The Difference Levels of Hepcidin and Interleukin-6 between Obese and Non-Obese Type 2 Diabetes Mellitus

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RESEARCH ARTICLE

The Difference Levels of Hepcidin and Interleukin-6 between Obese and Non-Obese Type 2 Diabetes Mellitus

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Abstract

BACKGROUND: Inflammation occurs in chronic diseases such as type 2 diabetes mellitus (DM). Insulin resistance and inflammation in type 2 DM with obesity can increase interleukin (IL)-6, causing an increase in hepcidin synthesis in the liver. Increased inflammation can exacerbate the course of type 2 DM. This study aims to prove that there are differences in the levels of hepcidin and IL-6 between obese and non-obese type 2 DM.

METHODS: This cross-sectional study was conducted on 61 patients with type 2 DM, consist of 22 male and 39 female with an age of more than 40 years. Type 2 DM subjects were obtained from a doctor's diagnosis and were divided into obese and non-obese groups based on body mass index (BMI). Hepcidin and IL-6 levels were examined using the Enzyme-Linked Immunosorbent Assay (ELISA) principle. The data were analyzed using an independent t-test and Mann-Whitney test.

RESULTS: The mean level of hepcidin in the obese with type 2 DM group was 25.32±11.54 ng/mL, and non-obese was 11.94±5.31 ng/mL. The median level of IL-6 in the obese with type 2 DM group was 11.9 (5-61) pg/mL, and non-obese 4.8 (1.5-9.8) pg/mL. There was a significant difference in hepcidin and IL-6 levels between the obese and non-obese groups (p=0.000).

CONCLUSION: Hepcidin and IL-6 levels in the obese group with type 2 DM were higher than non-obese group.

KEYWORDS: type 2 diabetes mellitus, obesity, hepcidin, interleukin-6

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Introduction

Type 2 diabetes mellitus (DM) according to The Indonesian Society for Endocrinology is a group of metabolic diseases characterized by hyperglycemia caused by abnormalities in insulin secretion, action, or both.(1) Obesity is one of the risk factors for type 2 DM. Reducing obesity is very important in patients with type 2 DM.(2) There was a positive relationship between Body Mass Index (BMI) and the risk of developing type 2 DM.(3) Obesity criteria are established by calculating BMI.(4) A research in Japanese, South Asian, and Southeast Asian ethnics shows the cut-off BMI 25 kg/m^2 is the threshold for diabetes risk.(5)

Hepcidin is a hormone that regulates iron homeostasis which is synthesized in the liver, fat tissue, pancreas, and intestinal cells. Hepcidin synthesis is influenced by inflammation and infection. Several pro-inflammatory cytokines such as interleukin (IL)-6, IL-1, IL-22, and interferon appear as regulators of hepcidin synthesis through the signal transducer and activator of transcription (STAT)-3 mechanism.(6) There are many differences of opinion regarding hepcidin levels in obesity. A study found an increase in serum hepcidin in respondents with type 2 DM and obesity.(7) In other study stated that serum prohepcidin was decreased in diabetic patients.(8) Meanwhile, hepcidin levels increased in type 2 DM patient with overweight but decreased in grade I obesity patients.(9)

IL-6 is a cytokine with multiple actions to regulate the body's metabolism. IL-6 triggers the synthesis of c-reactive protein (CRP), serum amyloid, fibrinogen, hepcidin, and inhibits albumin production.(10) IL-6 interfere the signal to produce insulin hormone by increasing suppressor of cytokine signalling (SOCS)-3 in adipose tissue. Suppressor of cytokine signaling-3 interact with insulin like growth factor-1 receptor, insulin receptor substrate 1,2 thereby blocking cytokines that mediate insulin receptor transcription factor.(11,12)

The severity in type 2 DM is influenced by inflammation. Type 2 DM activated innate and adaptive immunity and play a role inflammation adipose tissue.(13) Obesity is one of triggering factors for inflammation in type 2 DM. Infiltration of immune cell in obesity causing inflammation, increasing oxidative stress, insulin resistance, organ failure and aging.(14) Hepcidin and IL-6 are acute proinflammatory factors. Hepcidin produced in the liver through IL-6 stimulation and also produced by β cell pancreas in insulin secretory granules so that its production influenced by blood glucose level.(15) Increasing hepcidin and IL-6 levels stimulating inflammation causes iron metabolism disorder and disturbance in organs such as β cell pancreas, blood vessels, liver and aggravating the disease. Studies on hepcidin and IL-6 in type 2 DM with obesity are limited. This study is expected to provide information about the risk of obesity to increase inflammation in type 2 DM.

