

HbA1c and interleukin-17a level inpatient at risk for metabolic syndrome in Yogyakarta

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HbA1c and interleukin-17a level inpatient at risk for metabolic syndrome in Yogyakarta

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Abstract

Metabolic syndrome resulted from complex metabolic abnormalities due to obesity, hypertension, dyslipidemia and hyperglycemia. Insulin resistance causes an increase in reactive oxygen species that will increase HbA1c levels. Meanwhile, oxidative damage leads to a rise of pro-inflammatory resulting in increased interleukin (IL)-17a. This study aimed to investigate the correlation between HbA1c and IL-17a levels in patients at risk for metabolic syndrome. A total of 89 patients at risk for metabolic syndrome who had met inclusion and exclusion criteria and agreed to fill in the informed consent were involved in this cross-sectional study. Determination of IL-17a level used enzyme-linked immunosorbent assay method. The HbA1c determination was performed using plasma glucose mean way.

The correlation between HbA1c and IL-17a levels was analyzed using bivariate correlation test with a 95% confidence level. There was no difference in HbA1c and IL-17a levels in the sample based on smoking behaviour and clinical manifestations of hypertension, hypertriglyceridemia, diabetes mellitus and obesity. Based on sex and age, there was no difference in IL-17a level, but there was a significant difference in HbA1c level. The conclusion of this study showed no correlation between HbA1c and IL-17a levels in patients at risk for metabolic syndrome.

Keywords: Stress oxidative, dyslipidemia, hyperglycemia, proinflammatory cytokine, cross-sectional.

Introduction

Metabolic syndrome occurs when multiple factors in humans are gathered such as hypertension, hypertriglyceridemia, hyperglycemia, dyslipidemia and obesity^{1,2}. The prevalence of metabolic syndrome varies in each country. The prevalence of metabolic syndrome around the world is about 15%–30% which is majorly¹¹ and in developing countries. Some studies suggest that metabolic syndrome is more common in women than in men^{3,4} especially among the 55–64 year age group. At that age, there is some decrease in organ functions such as pancreatic endocrine function, muscle mass and mitochondrial activity.^{5,6} Several lifestyles significantly affect a person being exposed to the risk of metabolic syndrome. One of them is smoking behaviour. Nicotine in cigarettes causes a

decrease in insulin sensitivity and an increase in insulin resistance. Also, the reduction in HDL levels and arterial blockage are prone due to smoking behaviour⁷.

In patients at risk for metabolic syndrome, there is an increase in interleukin (IL)-17a levels caused by the rise in pro-inflammatory that is initially started by stress oxidative^{8,9}. HbA1c levels also increase as blood sugar increases when the glucose in the body is excessive. The condition of hyperglycemia causes an increase in HbA1c levels, resulting in a decrease in the capacity of haemoglobin to bind oxygen and also generates an accumulation of advanced glycation end product (AGE) compounds, reactive substances that are harmful to the body^{10,11}. Until now, research on factors related to elevated levels of HbA1c and IL-17a in patients at risk for metabolic syndrome has not been widely practised in Indonesia. This study aims to determine the factors associated with HbA1c and IL-17a levels in patients at risk of metabolic syndrome.

Material and Methods

The type of this research was observational analytic with a cross-sectional design. The study was conducted from December 2017 until January 2018 in Public Health Center, Jetis 1, Bantul, Yogyakarta. The informed consent and study protocol sheet had been reviewed and validated by Ahmad Dahlan University Research Ethics Committee with number 011801018.

Material: The tool used in the research was the questionnaires form to determine the demographic and clinical characteristics of the subject. Measurement of HbA1c levels used spectrophotometric devices. Analysis of IL-17a levels used enzyme-linked immunosorbent assay (ELISA) (stressMarq from Biosciences Inc.) methods. Standard solutions, wash buffers, biotinylated anti-human IL-17a antibody solutions and Avidin-Biotin-Peroxidase Complex (ABC) solutions are all prepared at room temperature for measurement of IL-17a levels in ng/mL.

Subject: The subjects of this study were patients at risk for metabolic syndrome at Public Health Centre, Jetis 1, Bantul, Yogyakarta. The minimum sample size was calculated using OpenEpi software (www.openepi.com). The inclusion criteria of the study subjects were patients who were clinically diagnosed with hypertension (blood pressure \geq 140/90 mmHg), hypertriglyceridemia (triglyceride level \geq 200 mg/dL, diabetes mellitus (random blood glucose level \geq 200 mg/dL) and obesity (body mass index \geq 25) by a primary care physician, aged >18 years and agreed to participate in

this study by signing informed consent. Exclusion criteria of the research subjects were pregnant women.

