found most research subjects were patients aged 41–60 years, both in the uncontrolled and controlled groups of T2DM patients. The results of this study are from a study conducted by Yuniarti *et al*. who reported that the average age of patients with T2DM was generally above 50 years old. In addition, a study conducted by Sunjaya also found that the age group with the most diabetes mellitus was the 45–52 years old age group.

Table 2 shows the comparison between the levels of IL-6 in the uncontrolled and controlled T2DM groups. The level of IL-6 in uncontrolled type 2 diabetes was higher (64.00±77.65 pg/mL) than in controlled type 2 diabetes (31.25±11.04 pg/mL). Although different, the results of statistical tests showed that the difference was not significant, with p =0.120.

Table 2 explains that the levels of IL-6 in the uncontrolled T2DM group were higher than in the controlled T2DM group, even though there was no statistically significant difference (p=0.120). Several experimental studies have shown that IL-6 inhibits glucose-stimulated insulin secretion from the pancreatic islets in experimental animals, but several studies have also found that acute exposure to IL-6 does not appear to affect pancreatic islet-cell function, so this is controversial. This is in line with what Dan *et al*. stated, that although a large number of previous studies have been carried out, the role of IL-6 in insulin resistance remains controversial. Phosat *et al.* also suggested that although the relationship between IL-6 and type 2 diabetes was revealed in several studies, the role of IL-6 as a predictive marker for type 2 diabetes is still being investigated. This is in contrast to the study proposed by Sari *et al*., which found that IL-6 levels in uncontrolled diabetic patients were higher than in poorly controlled and controlled diabetic patients (p<0.05). Several factors that can affect IL-6 levels, such as malignancy, cardiovascular disease, kidney disease, and acute infections, have been ruled out in this study, but the measurement of Body Mass Index (BMI) was not carried out in this study, so obesity in T2DM patients could still be a confounding factor in this study. [5,13-16]

Interleukin-6 is a multifunctional cytokine with complex roles and is expressed in many cells, including immune cells, skeletal muscle cells, and pancreatic cells. Interleukin-6 has a dual role in modulating insulin sensitivity, which can act as either an enhancer or an inhibitor of insulin action. In addition, IL-6 is responsible for the recruitment of macrophages to adipose tissue in obesity, which leads to the development of inflammation, insulin resistance, and type 2 diabetes. Insulin-stimulated tyrosine phosphorylation and insulin-stimulated glucose transport lead to impaired insulin signaling and action. [17,18]

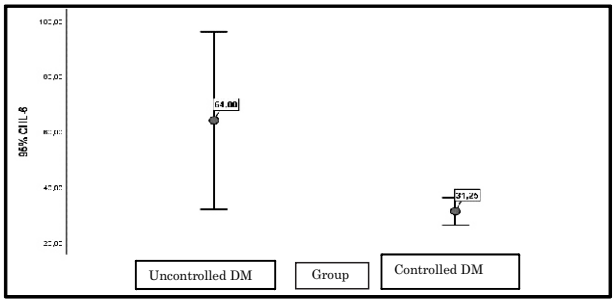

Figure 1. IL-6 levels between uncontrolled and controlled T2DM

A Spearman's test was performed to assess the correlation between IL-6 levels and HbA1c levels. The results of the Spearman correlation test (Table 3) showed no significant correlation was found between HbA1c levels and IL-6 (r =0.230, p=0.125).