Methods

Study Design

This research is an analytic observational study with a cross-sectional approach. The research was carried out in February to April 2021 at the Kedungmundu Public Health Center in Semarang. Research was conducted after obtaining an ethical approval from Health Research Ethics Committee, Faculty of Medicine, Diponegoro University, Semarang, (No. 03/EC/KEPK/FK-UNDIP/2021).

Sample Collection

All patients were asked written informed consent and their identities were kept confidential. Sampling was carried out from February to April 2021. Research subjects of this study were diagnosed with type 2 DM by clinician in Kedungmundu Public Health Center. Inclusion criteria were male and female over 40 years, normal body temperature, normal oxygen saturation (SaO_2), haemoglobin (Hb) more than 7 g/dL, normal white blood count (WBC), good glycemic control. Glycemic control according to Perkeni's criteria (2019) using an average fasting blood glucose (FBG) for the last 3 months 152 mg/dL at the age <60 years old, while the age ≥60 years old is 178 mg/dL.(1) Exclusion criteria were pregnancy, history of autoimmune, malignancy, chronic hepatitis, iron supplementation.

There were 61 patients with type 2 DM who met the inclusion and exclusion criteria and gave consent to participate in the study. Sample was divided into two groups, 31 patients were included in the obese group, and 30 patients included in the non-obese group. Body mass index was calculated as body weight in kilogram divided by square of height in meters (kg/m^2). Obesity was defined as BMI ≥25 kg/m^2 according to Japan Society for the Study of Obesity (JASSO) criteria.(16)

Analysis Method

Basic data were collected through history taking (history of autoimmune, malignancy, chronic hepatitis, iron supplementation), physical examination (body weight, height, temperature, oxygen saturation), and medical record (levels of FBG for the last 3 months). Five mL of venous blood was taken, 3 mL for complete blood count, and 2 mL for serum and stored at -80°C until the time of examination.

Hepcidin Serum Level Test

Hepcidin serum level test were measured using sandwich ELISA (Elabscience, Houston, TX, USA). A micro-plate ELISA coated with specific antibody to human hepcidin. Samples combined with the specific antibody then a biotinylated detection antibody and Avidin-Horseradish Peroxidase (HRP) conjugate added and incubated. The substrate solution is added to each well. The wells that contain hepcidin, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured with spectrophotometri at 450 nm±2 nm and OD value proportional to hepcidin level. Detection range for human hepcidin serum is 0.156-10 ng/mL with sensitivity 0.09 ng/mL.

IL-6 Serum Level Test

IL-6 serum level test were measured using sandwich ELISA principle (Elabscience, Houston, TX, USA). Micro-plate ELISA has been pre-coated with an antibody specific to human IL-6. Samples are added to the micro-plate wells and combined with the specific antibody. Biotinylated detection antibody specific for human IL-6 and Avidin-Horseradish

Peroxidase (HRP) conjugate added and incubated. The substrate solution added to each micro-plate well. Only those wells that contain human IL-6, biotinylated detection antibody and Avidin HRP conjugate will appear in blue. The enzyme-substrate reaction terminated after addition of stop solution and turns yellow. The OD is measured spectrophotometrically at 450 nm±2 nm and OD value proportional to concentration of Human IL-6. Detection range for IL-6 serum is 1.56-100 pg/mL with sensitivity 0.94 pg/mL.

Statistical Analysis

Data were analyzed by Statistical Package for the Social Sciences (SPSS) 23 (IBM Corporation, Armonk, NY, USA). The results of the study with normal data distribution were presented in the form of mean±standard deviation, followed by the independent t-test. The distribution of abnormal data is presented in the median form, followed by Mann-Whitney test. Statistical significance was assigned to $p<0.05$.

Results

The results of the study were statistical tests to determine the normality of the data using the Shapiro Wilk test. Data with normal distribution included age, weight, average FBG for the last 3 months, WBC, hepcidin. Data with abnormal distribution consisted of height, BMI, duration of type 2 DM, systolic, diastolic, respiratory rate (RR), heart rate (HR), temperature, SaO_2, Hb, IL-6.

The mean age subjects of the study was 58.71±8.22 years old in obese group, and 62.3±6.57 years old in non-obese group. The median value BMI of the research was 29.1 (26-34) kg/m^2 in obese group, and 23.8 (18.1-24.9) kg/m^2 in non-obese group. General results of the characteristics of the research subject data can be seen in the Table 1.

