ACTA BIOCHIMICA INDONESIANA

RESEARCH ARTICLE

A SIGN OF ACUTE INFLAMMATION IN TYPE 2 DIABETES MELLITUS PATIENTS IN KOTA BARU AND KALIBARU SUBDISTRICTS, BEKASI

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ABSTRACT

Background: During the development of chronic type 2 diabetes mellitus (T2DM), inflammatory signals are elevated which can cause microvascular damage. C-Reactive Protein (CRP) is one of acute phase proteins stimulated under inflammatory conditions and creatinine is a waste product used to measure the glomerular filtration rate (GFR). Both of these compounds are considered as biomarkers of acute kidney damage among people with T2DM.

Objective: The purpose of this study was to determine relationship between CRP and creatinine levels in T2DM patients.

Methods: We conducted analytic cross-sectional study in Kota Baru and Kalibaru sub-districts, Bekasi, from January until February 2019. Creatinine was measured using the jaffe method and CRP was measured using a latex agglutination technique. The correlation between CRP and creatinine was analyzed with Spearman test.

Results: Spearman correlation test from 55 samples showed a weak positive correlation ($r = 0.289$; $p < 0.05$) between CRP levels and creatinine levels. These results indicate that high CRP levels are directly proportional to creatinine levels in the serum of T2DM patients. Creatinine and CRP levels can be used as clinical parameters as biomarker for acute microvascular damage in nephron cells that can develop into complications due to T2DM.

Conclusion: There was a significant, weak positive correlation between CRP levels and creatinine levels in T2DM patients in Kota Baru and Kalibaru districts, Bekasi

Keywords : C-Reactive Protein, Creatinine, Inflammation, Kidney, Type 2 Diabetes Mellitus

Received Dec 10, 2019 ; Revised Jan 28, 2020 ; Accepted Jan 30, 2020

INTRODUCTION

Globally, the number of people with diabetes mellitus has quadrupled in the past three decades, and diabetes mellitus is the ninth major cause of death. Asia is major area of the rapidly emerging T2DM global epidemic, with China dan India the top two epicetres.[1] Indonesia was ranked as the seventh highest diabetes prevalence, with 10-20 million of total number of adults (20-79 years) were estimated to be living with diabetes mellitus and is predicted to increase to sixth with 16.2 million diabetics in 2040. Type 2 diabetes mellitus is caused by metabolic disorders and insulin resistance.[1,2] Insulin resistance is a condition of decreased responsiveness of cells or tissues to insulin. As a result of decreased cell response to insulin, glucose in the blood can not be taken into cell, causing hyperglycemia. Hyperglycemia causes cells to produce superoxide oxidants (O_2^-) which can cause cell damage. As a sign of inflammation, inflammatory cytokines such as IL-6 will be activated during cell damage.[3]

Cytokines IL-6 is a proinflammatory cytokine produced by liver cells that can stimulate acute phase proteins such as C-Reactive Protein. C-Reactive Proteins (CRP) can bind to damaged cells using phosphatidylcholine binding site and the membrane binding process can initiate the complement immune response and assists the clearance of apoptotic cells.[4] CRP can activate the classical pathway complement system with C1q molecules and alternative pathways with C3b molecules.[5] In addition, CRP can stimulate the production of proinflammatory cytokines such as IL-6, IL-4, TNF-α and IL-17.[6] The presence of proinflammatory cytokines can increase ability of phagocytic cells such as neutrophils, macrophages to clear destroyed cells in inflamed area.[7] Therefore, an increase in CRP levels is used as a sign of inflammation in the body. If this inflammatory state continues in people with type 2 diabetes mellitus, it can develop into organ damage due to the inflammatory response.[8] Changes in extracellular matrix protein synthesis in conjunction with advanced glycation end-products cause oxidative stress that can lead to complications.[9]

The complications of diabetes mellitus have been divided into macrovascular complications, for example cardiovascular disease (CVD), and microvascular complications, for example nephropathy, retinopathy, and neuropathy.[1] Diabetic kidney disease develops in approximately 40% of diabetic patients.[10] One of the earliest organs to be damaged in people with type 2 diabetes mellitus is the kidney.[8] The natural history of diabetic kidney disease includes glomerular hyperfiltration, progressive albuminuria, reduce glomerular filtration rate (GFR), and finally, end-stage renal disease (ESRD). Metabolic changes associated with diabetes induce to glomerular hypertrophy, glomerulosclerosis, tubulointerstitial inflammation and fibrosis.[10]

Kidney is an organ that functions for excretion of metabolic waste in urine while reabsorption of necessary molecules such as glucose. Hyperglycemia increases blood viscosity thus increases kidney burden to filter and excrete metabolic waste from blood. Disruption of kidney function can be identified by creatinine levels. Creatinine is a breakdown product of creatine phosphate used as a parameter of kidney damage.[8] Therapy has been available to reduce the risk of complications in diabetes kidney disease.

