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NEGATIVE CORRELATION BETWEEN LEUKOCYTE COUNT AND HIGH DENSITY LIPOPROTEIN IN TYPE 2 DIABETES MELLITUS PATIENTS IN SANGLAH GENERAL HOSPITAL, BALI

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ABSTRACT

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Type 2 diabetes mellitus (T2DM) is group with high cardiovascular disease (CVD) risk. Inflammation which is reflected by increasing leukocyte number, CRP, and lipid profile. Despite that, infection as one of diabetic complication commonly occurs in patient with T2DM. The dyslipidemia status reflected by lipid profile composition. So here, we aim to evaluate the relationship between leukocyte count with lipid profile to know the association between traditional and non-traditional risk factor of CVD in T2DM patient. The type of this research is *cross sectional analytic research* with 94 samples was enrolled into this study. The data is obtained initially by collecting from the quistionare and from laboratorium examination. Mean of samples' age is 55 years and large number of patients had leukocytosis as reflected by high value of its mean (15.02±8.78 x $10^3/\mu L$). We found that 56.4% of the subjects fall into worst category of glycemic control (Hb_{A1C}). Only 24.5% of the subjects had good glycemic control. Further evaluation showed that there were significant strong negative correlations between leukocyte count with HDL (r= -0.555; p=0.000). Inflammation which mainly related to infectious disease is common among T2DM patients which increase CVD risk by lowering HDL and worsening glycemic control.

Keywords: *Type 2 Diabetes Mellitus, lipid profile, leukocyte count*

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic progressive disease which mainly marked by impairment of glucose utilization in the body. The underlying metabolic causes of T2DM are the combination of impairment in insulin-mediated glucose disposal (insulin resistance) and defective secretion of insulin by pancreatic ß-cells.[1] In the last two decades, T2DM has become pandemic and one of the major medical problems in the world. The

prevalence of diabetes mellitus is rising at an alarming rate. In the United States, 23.6 million people, or 7.8% of the population, have diabetes mellitus, with 1.6 million new cases diagnosed annually.In 2011, its prevalence reaches 346 million worldwide and projected to increase up to 486 million in 2030.[1,2] T2DM prevalence is also associated with increasing prevalence of obesity and metabolic syndrome (MS).[2]

Complex pathogenesis in T2DM eventually leads to the emergence of various complications, especially cardiovascular disease (CVD).[2] CVD contribute to most of mortality and morbidity in T2DM. The pathophysio- logy of CVD in diabetes involves traditional and novel cardiac risk factors, including hypertension, dyslipidemia, smoking, genetics, hyperglycemia, insulin resistance or hyperinsulinemia, glycoxidative stress, inflammation, endothelial dysfunction, procoagulant state, and myocardial fibrosis. Patients with diabetes mellitus have a 2 to 4 time's higher risk of cardiovascular disease and up to a 3 times increase in mortality than non-diabetics people.CVDs are listed as the cause of death in 65% of persons with diabetes.

Infection is one of complication related to T2DM and often acts as precipitator of acute diabetic complication, such as diabetic ketoacidosis (DKA) and hyperglycemia hyperosmolar state (HHS).[3] It is widely accepted by both the medical profession and the general public that diabetic have an increased propensity to develop infections. Both systemic and local host factors are also contribute to infections. On the other hand, infection has also been associated with CVD either directly or indirectly.[4,5,6]

Inflammation following the infection has the potential hazard to destabilize the plaque and precipitate CVD. Indonesia's status as a developing country, infectious diseases are still commonly found and contribute to large percentage of mortality. Concurrent infection in T2DM may worse the prognosis which is mainly related to CVD.

Currently, there are only a few researches that evaluate the effect of the inflammatory state, especially acute inflammation related with infection, toward the risk of CVD.[7,8] Laboratory and pathological data support the idea that inflammation has a role in both the initiation and the progression of atherosclerosis.[4] However, there are few data to indicate whether inflammation increases the risk of first myocardial infarction, stroke, and venous thrombosis.