Table 3. IL-6 level correlation test with HbA1c levels

Parameter	IL-6 (pg/mL)	
	r	p*
HbA1c (%)	0.230	0.125

Description: *Spearman test

Table 2. Comparison of IL-6 levels in uncontrolled and controlled T2DM groups

IL-6 (pg/mL)	T2DM is not Controlled (n=25)	Controlled T2DM (n=21)	p
Mean±SD	64.00±77.65	31.25±11.04	0.120*
Median (min-max)	31.09(13.98-324.28)	26.63(22.68-64.32)	

Description: *Mann-Whitney test

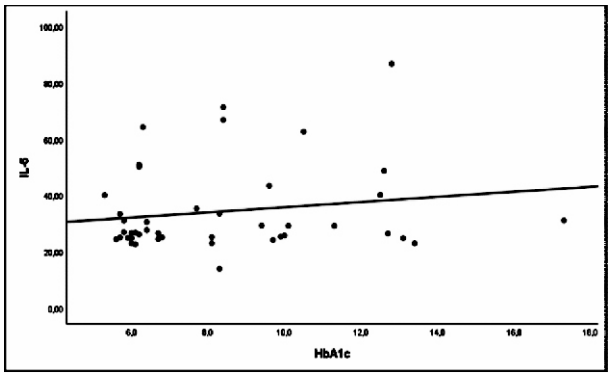

Figure 2. Correlation of HbA1c levels with IL-6

The points in Figure 2 are the intersections between the HbA1c and IL-6 values for each subject. The green line is a trend line (tendency) that appears to be going up, which means (if the test results are significant) the higher the HbA1c, the higher the IL-6. In this study, showed no significant correlation was found between HbA1c levels and IL-6 (r =0.230, p=0.125). Optimal glycemic control is needed to control DM and prevent the worsening of DM so that the increase in proinflammatory cytokines and antioxidant imbalance due to oxidative stress can be controlled. Glycated hemoglobin (HbA1c) is used as a biomarker of glycemic control in diabetic conditions because it describes blood glucose levels in the last 60-90 days. The results of the study by Qiao *et al.* found that HbA1c levels were positively correlated with cytokine levels. In addition to describing the history of blood glucose control in DM, HbA1c is also used as a prognostic indicator in the development of complications in DM. Several studies have found that HbA1c is significantly correlated with cytokine concentrations in diabetic nephropathy.[19,20] Krystallenia *et al*. reported that the different inflammatory states in some patients with type 2 diabetes may indicate a variety of different clinical histories. Their findings suggest that the level of inflammation can influence the level of inflammatory cytokines in DM patients.[20,21]

This study has several limitations. The patients who participated in this study were not screened for BMI measurements to determine obesity status, which could be a confounding factor and could affect IL-6 levels. The lack of previous research studies similar to this study is also another limitation of this study.

CONCLUSIONS AND SUGGESTIONS

This study found a tendency of increased IL-6 in type 2 diabetes with high severity rather than low severity, although statically insignificant. Further research must be done using samples that are more clinically homogeneous and in various hospitals. Further research related to IL-6 with different research subjects and from various characteristics/variables is also needed.

