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Interleukin-6 Associated with Insulin Resistance in Non-Diabetic Predialysis Chronic Kidney Disease Patients

Rendy Revandana Bramantya[1], Chandra Irwanadi Mohani[2], Soebagijo Adi Soelistijo[3]

[1]Departement of Internal Medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia

[2]Nephrology-Hypertension Division, Department of Internal medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia

[3]Endocrine and Metabolic Division, Department of Internal Medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131, Indonesia

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ABSTRACT

IL-6 is a proinflammatory cytokine that is commonly obtained in chronic kidney disease (CKD) patients and has a role in insulin resistance. This study aimed to determine the correlation between IL-6 and insulin resistance in non-diabetic predialysis CKD patients.

Methods: The cross sectional observational analytic study involved 35 subjects as CKD patients with non-diabetic predialysis who were diagnosed based on KDOQI criteria. Examination of serum IL-6 levels was carried out using the ELISA method, and insulin resistance was calculated using the HOMA-IR formula.

Results: Thirty-five non-diabetic predialysis CKD patients were enrolled in this study, consisting of 25 (71.4%) males and 10 (28.6%) females. The mean age was 52.5 years (range of 31-60 years). Subjects with stage 3 CKD was 11 patients (31.4%), stage 4 was 6 patients (17.14%), and stage 5 was 18 patients (51.43%). HOMA-IR level was 1.61±1.13 with a median of 1.15 (0.32-4.59). Levels of IL-6 had a mean of 9.03 ± 7.01 and a median of 8.13 (1.01-24.82). There was a significant positive correlation between IL-6 levels and insulin resistance (p = 0.031, r = 0.366)

Conclusion: Serum IL-6 levels had a significant positive correlation with insulin resistance in CKD patients with non-diabetic predialysis.

Keywords: Chronic Kidney Disease, insulin resistance, IL-6.

Correspondance:
Soebagijo Adi Soelistijo, MD
Department of Internal Medicine, Faculty of Medicine-Dr. Soetomo Teaching Hospital, Universitas Airlangga, Surabaya 60131
Indonesia
Jalan Prof. Dr. Moestopo 4-6, Surabaya
E-mail: soebagijo@yahoo.com

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INTRODUCTION

Chronic Kidney Disease (CKD) is a global health problem with increasing numbers of patients. Both history and/or complication of cardiovascular disease is the highest cause of death in CKD.[1,2] The high incidence is still not well explained by traditional risk factors, such as diabetes mellitus, hypertension, and hypercholesterolemia. Strict reduction in cholesterol and blood pressure control do not provide satisfactory results in several studies, so several non-traditional risk factors are sought after including inflammation and insulin resistance.[3–5]

In recent years, insulin resistance has become one of the most studied non-traditional risk factors and is expected to explain the high risk of numbers cardiovascular events in CKD. Cardiovascular disease increases mortality and lowers life expectation of CKD patients. Previous studies showed that insulin resistance is an independent predictor for cardiovascular events in patients with end-stage CKD.[6] Various factors influence insulin resistance in CKD, one of the most prominent is chronic inflammation.[4,6–8] This chronic inflammatory processes cause the low value of phase angle (PhA) in chronic hemodialysis patients.[9]

IL-6 is a pleiotropic cytokine with various biological activities, such as immune regulation, acute phase inflammation, and hemopoiesis. IL-6 also plays a role in several chronic disease, such as various carcinoma and autoimmune disease that is often associated with infection disease.[10] IL-6 is also one of the most prominent proinflammatory cytokines that can be found in CKD patients. Various studies has shown that IL-6 levels increased in CKD, especially in later stage and in dialysis patients. Il-6 can influence insulin resistance through JAK/STAT pathway that affects insulin receptor substrate (IRS).[11–15]

Insulin resistance in non-diabetic patients has not been widely studied, and dialysis conditions are one of the factors that significantly increases cardiovascular risk, so early prevention and treatment in predialysis patients become much more important. IL-6 as major proinflammatory cytokine can be useful as a marker and target of biological treatment in the future.[5,13] Therefore, this study aimed to determine the correlation between IL-6 serum and insulin resistance.