The effect of inflammation on metabolic profile of T2DM especially CVD risk factors are also not well understood. Because of the high prevalence and incidence of infectious diseases in Indonesia, high vulnerability of patients with T2DM toward infection. The fact that inflammation following an infection may precipitate CVD, we aim to evaluate the correlation of leukocytosis with lipid profiles and Hb_{A1C} among patients with T2DM in Sanglah General Hospital. We also aim to reveal any relationship between leukocytosis with lipid profiles and Hb_{A1C} in order to elucidate its potential role in altering CVD risk.

METHODS

Type of Research

The type of this research is *cross sectional comparative analytic* which evaluates the prevalence of leukocytosis among patients with T2DM and its correlation with HDL level and Hb_{A1C}.

Study sites and Participants

Whole research process is taken in Sanglah General Hospital especially at Diabetes Center for collecting the data. The statistical analysis study is conducted in Endocronology Department of Internal Medicine, Sanglah General Hospital for ± 1 month, from Januari, 24th 2011 until January, 9th 2012. The participants of this study are diabetic patients in Angsoka installation. There are 174 patients with primary diagnosis T2DM, which were taken from January 2011 until Januari 2012.

Population of the Research

The population of this research is as follows:

Target Population

The target population in this research is T2DM patients.

Sample

Samples in this research is patients with T2DM at Sanglah General Hospital.

Inclusion and Exclusion Criteria

Inclusion Chriteria

1. T2DM Patients with no acute complication
2. Included in age's range criteria (< 85 years old).
3. Had never been diagnosedwith acute diabetic complications, such as DKA and HHS.

Exclusion Chriteria

1. Medical record which belongs to other types of diabetes.
2. Diabetes with malignancy.
3. Suffering from acute systemic complication such as sepsis.

Sample size

The sample size was calculated to get at least 90% power to detect a 10% difference of the risk of deviation so it can get 5% significance level. Epecting a dropout rate of not more than 10%, the targeted number of participants in each study arm was 94. We count the minimal total sample with the formula:

$$N = \frac{\{(Z\alpha + Z\beta)^2\}}{\{0.5 ln\,[\frac{1+r}{1-r}]^2\}} + 3$$

Type I mistake that can be tolerated is decided to be 5% , Zα = 1.64.

Type II mistake is decided to be 10%, so Zβ = 1.28.

Previous study showed that relationship ratio between leukocyte count and HDL equals 0.5.

$$N = \frac{\{(1.64+1.28)^2\}}{\{0.5 ln\,[\frac{1-0.5}{1+0.5}]^2\}} + 3$$

$$N = 94$$

So, the minimum number of sample is 94.

Variabel of the Research

Dependent Variable :

- Lipid profile (HDL, cholesterol, LDL, and Triglyceride), HbA1C

Independent Variable :

- Leukocyte count

Operational Definition of Variable

- Leukocyte:
  The numbers of leukocyte take from routine Complete Blood Count (CBC).
- Lipid profile :
  Pattern of lipids in the bloods that take from blood laboratoryanalysis that recorded in patient medical records (consist oftriglyceride, cholesterol, HDL, LDL).
- HDL:
  High density lipoprotein which is assessed from lipid profile examination data. According to American Diabetes Association (ADA, 2007), HDL level can divided into several categories : Low (< 35 mg/dL), Borderline (35 – 45 mg/dL), Normal (> 45mg/dL).
- CVD :
  Cardiovascular disease is defined by secondary diagnosis of the patient in medical record.

Research Procedure

Data Selection

Data of the patients are obtained from Endocrinology Departement of Sanglah General Hospital. The data are selected based on inclusion and exclusion criteria. Then, baseline characteristic of the sample is analyzed by SPSS 16. We selected medical records which belongs to T2DM patients and exclude any medical record from patients which is incomplete, too old (>85 years old), had diagnosis of acute diabetic complication such as DKA and HHS, and who suffer from acute systemic complication such as sepsis. Finally, 94 medical records which met the criteria were enrolled in this study. Demographic data such as age, leukocyte count (cut off value: 12000/μL), lipid profile (cholesterol, triglyceride, LDL, and HDL), and Hb_{A1C} were taken from patient diabetes register as baseline characteristics.