Research Procedure

Subject recruitment: In summary, subject recruitment procedures are: Participant candidates who matched the inclusion criteria were contacted one by one to be asked for their willingness to be the subjects of the research. Participant candidates who stated their desire were then told about the purpose, benefits and consequences as respondents. Participant candidates who voluntarily indicated their willingness to follow the research after listening to the explanation were then asked to sign the agreement. Patients at risk for metabolic syndrome who had filled in the informed consent were then subjected to blood sampling.

Determination of HbA1c level: Determination of HbA1c levels was performed with the following concise procedure: peripheral blood was taken from the cubital vein by a trained analyst. Random blood glucose level was measured by spectrophotometry 5010 and used for plasma glucose mean method to get HbA1c level. Patient's plasma blood sugar data were then converted to HbA1c levels using the formula: %HbA1c = (plasma glucose+77.3/35.6).

Determination of IL-17a level: IL-17a levels measurement was performed using ELISA method. The procedure for examining IL-17a levels is carried out according to the instructions on the stress Marq reagent (Biosciences Inc.).

In summary, the process for IL-17 levels determination is as follows: A total of 0.1 ml of the sample and standard solution were added separately to the well. The well was closed and incubated at 37 °C for 90 minutes. After the incubation is complete, the cover of the well is opened, then the contents of the well are removed and dried by tapping the rest on clean tissue paper. A total of 0.1 biotinylated anti-human IL-17a antibody working solutions were added to each well. After that, the well was closed and incubated at 37 °C for 60 minutes. The solution was removed and washed three times with 0.01 M phosphate buffered saline (PBS). A total of 0.3 ml 0.01 M PBS per washing is put into each well using a multichannel pipette. After the last rinse, the remaining PBS is removed and the well is dried by tapping the rest on clean tissue paper. Avidin-biotin-peroxidase complex (ABC) working solution as much as 0.1 ml is added to each well, then the well is closed and incubated at 37 °C for 30 minutes.

The solution was removed and washed five times with 0.001 M PBS. A total of 0.3 ml of 0.001 M PBS per rinse is inserted into each well using a multichannel pipette. After the last rinse, the remaining PBS is removed and the well is dried by tapping the rest on clean tissue paper. 3,3',5,5'-Tetramethylbenzidine (TMB) colour developing agent as much as 90 ml was added to each well, then the well was covered with aluminium foil and incubated the plate at 37 °C in the dark for 20-25 minutes. 3,3',5,5'-

Tetramethylbenzidine (TMB) colour developing agent as much as 90 ml was added to each well, then the well was covered with aluminium foil and incubated the plate at 37 °C in the dark for 20-25 minutes. After the incubation is complete with 0.1 ml of TMB stop solution into each well, then read at a wavelength of 450 nm a maximum of 30 minutes after the plate is added stop solution. IL-17a levels are expressed as ng / mL.

Data analyzed: Data on demographic characteristics (sex, age and smoking behaviour) and clinical manifestations (hypertension, hypertriglyceridemia, diabetes mellitus and obesity) were presented descriptively. Mann-Whitney test was performed to find out the correlation of patient characteristics with HbA1c and IL-17a levels. A bivariate correlation test (Spearman correlation test) was conducted to determine the relationship between HbA1c and IL-17a levels in patients at risk for metabolic syndrome. Statistic test used SPSS 16.0 for Windows software with a 95% confidence level.

Results and Discussion

Table 1 represents the demographic characteristics of patients. From table 1, it can be seen that the number of female patients is higher than male patients (75.2% vs 24.7%). The age group data indicated that the participants aged <60 years (65.1%) were more than those aged ≥60 years (34.8%). Most participants had a clinical diagnosis of hyperglycemia (97.8%) and hypertension (61.8%). Also, some participants had hypertriglyceridemia (58.4%) and obesity (8.9%).

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Table 1

Characteristics of patients at risk for metabolic syndrome at Public Health Center, Jetis 1, Bantul in 2018.

Patient characteristics	Total	Percentage (%)
Sex		
Man	22	24.7
Woman	67	75.2
Age		
≥60 years	31	34.8
<60 years	58	65.1
Lifestyle		
Smoking behaviour	11	12.4
Clinical manifestations		
Hypertension	55	61.8
Hypertriglyceridemia	52	58.4
Hyperglycemia	87	97.8
Obesity	8	8.9

Based on these characteristic [16](#) it can be seen that women aged <60 years are at more risk for metabolic syndrome. This condition is by the results of previous [14](#) arch conducted by Yu et al⁴ and Beigh and Jain¹² that the prevalence of metabolic syndrome is higher in women by 56.3% compared to men of 32.6%. Also, in women aged 55-

64 years, there is an increased risk for metabolic syndrome compared with men of the same age group⁴. Changes in the menopausal cycle in women are associated with the increased component of the metabolic syndrome such as obesity, hypertension, changes in lipid profile and hyperinsulinemia. Most of the participants of this study had hyperglycemia and hypertension with a percentage of 97.8% and 61.8% respectively.