MATERIALS AND METHODS

This was an analytic observational study using cross sectional design. The study was conducted at outpatient nephrology clinic, dr. Soetomo General Hospital in Surabaya. The samples studied were all non-diabetic predialysis CKD patients taken with consecutive sampling. The inclusion criteria were men or women from 18 to 60 years of age, diagnosed with CKD according to KDIGO criteria, no history of diabetes and dialysis, and subjects agreed to participate in the study. Patients with obesity, hepatitis C, acute infection, malignancy, autoimmune disease and who took hyperglycemic medicine were excluded. This study was approved by local ethical committee of dr. Soetomo Hospital. All subjects underwent the same examination and assessment process, including history taking, physical examination and laboratorium data. Insulin resistance was measured using static method of HOMA-IR equation (fasting plasma insulin x fasting plasma glucose/405)

Sample Collection and Assay

Blood samples were obtained by trained laboratory employee, taken after at least 8 hours of overnight fasting. Fresh blood from each subjects was collected in serum separator tube and allowed to clot or 30 minute at room temperature before centrifugation for 1 minutes at 1000 x g. Samples were stored at 20^{o}C until the next process for enzyme-linked immunosorbent assay *(Elisa)*.

Serum Level Assay of Il-6

This examination was performed to all subjects by Prodia Surabaya Laboratory using ELISA Human IL6 Immunoassay with procedure according to manufacturer (Quantikine HS ELISA, catalog number HS600C).

Statistical Analysis

All collected data were arranged in table form and processed statistically using SPSS 23.0 program. Data distribution was analyzed using Saphiro Wilk normality test. Correlation between IL-6 and insulin resistance was analyzed using Pearson correlation test if normally distributed and with Spearman correlation test if not normally distributed. p value < 0.05 was considered significant, and the correlation strength was based on correlation coefficient (r).

RESULTS

The samples in this study were non-diabetic patients with stage 3 to 5 CKD who had not yet undergone dialysis with ages of 18-60 years who came to the Internal Medicine Outpatient Clinic at dr. Soetomo Hospital, Surabaya from 1 January to 28 February 2019 with a total of 35 samples (Table 1). It consisted of 25 males and 10 females with mean age of 52.5 ± 6.87 years old, with the youngest age of 31 years old and oldest at 60 years old. The nutritional status of subjects were measured according to body mass index (BMI) with means of 20.82 kg/m^2 ± 1.21.

The clinical characteristics are shown in Table 2. Based on eGFR from Cockroft-Goult equation, the subjects were divided into stages 3-5 of CKD. More than half of the patients were in stage 5 (51.43%) with eGFR means of 8.56 ± 3.06 ml/minute/1.73 m^2. The other half consisted of stages 3 (31.43%) and 4 (17.14) with means of 43.01 ± 10.6 ml/minute/1.73 m^2 and 19.64 ± 5.76 ml/minute/1.73 m^2, respectively. The major components for HOMA-IR equation were fasting glucose and fasting insulin, in which they were measured after overnight fasting. Fasting glucose had mean of 98 ± 8.013 mg/dl, and fasting insulin had 6.34 ± 4.75 mU/ml.

All subjects were examined for serum IL-6 levels. The results are shown in Table 3. The results showed IL-6 median of 8.13 pg/ml, with the highest value of 24.82 pg/ml and the lowest value of 1.01 pg/ml. The distribution of IL-6 values among CKD stadium showed that higher level of IL-6 found in the late stage, with stage 3 having median of 3.71 pg/ml, stage 4 with 5.30 pg/ml, and the highest in stage with 10.61 pg/ml. The total median across all stadiums was 8.13 pg/ml.

Insulin resistance was measured using HOMA-IR equation of (Fasting insulin x Fasting glucose/405) for mg/dl unit (Table 4). The median of HOMA-IR in all subjects was 1.15, with the highest value of 4.59 and the lowest value of 0.32. The distribution followed a similar pattern to IL-6, with the latest stage of CKD having higher HOMA-IR level. Stage 3 had median of 0.67, stage 4 with 0.78, and stage 5 with 2.45.