Evaluation of Correlation between Leukocyte Level and HDL Parameters

The correlation of leukocyte level and HDL then evaluated. First, we evaluate the distribution of the data by normalization test to determine the method that will be used to evaluate correlation coefficient. Then, correlation between leukocyte count and HDL is analyzed either by Pearson Correlation or Spearman Correlation based on the result of the normalization test.

The prevalence of leukocytosis and its causes, profile of glycemic control (Hb_{A1C}), and lipid profile were assessed to provide the description of samples'metabolic status. Component of lipid profiles (cholesterol, triglyceride, HDL, and LDL) are also categorized independently.

Cholesterol level is categorized into desirable, borderline high, and high. Triglyceride level is described as normal, borderline high, high, and very high. LDL level is categorized into optimal, above normal, borderline high, high, and very high. And HDL level is describe as low (<35 mg/dl), borderline (35-45 mg/dl), and normal (>45 mg/dl). Meanwhile HbA1C is classify as good (<7%), poor (7-9%) and worst (>9%). Then, the normality of data from leukocyte count, systolic blood pressure (SBP), lipid profile, and Hb_{A1C} were evaluated and continued by evaluation of correlation between leukocyte count with SBP, lipid profile, and Hb_{A1C} to assess the effect of leukocytosis toward CVD risk in patients with T2DM. Research profile could be seen in figure.

Statistical Analysis

Per-protocol analysis included all patients who match all inclusion criterias, were properly evaluated for the leukocyte count. Then the result of leukocyte count will be evaluated with other parameters especially HDL and Hb_{A1C} to know whether there is direct strong, inversely, or no relationship between those parameters. All

Variables	Mean±SD
Age	55±11.4 years
Leukocyte Count	15.02±8.78
Cholesterol	165.36±70.36
Triglyceride	146.20±11.36
HDL	28.10±14.28
LDL	109.05±57.4
HbA1C	10.11±4.93

statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) 16.

RESULT

Baseline Characteristic and Profile of Leukocyte Count, Cholesterol, Triglyceride, LDL, HDL, and HbA1C

Initially, samples' baseline characteristics were obtained as shown in table 1. Mean of samples' age is 55 years. Most of samples have poor glycemic controls which are reflected with high mean of Hb_{A1c}.

Variables	Kolmogorov-Smirnov (p-value)
Leukocyte	0.000
Cholesterol	0.200
Triglyceride	0.000
LDL	0.200
HDL	0.200
HbA1C	0.002

Table 1. Sample's Baseline Characteristic

Most of samples also have low level of HDL (<35 mg/dl).Then we examine the profile of leukocyte, lipid profile, and Hb_{A1C}. From 94 subjects being enrolled, 59 (62.8%) had leukocytosis and 35 (37.2%) were normal.

Variables	Correlation Coefficient	p-value
	Leukocyte Count	
Cholesterol	0.133	0.292
Triglyceride	0.145	0.159
LDL	0.164	0.362
HDL	-0.555	0.000
HbA1C	0.232	0.023

Table 2. Cause of leukocytosis In Samples

The causes of leukocytosis are obviously infectious diseases which compose of diabetic foot (31 cases), pneumonia (25 cases), urinary tract infection (UTI) (6), tuberculosis (TB) (5), abscess (4), erysipelas (1), and idiopathic (15).

There are 7 (7.44%) who experienced more than two infections. Then, we examine the lipid profile of the subjects. Most of the subject (69.1%) had desirable level of cholesterol with only 19% had borderline high and 12% high.

Causes of Leukocytosis	Frequency of Cases
Diabetic Foot	30
Pneumonia	25
Urinary Tract Infection (UTI)	6
Tuberculosis (TB)	5
Abscess	4
Erysipelas	1
Unknown	15

The same pattern also appears in triglyceride and LDL profile. 70.2% of the patients had normal level of triglyceride with 16% had borderline high, 11.7% high, and 2.1% with very high level of TG. For LDL, 47.9% subjects had optimal level of LDL, 20.2% above normal, 12.8% borderline high, 13.8% high, and 5.3% with very high level of LDL.