The mean hepcidin level in obese group was 25.32±11.54 ng/mL, while the non-obese group was 11.94±5.31 ng/mL. Analysis of differences between groups in hepcidin using independent t-test found significant difference (p=0.000) (Figure 1A). The median IL-6 level in the obese group with type 2 DM was 11.9 (5-61) pg/mL, while in the non-obese group was 4.8 (1.5-9.8) pg/mL. Analysis between groups in IL-6 using Mann Whitney test showed there was a significant difference (p=0.000) (Figure 1B). Comparison hepcidine and IL-6 in obese and non-obese group with type 2 DM can be seen in table 2.

Discussion

This study included 61 patients with type 2 DM, subject type 2 DM non-obese consisted of 11 male (36.67%) and 19 female (63.33%). Subject type 2 DM with obese consisted of 11 male (35.4%) and 20 female (64.51%). Estrogen hormone in female plays role in obesity, increasing

Table 1. Research subject's characteristics.

Variable	Obese (n= 31)	Non-Obese (n= 30)	*p*-value
Age (year)	58.71±8.22	62.3±6.57	0.065
Weight (kg)	72.35±9.94	54.93±6.22	0.000
Height (cm)	155 (145-175)	153 (140-167)	0.269
BMI (kg/m^2)	29.1 (26-34)	23.8 (18.1-24.9)	0.000
Duration (year)	4 (1-23)	5 (1-25)	0.541
Sistol (mmHg)	130 (110-170)	130 (100-170)	0.801
Diastol (mmHg)	80 (60-100)	80 (60-100)	0.957
RR (x/menit)	20 (18-22)	20 (20-22)	0.343
HR (x/menit)	80 (80-88)	82 (78-88)	0.126
Temperature (°C)	36.2 (35.9-36.8)	36.2 (35-36.7)	0.804
SaO_2 (%)	99 (97-99)	98 (97-99)	0.322
Average FBG for the last 3 months			
Age <60 years (mg/dL)	119.38±16.55	113.63±21.71	0.655
Age ≥60 years (mg/dL)	125.78±25.80	115.96±26.15	0.243
Hb (g/dL)	13.05 (9.3-15.9)	12.9 (7.9-15.3)	0.568
WBC (x10^3/uL)	7.97±1.57	7.47±1.48	0.209

DM: Diabetes Mellitus; BMI: Body Mass Index; FBG: Fasting Blood Glucose; Hb: hemoglobin; HR: heart rate; BMI: body mass index; RR: respiratory rate; SaO_2: oxygen saturation.

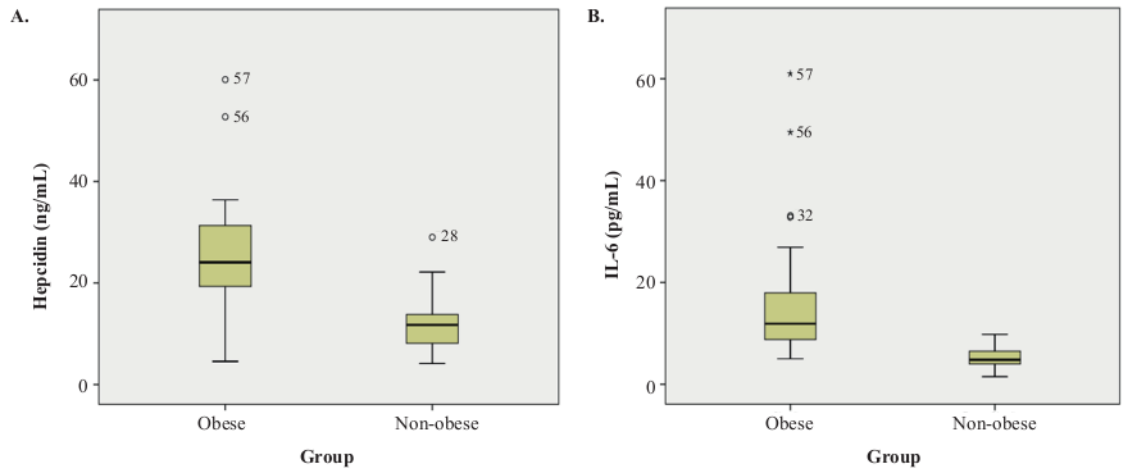

Figure 1. Boxplot graph of hepcidin levels (A) and IL-6 levels (B) in type 2 DM obese and non-obese group.