Screening of CRP and creatinine levels in diabetic patients who have not been affected by kidney damage can be used as a preventive measure to reduce the risk of diabetic kidney disease. C-Reactive Proteins can indicate inflammation in diabetics in general and creatinine levels can indicate damage to the kidneys, so that both parameters can be used as initial biomarkers to detect complications in diabetics.

MATERIAL AND METHODS

We conducted an analytic cross-sectional study. The sample was selected using a purposive technique. The research was conducted on behalf of Dinas Kesehatan Kota Bekasi with letter number 070/69B/Dinkes.SDK. The study sample was 55 patients with inclusion criteria such as people with type 2 diabetes mellitus, aged > 36 years, and residents of Kota Baru or Kalibaru sub-district, Bekasi. The specimen used was serum.

C-Reactive Protein was measured using latex agglutination method (CRP AIM test kit) with a cut-off value of 0.8 mg/dL. C-Reactive Protein examination was carried out in two steps. Firstly we conducted a qualitative examination. If the qualitative CRP examination showed positive result, analysis then proceeded with a semi-quantitative examination to determine CRP level.

Qualitative CRP analysis was conducted by dropping 50µl of undiluted serum into the circle on the glass slide with positive and negative controls on the other circles. The serum and controls were mixed with one drop of previously homogenized the AIM CRP Latex Test reagent. The mixtures were homogenized using a stirring rod then shaken for 3 minutes. Positive results are indicated by agglutination in the sample. The semi-quantitative CRP examination aims to determine the levels of serum CRP. First, the serum sample was diluted by stratified dilution. Then, 50µl of saline was dropped in circles 1, 2, 3, and 4. Fifty µl of the serum sample was dropped in circle 1. Solution in circle 1 was mixed, and then 50µl of liquid in circle 1 was transferred to circle 2 and so on. The last dilution series liquid was discarded. Air bubbles formation was avoided during the dilution. After sample dilution was ready, 50µl of AIM CRP reagent was mixed into each circle and shaken for 3 minutes. Positive results are indicated by agglutination in the last circle with titer 1 (1: 2), circle 2 (1: 4), circle 3 (1: 8), and circle 4 (1:16). If the final agglutination is shown in circle 3, then the way to determine serum CRP levels is 1: 8 multiplied with a reagent cut-off value of 0.8 mg / dL with a result of 6.4 mg / dL.

Creatinine was evaluated using the Semi-Auto Chemistry Analyzer BA - 88A based on the standards of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Assessment criteria were based on the Decree of the Minister of Health of the Republic of Indonesia Number 1792/MENKES/SK/XII/2010 using the Jaffe method. Principle of the reaction was creatinine reaction with alkaline picrate solution to form a reddish-orange complex. The intensity of the resulting color is directly proportional to the concentration of creatinine in serum and can be measured photometrically at λ 500-560nm. Two test tubes was prepared for creatinine measurement, labelled as blank and sample tubes. Ninety µl of reagent A was transferred into both tubes. Then, 9µl of aquadest was transferred into the blank tube while the 9µl of serum was transferred into the sample tube. Tubes

were homogenized and incubated for 1 minute at 37ºC. After that, 90µl of reagent B was transferred into both tubes. Tubes was further homogenized and incubated at 37ºC for 30 seconds. Results were read using the semi-auto chemistry analyzer BA - 88A based on kit creatinine Mindray. The normal range of serum creatinine is 0.6-1.2 mg/dL for man and 0.5-1.1 mg/dL for woman.

The data was presented descriptively. To find out the correlation between CRP and creatinine, statistical tests were performed using the Spearman correlation.

RESULTS

Table 1 shows that respondents were predominantly female (78%) with the mean age of 56 ± 7.7 years. The median of C-reactive Protein in type 2 diabetics was <0.8 mg/dL and creatinine level was 0.55 mg/dL. Both of these data were not normally distributed, so we analyzed the correlation between CRP and creatinin levels using Spearman test.

Tabel 1. Univariate analysis

Variable	Result (n=55)	
Sex	Female = 43 (78%)	Male = 12 (22%)
Age	56 ± 7.7 years (min 36 - max 70)	
CRP	<0.8 mg/dL (min<0.8 - max 12.8)	
Creatinine	0.55 mg/dL (min 0.2 - 2.3)	

Results of the C-Reactive Protein semiquantitative test are presented in Table 2. The level of C-Reactive Protein in 28 samples is in the normal value (<0.8 mg/dL) and 27 samples above normal. Based on the range of normal creatinine serum values obtained 4 patients with type 2 diabetes have values above the normal limit. Rank Spearman correlation statistical analysis is presented in table 3. There was a significant relationship between CRP and creatinine levels in T2DM patients with a weak positive correlation (r value=0.289).