Inversely, we found that 64.9% subject had low level of HDL with only 14.9% with normal level and 20.2% had borderline level. Then we examined the profile of Hb_{A1C} of the subject to examine their glycemic controls. We found that 56.4% of the subjects fall into worst category of glycemic control (Hb_{A1C}>9%). Only 24.5% of the subjects had good glycemic control (Hb_{A1C}<7%) and the remaining 19.1% had poor glycemic control (Hb_{A1C} between 7% and 9%).

Correlation between Leukocyte Count with Lipid Profile and HbA1C

Before we assess the correlation of leukocyte with lipid profile and HbA1C, we examine the normality of the data by using Kolmogorov-Smirnov test. We found that the data of leukocyte count, TG, and HbA1C was not normally distribute (p = 0.000).

Table 3. Result of Normality Test of Research's Variables

Based on the result of normality test, we examined correlation ratio between leukocyte count with lipid profile and HbA1C by using spearman's correlation test.

Table 4. Correlation of Leukocyte Count with Several Variables

The result shows there were significant strong negative correlations between leukocyte count with HDL (r= -0.555; p=0.000) and significantly moderate positive correlation with HbA1C (r=0.232; p=0.023). Meanwhile there is no significant correlation between leukocyte count with cholesterol, TG, and LDL.

DISCUSSION

Inflamatory state, which often related to infection, has been proved to be independent risk factor of CVD which is the main cause of diabetic mortality and morbidity.[7,8] Inflammation plays an important role in the development and progression of a variety of cardiovascular conditions, most notably coronary atherosclerosis and congestive heart failure. In this study, we found several findings that may elucidate the importance of infection prevention as well as evaluation of treatment of T2DM related with CVD prevention. First we found that more than half of patients with T2DM attending with leukocytosis which indicates inflammatory state, presumably acute

inflammation. Second, there are several causes of inflammation with diabetic foot, pneumonia, and UTI being the most prominent ones.

Third, most of the subjects have favorable level of cholesterol, TG, and LDL but more than 60% have low level of HDL. The finding of glycemic control also yield similar result that 54.6% of subjects have the worst glycemic control (Hb_{A1C}>9%). And the last, there are significant strong negative correlation between leukocyte count with HDL and moderate positive correlation with HbA1C. Infection has been known as one complication of T2DM but the fact that it is common among diabetic patients should raise concern about it contribution to prognosis of diabetic patients.

In T2DM, infection usually acts as precipitating factor of diabetic related complication such as DKA and HHS.[3] But there are several evidences that support its role as independent risk factor of CVD.[4,5,6] Infectious agents such as influenza, C pneumonia, H pylori, and Human papillomavirus (HPV) have been proposed to have direct relationship with atherosclerosis formation. Deoxyribonucleic acids (DNA) of C pneumonia, HPV, and influenza also have been extracted from atherosclerotic plaque. More recent studies reveal that inflammatory process in response to infection may have direct effect on atherosclerosis progression.[9]

Infection usually elicit type I immune reactions that eliminate offending agent by phagocytic process and terminate with type II immune reaction with subsequent antibody formation. Inflammatory process involved in atherosclerosis also Th1 based immune response and the presence of infection in such patients will result in plaque destabilization and even rupture leading to CVD manifestation such as myocardial infarction.

Elevation of the leucocyte count can be a marker for the prediction of the disease. Formed partially in the bone marrow and partially in the lymph glands, their site of action is the areas of serious infection and inflammation. Hence, they can be considered as an important inflammatory marker. Leucocytes are of different types, each having a discrete function. The ubiquitous monocyte, the precursor of macrophages in all tissues, is present in every phase of atherogenesis. Monocyte derived macrophages are scavenging and antigen presenting cells, and they secrete cytokines, chemokines, growth regulating molecules, and other hydrolytic enzymes. The ability of macrophages to produce cytokines, proteolytic enzymes and growth factors may be critical in the role of these cells in the damage and repair that ensue as the lesion progress. Monocytes and granulocytes protect the body against invading organisms by phagocytosis. Lymphocytes and plasma cells neutralize the antigens by producing antibodies.