The HOMA-IR and IL-6 level data were not evenly distributed, so non-parametric test of Spearman was used to calculate the correlation. The results can be seen in Figure 1, with r value 0.366 and p = 0.031. These results showed a positive and statistically significant correlation between IL-6 and insulin resistance in non-diabetic predialysis CKD patients.

DISCUSSION

Cardiovascular event is closely associated with CKD and increased mortality almost ten times higher compared to normal population. We found a significant positive correlation between IL-6 serum and insulin resistance in this study. Insulin resistance is one of the non-traditional risk factors for cardiovascular event in CKD patients. A recent study showed that insulin resistance is independent risk factor for cardiovascular.[11] The exact pathogenesis is still unknown, but the mechanism is thought to be because of disturbance in P13K-Akt pathway that disrupts production of nitric oxide.[16–18] This is consistent with previous study, which was a cross-sectional study of 44 CKD patients who underwent chronic hemodialysis in Turkey, to observe the correlation of insulin resistance with various clinical parameters, such as inflammation, malnutrition, echocardiography, and 24-hour blood pressure. One of the inflammatory parameters measured was IL-6. The results were a positive correlation with a correlation coefficient of 0.371.[19]

The concept of insulin resistance in CKD using hyperinsulinemia euglycemic clamp method found that majority patients with uremic have insulin resistance. This dynamic method serves as gold standard but needs more manpower, time, and cost to implement. HOMA-IR is a static method to measure insulin resistance using equation of (Fasting insulin x Fasting glucose/405) for mg/dl unit. This method is correlated well with euglycemic clamp and can be used in large scale study.[20,21]

Insulin resistance in CKD can be induced by variety of cause, one of the most prominent factor is inflammation.[8] Low grade inflammation can be found in almost all stages of CKD that may be linked to glycemic control, genetic, and dietary factor,[22] with later stage and dialysis patients having higher level of inflammation. Some of the inflammatory factors in CKD include reduced cytokine clearance, oxidative stress, recurrent infections, intestinal dysbiosis, metabolic acidosis, and vitamin D deficiency. Various factors affect insulin resistance in CKD, such as uremic toxin, metabolic acidosis, and increased angiotensin II.[17,21,23–25] This is related to risk to renal function.[26]

Various pro inflammation cytokine is expressed in CKD, such as IL-6, TNF-α and CRP. Among them, IL-6 is said to be the most representative for systemic inflammation. IL-6, which was initially identified as a B cell differentiation factor, is a multifunctional cytokine that regulates the immune response, hemopoiesis, chronic phase response, and inflammation. IL-6 will inhibit insulin receptor substrate (IRS) at the post-receptor level via the Janus Kinase (JAK) activation pathway, which is continued through Signal transducer and activator of transcription (STAT). Activation of this pathway will induce Suppressor of cytokine signaling (SOCS) 1 and 3, which then inhibits phosphorylation of the IRS and ultimately, inhibits the insulin signaling pathway.[12,14] Increased production of IL-6 in CKD can be caused by uremic stress, which activates inflammation and decreases renal clearance from proinflammatory cytokines. Another

study showed an increase of 2 to 3 times IL-6 levels in subcutaneous adipose tissue in patients with CKD. The production of IL-6 in adipocytes is stimulated by proinflammatory cytokines, such as TNF and IL-6 itself, as well as other pathways through stress exposure, such as uremic imbalance that is higher and causes renal dysfunction.[27–29]

Different results obtained in another study which examined the correlation between body mass index (BMI) and fat mass with insulin resistance in patients with stages 3-4 non-diabetic CKD. The study was conducted in the United States involving 95 nephrology patients at the hospital. The result was a negative correlation coefficient of -0.057 (p = 0.001). This negative correlation can be caused by the selection of research subjects of onlystages 3 and 4 CKD patients, in which the inflammation was not severe.[30]

Studies that directly correlate between IL-6 and HOMA-IR in non-diabetic predialysis setting are still very scarce. We believe that the exclusion of diabetes and hemodialysis from our study will make more accurate correlation because of the absence of confounding factors. However, in this study, there were several limitations. The design was cross sectional so it could not follow how the levels of HOMA-IR and IL-6 had been fluctuated since the early stages of CKD and the calculation of insulin resistance uses the HOMA-IR method, which is not a gold standard because of limited time, facilities and costs. Other factors that can affect insulin resistance such as uremic toxin, metabolic acidosis and increased angiotensin II cannot be controlled because these conditions found in most CKD patients. This study also did not examine the differences in each stage for levels of IL-6 and HOMA-IR.