Table 2. Results of the C-Reactive Protein semiquantitative test

	Level CRP (mg/dL)				
	<0.8	1.6	3.2	6.4	12.8
Frequency	28	6	8	10	3

Table 3. Correlation between C-Reactive Protein and Creatinine levels

		Creatinine Levels
CRP Levels	r	0.289
	p	0.033
	n	55

DISCUSSION

Table 1 show a higher percentage of women with diabetes mellitus. Women have a greater risk of developing diabetes mellitus, especially during postmenopause.[11,12] The derivation of estrogen in postmenopausal women catalyzes the development of insulin resistance and type 2 diabetes mellitus. Estrogen and progesterone hormone therapy increases the response of insulin receptors in postmenopausal.[13] Table 1 also shows the average age of respondents is 56 ± 7.7 years with the youngest and oldest age was 36 years old and 70 years old, respectively.

Aging can cause decreased organ function, including the endocrine system. The performance of the endocrine glands will decrease thereby reducing the production of hormones including estrogen and progesterone. Activated estrogen receptor (ER) and estradiol receptor (ER) genes cause metabolic processes. These genes work together in insulin sensitivity and increased transport of blood glucose into cells. Increased age will reduce the expression of these genes so thus decrease

insulin sensitivity which causes the pathophysiology of type 2 diabetes mellitus.[12]

There were 28 samples that had the CRP levels of <0.8 mg/dL. This result indicated that there was no inflammatory process. Basically C-Reactive Protein is normally produced at < 3 mg/L level or equivalent to 0.3 mg/dL. C-Reactive Protein is an acute-phase protein which level will rapidly increase as a sign of inflammation and is not influenced by age or sex.[14] C-Reactive Protein levels will usually increase from 5-50 mg/dL. The time needed to increase CRP levels is 6-8 hours after inflammation and reaches its peak after 48 hours.[14,15] The remaining 27 people were in an inflammatory condition. They were estimated to have experienced hyperglycemia for longer time, which can cause complications from type 2 diabetes mellitus.[16]

Increased C-Reactive Protein level begins with hyperglycemia which causes cells to not get a supply of glucose as a source of energy. This causes breakage in the mitochondria, so the Reactive Oxygen Species (ROS) production in cells increases. Increased reactive oxygen species causes macrovascular and microvascular damage.[3] This damage increases in diacylglycerol (DAG) synthesis which subsequently increases Protein Kinase-C (PKC) expression level in cells as well. The increase in PKC activation is followed by an increase in Nf-kB which functions as transcription factor for pro-inflammatory cytokines.[4] Several proinflammatory cytokines that can increase CRP levels are IL-6, IL-1β and TNF-α.[6]

Table 3 shows the correlation between C-Reactive Protein and creatinine levels is weak positive (r value=0.289). Serum hs-CRP was also associated with serum creatinine concentration in diabetic nephropathy, but no association was observed in type 2 diabetic patients without any complications.[17] In this study, we used type 2 diabetic patients without complications. The results of a weak relationship between CRP and creatinine are thought to indicate acute inflammation which signal kidney damage. This shows that early screening of CRP and creatinine in diabetic patients without complications can be used as biomarkers for early detection of diabetes nephropathy. C-reactive protein (CRP) is related with advanced diabetic nephropathy in patients with type-2 diabetes. However, role of CRP in type 2 diabetes remains unclear. CRP promoted renal fibrosis by a CD32b-Smad3-mTOR pathway because blocking mTOR signaling with rapamycin inhibited CRP-induced CTGF and collagen I expression CRP may induce CD32b- NF-κB signaling to facilitate renal inflammation.[18]

Creatinine is a product of muscle metabolism that is associated with type 2 diabetes mellitus.[19] Creatinine is an indicator of good kidney function due to its role in maintaining creatinine at normal levels. There were 23 samples who showed low creatinine levels below normal values. Low creatinine levels indicate reduced muscle cell mass. Muscle cells are storage areas for glucose in the form of glycogen.[19] In type 2 diabetes mellitus, insulin resistance condition is associated with the breakdown of glycogen in muscle cells as a substitute for energy sources in muscle cells. Otherwise, increased creatinine level is a marker of impaired kidney function. One of the organs that can be damaged in diabetic patients is the kidney.[8] We assumed that an increase in CRP in patients with type 2 diabetes without complications can trigger kidney cell damage which is characterized

by an increase in serum creatinine. The level CRP and creatinine serum are thought to be able to be used as monitoring parameters for type 2 diabetes without complications progress into complications.

The limitation of this study was limited sample size and possible effect from type 2 diabetes duration. Therefore, in future research, it is suggested to further classify the diabetes duration so increased CRP and creatinine levels can be analysed with time post type 2 diabetes diagnosis.

CONCLUSION

There was a significant relationship between CRP and creatinine levels in T2DM patients with weak positive correlation levels. Based on this we assumed the presence of inflammation which indicates damage to muscle cells and impaired kidney function in regulating creatinine levels in the serum of type 2 diabetes mellitus patients.

Acknowledgment

We would like to thank the Dinas Kesehatan Bekasi, Puskesmas Kota Baru and Kalibaru for their collaboration in this research.

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