Inflammation is also reported to alter lipid profile mainly by increasingcholesterol, LDL, triglyceride level and decreasing HDL level.[10] Researches on patient with systemic lupus erythematous have been revealing the effect of inflammation on lipid profile.[11] Such patients tend to have low level of HDL with elevation in cholesterol, triglyceride, and LDL. Inflammation reduces plasma HDL levels by blocking the synthesis and secretion of HDL in the liverThe proposed mechanism pointed toward Tumor Necrosis Factor- α (TNF-α) as central player. TNF-α and the other inflammatory cytokines like Interleukin-1β (IL-1β) and Interleukin-6(IL-6) are known to have metabolic effect by inducing insulin resistance.[12]The IL-1 gene family consists of 3 proteins, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra).

They are also predicted that inflammation decrease adiponectine secretion by adipocyte which also play a role in insulin resistance.[13] Increasing insulin resistance and lowering plasma adiponectine level have been shown to inversely associated with plasma HDL concentration and directly correlate with cholesterol, triglyceride, and LDL level. Unique pattern of lipid profile of the T2DM subjects in this study may be caused by extensive use of statin as lipid lowering agent. Despite of that, low plasma HDL level proof that increases cardiovascular risk due to inflammation may still persist despite the usage of lipid lowering agent. Moreover, some researches have reveal that inflammation may convert HDL from antiatherogenic into atherogenic which may aggravate the risk of CVD.[14] Like described above, inflammation also aggravate insulin resistance status in T2DM which explain relatively poor glycemic index among T2DM subjects in this study. Like lipid profile, plasma glucose and HbA1C level also act as independent risk factor of CVD in diabetic subject and in fact, lowering post prandial glucose level and HbA1C has been related to reduction in CVD mortality in T2DM.[1] Inflammatory cytokine release during infection process may contribute to insulin resistance. TNF-α, IL-6, resistin, and undoubtedly other proinflammatory or antiinflammatory cytokines appear to participate in the induction and maintenance of the subacute inflammatory state

associated with obesity. Monocyte Chemotactic Protein-1 (MCP-1) and other chemokines have essential roles in the recruitment of macrophages to adipose tissue. These cytokines and chemokines activate intracellular pathways that promote the development of insulin resistance and T2DM. Role of inflammation in inducing insulin resistance is shown in figure 9 below.

This research elucidates several points of consideration in CVD prevention in T2DM and possibly in general population. First, it is important to take infection and inflammation into consideration when deciding proper management of T2DM in order to achieve better degree of glycemic control and lipid profile. Thus, rigorous attempt should be made to treat infection in diabetic patients in order to lower CVD related mortality and morbidity as well as enhance patient survival. Third, prevention of infectious disease in general population may prevent the surge of CVD mortality as may be experienced by our country as we advanced into third phase of CVD development as chronic disease and infectious disease coincides and may also interact.

Despite of its finding and implication, this study has several handicaps. First, since this is cross sectional study, there is no precise confirmation of the real connection between inflammation with lipid profile and HbA1C which can be elucidated in follow up study. Second, despite higher than minimally required, the number of sample in this study is still considerably small.

CONCLUSION

Inflammation which mainly related to infectious disease is common among patients with T2DM which increase CVD risk by lowering HDL and worsening glycemic control. The effect of inflammation toward HDL also cannot be improved by lipid lowering agent which may require appropriate infection and inflammation management. Prevention of infection with subsequent inflammation may have beneficial effect on patients with T2DM by lowering CVD risk as well as mortality and morbidity related to CVD. At last, it is necessary to conduct large and more precise studies to reveal the effect of inflammation and infection on CVD risk especially in Indonesia since prevention of infection may prevent the future surge of CVD mortality as we advanced into the third stage of CVD evolution.

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