REFERENCES

1. Fatimah RN. Diabetes melitus tipe 2. J Major, 2015; 4(5): 74-9.
2. Soelistijo SA, Novida H, Rudijanto A. Pedoman pengelolaan dan pencegahan diabetes melitus tipe 2 di Indonesia. Perkeni, 2015; 133.
3. Rodrigues KF, Pietrani NT, Bosco AA, Campos FMF, Sandrim VC, Gomes KB. IL-6, TNF-α, and IL-10 levels/ polymorphisms and their association with type 2 diabetes mellitus and obesity in Brazilian individuals. Arch Endocrinol Metab, 2017; 61(5): 438-46.
4. Soelistijo Soebagijo Adi, Suastika Ketut, Lindarto Dharma, Decroli Eva, Permana Hikat, *et al*. Pengelolaan dan pencegahan diabetes melitus tipe 2 dewasa di Indonesia. Perkumpulan Endokrinol Indonesia, 2019; 133.
5. Rehman K, Akash MSH, Liaqat A, Kamal S, Qadir MI, Rasul A. Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. Crit Rev Eukaryot Gene Expr, 2017; 27(3): 229-36.
6. Al-Awaida WJ, Hameed WS, Al-Hassany HJ, Al-Dabet MM, Al-bawareed O, Hadi NR. Evaluation of the genetic association and expressions of Notch-2 /Jagged-1 in patients with type 2 diabetes mellitus. Medical Archives journal of Academy of Medical Sciences of Bosnia and Herzegovina, 2021; 75(2): 101-108.
7. Decroli E. Diabetes melitus tipe 2. Fakultas Kedokteran Andalas, 2019; 4-8.
8. Yuniarti E, Putri DH, Yuni S, Puja A, Sonata D, Biologi J, *et al.* Correlation of fasting blood glucose with IL-6 levels in type 2 diabetes mellitus ethnic Minangkabau. Bioscience, 2018; 2(1): 11-21.
9. American Diabetes Association. Standards of medical care in diabetes - 2020 abridged for primary care providers. Am Diabetes Assoc, 2020; 38(1): 10-38.
10. Bowker N, Shah Rupal L, Sharp Stephen J, Luan J, Stewart Isobel D, *et al*. Meta-analysis investigating the role of interleukin-6 mediated inflammation in type 2 diabetes. EBioMedicine, 2020; 61: 1-10.
11. Kurauti MA, Costa-júnior JM, Ferreira SM, Santos GJ, Sponton CHG, *et al.* Interleukin-6 increases the expression and activity of insulin-degrading enzyme. Sci Rep. 2017; 7: 1-12.
12. Yasa I Wayan PS, Wande I Nyoman, Susila Ni Ketut N, Budhiastra Putu, Cokorda Istri D, Herawati Sianny. Kadar IL-6 plasma pasien diabetes melitus dengan dan tanpa pengidap retinopati diabetika. Indonesian Journal of Clinical Pathology and Medical Pathology, 2018; 21(1): 1-4.
13. Susantiningsih T, Mustofa S. Ekspresi IL-6 dan TNF-α pada obesitas IL-6 and TNF-α expression in obesity. JK

Unila, 2018; 2(2): 174-80.
14. Thamer MM, Wong CF, Ghali ZH. Estimation of Interleukin-4 (IL-4) and Interleukin-6 (IL-6) levels in sera from patients with type 2 diabetes mellitus. Indian J Forensic Med Toxicol, 2021; 15(1): 1732-9.
15. Rea IM. Age and age-related diseases: Role of inflammation triggers and cytokines. Front Immunol, 2018; 9: 1-28.
16. Su Jian H, Luo Meng Y, Liang Na, Gong Shao X, Chen Wei, *et al.* I Interleukin-6: A novel target for cardio-cerebrovascular diseases. Frontiers in Pharmacology, 2021; 12(745601): 1-13.
17. Phosat C, Panprathip P, Chumpathat N, Prangthip P, Chantratita N. Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural Thais: A cross-sectional study. BMC Endocr Disord, 2017; 17(44): 1-8.
18. Sari MI, Tala ZZ, Wahyuni DD. Association between glycated hemoglobin with the levels of serum proinflammatory cytokines and antioxidants in patients with type 2 diabetes mellitus in Universitas Sumatera Utara Hospital. Basic Sci, 2019; 7(5): 715-20.
19. Shi J, Fan J, Su Q, Yang Z, Hospital X, Digestion C, *et al.* Cytokines and abnormal glucose and lipid metabolism in type 2 diabetes. Front Endocrinol (Lausanne), 2019; 10: 703.
20. Qiao Y, Shen J, He L, Hong X, Tian F, *et al.* Changes of regulatory t cells and of proinflammatory and immunosuppressive cytokines in patients with type 2 diabetes mellitus?: A Systematic review and meta-analysis. J Diabetes Res, 2016; 2016: 1-12.
21. Daryabor G, Atashzar MR, Kabelitz D, Meri S. The effects of type 2 diabetes mellitus on organ metabolism and the immune system. Front Immunol, 2020; 11: 1582.