Table 2 shows the results of HbA1c and IL-17a levels based on patient characteristics. The results of HbA1c levels showed that there were differences by sex and age group. There was a difference between men and women ($p=0.025$) and there was a difference between the age group ≥ 60 and <60 years ($p=0.041$). All of the characteristics (sex, age group, smoking behaviour and clinical manifestations) showed no difference in IL-17a level with a p-value higher than 0.05. There is a difference in HbA1c levels between men and women. It is because women are more likely to experience hyperglycemia.

Based on this research, Kumar et al²¹ explained that women are about 1.3 times more easily suffer from diabetes mellitus. Basic Health Research results found that the prevalence of diabetes mellitus is higher in women by 9.56 (7.54–11.41)% compared with men 7.46 (6.11–11.27)% ($p<0.05$). We suggest women are more likely to develop hyperglycemia with the HbA1c value more elevated than usual. Research in

China shows that women often experience low haemoglobin during menstruation. This menstrual cycle is thought to be the cause of HbA1c differences between men and women¹³.

Based on the age group, there is a difference in HbA1c levels between the age group ≥ 60 and <60 years. The age of 60 years in Indonesia is classified as geriatric. Geriatric and non-geriatric have different HbA1c levels because the function of human organs decreases due to ageing process. Most geriatric have the potential for hyperglycemia^{14–16}. Lifestyle (smoking) and clinical manifestations show no difference in HbA1c levels. Between smokers and non-smokers, there is no difference in HbA1c levels. Among all clinical signs such as hypertension, hypertriglyceridemia, diabetes mellitus and obesity, there is no difference in HbA1c levels^{17,18}.

There is no difference in IL-17a levels for all patient characteristics. If it is deeply traced, there may be several factors that cause this result. By the research conducted by Evia et al¹⁹, metformin increases anti-inflammatory activities, one of them is IL-17a. Therefore, there is no difference in all patient characteristics.

Table 3 shows no correlation between HbA1c and IL-17a levels. This result is as same as by the research conducted by Wu et al²⁰ who stated that there is no relationship between HbA1c levels and IL-17a levels.

Table 2
Level of HbA1c and IL-17a measurement based on patient characteristics (N = 89).

Characteristics	HbA1c (%)	p-value	IL-17a (IU)	p-value
Sex		0.025*		0.419
Man (n = 22)	7.46 (6.11–11.27)		7.54 (3.72–18.41)	
Woman (n = 67)	9.56 (7.54–11.41)		10.75 (5.38–16.18)	
Age		0.041*		0.990
≥ 60 years (n = 31)	7.73 (6.41–11.05)		10.88 (5.03–15.88)	
<60 years (n = 58)	9.83 (7.67–11.62)		9.84 (5.17–17.95)	
Lifestyle		0.876		0.186
Smoking behavior (n = 11)	9.90 (6.44–12.09)		4.94 (2.12–18.16)	
Not smoking (n = 78)	9.08 (7.39–11.14)		10.81 (5.36–16.73)	
Clinical manifestations				
Hypertension		0.342		0.661
Yes (n = 55)	9.33 (6.86–11.27)		10.75 (4.91–16.59)	
No (n = 34)	8.90 (7.67–12.12)		8.49 (5.36–18.25)	
Hypertriglyceridemia		0.281		0.283
Yes (n = 52)	9.74 (7.53–11.56)		11.84 (9.26–23.01)	
No (n = 37)	7.87 (6.84–11.18)		9.31 (5.15–14.78)	
Diabetes mellitus		0.618		0.719
Yes (n = 87)	9.19 (7.37–11.27)		10.75 (5.12–17.16)	
No (n = 2)	8.52 (5.37–11.67)		8.45 (6.52–10.37)	
BMI		0.785		0.601
≥ 30 (n = 8)	10.29 (6.48–10.67)		9.29 (3.51–14.15)	
<30 (n = 81)	8.97 (7.28–11.51)		10.7.2 (5.15–17.53)	

1 Table 3
Results of the Spearman Analysis between HbA1c levels and interleukin-17a levels
in patients at risk of metabolic syndrome in the PHC Jetis I Bantul

	HbA1c level
interleukin-17a level	r = 0,030 p = 0,782 N = 89

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 Research in Canada on the relation of inflammatory cytokines (IL-6, IL-17a and TNF- α) to the comorbid of cardiovascular disease involving 21,327 subjects explains that IL-17 levels cannot be used to predict HbA1c levels²⁰. The study showed that there was no relationship between HbA1c and IL-17a levels. Contrast with a study conducted by Kumar et al²¹, that as HbA1c levels increased, there was also an increase in blood sugar along with an increase in IL-17a. High blood glucose was said to be able to regulate IL-6 and IL-17 cytokines level by transcription through oxidative stress.

7 Conclusion

Based on the results of this study, it can be concluded that there is no correlation between HbA1c and IL-17a levels in patients at risk for metabolic syndrome.

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