CONCLUSION

The results of this study revealed that IL-6 serum levels were positively correlated with insulin resistance (calculated with HOMA-IR). This finding supported that IL-6 acts as one of the major factor for insulin resistance in CKD patients and has potential to become future treatment target.

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TABLES

Table 1: Demographic data of subjects

Category	Frequency	Percentage (%)
Sex		
Male	25	71.4%
Female	10	28.6%
Age (years)		
Mean ± SD	52.54 ± 6.87	
Range	31-60	
BMI (kg/m^2)		
Mean ± SD	20.82 ± 1.21	
Range	18.75-22.72	
CKD Stage		
Stage 3	11	31.43%
Stage 4	6	17.14%
Stage 5	18	51.43%

Table 2: Clinical data of subjects

Clinical data	Mean ± SD	Range
GFR (ml/minute/1.73 m^2)	21.27 ± 16.77	4.07 - 59.88
GFR Based on Stage		
Stage 3	43.01 ± 10.6	
Stage 4	19.64 ± 5.76	

Stage 5	8.56 ± 3.06	
Fasting Glucose (mg/dl)	98 ± 8.013	87 – 120
Fasting Insulin (mU/ml)	6.34 ± 4.75	1.33 - 20.21

Table 3: IL-6 in CKD patients

Stage	IL-6 Serum		
	Mean ± SD	Median	Range
Stage 3	3.69 ± 1.67 pg/ml	3.71 pg/ml	1.56-7.0 pg/ml
Stage 4	6.14 ± 5.29 pg/ml	5.30 pg/ml	1.01-14.52 pg/ml
Stage 5	13.25 ± 6.93 pg/ml	10.61 pg/ml	3.21-24.82 pg/ml
Total	9.03 ± 7.21 pg/ml	8.13 pg/ml	1.01-24.82 pg/ml

Table 4: HOMA-IR in CKD patients

Stage	HOMA-IR		
	Mean ± SD	Median	Range
Stage 3	0.814 ± 0.56	0.67	0.32-2.34
Stage 4	0.87 ± 0.49	0.78	0.48-1.83
Stage 5	2.35 ± 1.05	2.45	0.85-4.59
Total	1.61 ± 1.13	1.15	0.32-4.59

FIGURE

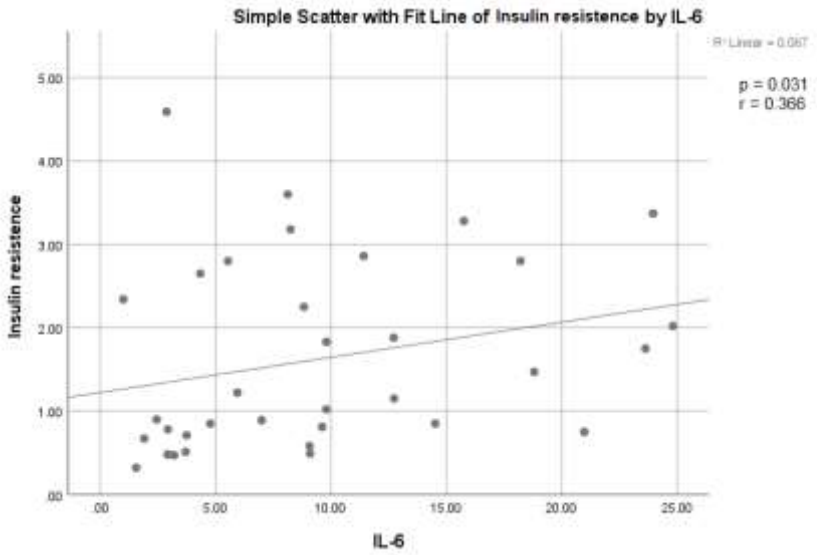

Figure 1: Correlation between IL-6 and insulin resistance in non-diabetic predialysis CKD patients