subcutaneous fat mass in glutofemoral. Decreased estrogen at menopause causes an increase in total adipose tissue and decrease body mass.(17) The median BMI for type 2 DM non-obese subjects was 23.8 (18.1-24.9) kg/m^2 and type 2 DM obese was 29.1 (26-34) kg/m^2. The measurement with BMI is easier to do, but cannot describe distribution of fat mass.(18)

Glycemic control in this study used conversion of HbA1c in to average FBG for the last 3 months according to Perkeni's recommendation. The result of the average FBG level in last 3 months in type2 DM with obese aged <60 years old was 119.38±16.55 mg/dL, higher than non-obese 113.63±21.71 mg/dL. Type 2 DM with obesity aged ≥60 years old was 125.78 ±25.80 mg/dL, higher than non-obese 115.96±26.15 mg/dL. Increasing age can cause dysfunction of islet leading to abnormal glucose metabolism.(19) A study stated that there was increasing release of nonesterified fatty acids (NEFA) in type 2 DM with obese inducing antilipolytic, peripheral insulin uptake and increasing blood glucose. Insulin resistance associated with BMI and increased weight gain.(20,21)

Type 2 DM is associated with chronic inflammation which will increase levels of inflammatory proteins and cytokines. In other study there was an increasing hepcidin in type 2 DM with obesity.(22) Hepcidin extrahepatic also produced by pancreatic cells so it can increase along insulin release.(23,24) Several studies have reported hepcidin expression in subcutaneous and visceral adipose tissue, although at much lower levels. Fat distribution in different regions have different levels of immune cells infiltration and inflammation.(25) Visceral adipose tissue through complex mechanism related to inflammation.(26) There is a relationship between visceral adipose tissue hepcidin expression and BMI, thereby increasing hepcidin levels. (27) Elevated hepcidin is associated with inflammation of adipokines that affect hepatic hepcidin transcription and hepcidin messenger ribonucleic acid (mRNA) expression via Janus Kinase (JAK)-STAT.(28) Type 2 DM can causes inflammation that occurs with low and prolonged levels. Subclinical and systemic inflammation can be characterized by elevated CRP, IL-6, and tumor necrosis factor (TNF)-α.(29)

IL-6 is one of the proinflammatory cytokines involved in the inflammatory process. IL-6 functions to regulate differentiation, migration, proliferation, and cell apoptosis. IL-6 produced by monocytes, endothelial cells, fibroblasts, and 10-35% is produced in adipose tissue.(11,30) These cytokines stimulate fatty acid synthesis from the liver and stimulate the production of acute-phase proteins. More inflammatory cells go to adipose tissue and β pancreatic

Table 2. Hepcidin and IL-6 levels of type 2 DM patients obese and non-obese.

Variable	Obese (n= 31)	Non-Obese (n= 30)	*p*-value
Hepsidin (ng/mL)	25.32±11.54	11.94±5.31	0.000
IL-6 (pg/mL)	11.9 (5-61)	4.8 (1.5-9.8)	0.000

cells. IL-6 suppresses insulin receptor autophosphorylation, thereby impairing insulin sensitivity, increasing hepatic glucose production, and insulin resistance in muscles. Impaired signaling in insulin production is also elicited by IL-6 by inducing IL-1β.(29,31)

Adipose tissue functions as an energy reserve by forming triglycerides, and produces various adipocytokine molecules such as IL-1, IL-6, IL-8, IFN-γ, TNF-α. The production of these molecules plus cellular destruction triggers chronic inflammation, alters the function, and causes other diseases.(32) Visceral fat on obesity individuals more infiltrated by macrophages thereby increasing secretion of IL-6 and hepcidin.(33) Increasing hepcidin through IL-6 stimulation inducing iron sequestration in tissue organ. Correlation between iron overload and insulin resistance still difficult to explain, it may be due to the presence of dysmetabolic iron overload syndrome (DIOS).(34) Iron overload in tissue organ can worsen DM. Iron affect β cell pancreas in insulin secretion and causing insulin resistance in liver, reducing glukosa oxidation in skeletal muscle. Iron load responsible for glucose homeostasis disorder.(35) The results of this study showed that the group of type 2 DM with obesity had an accumulation of inflammation so that the mean levels of hepcidin and IL-6 were higher than the group without obesity.

This study did not measure body anthropometry of subjects and did not consider sex difference, so the distribution of body fat can not be describe precisely. Waist circumference (WC), waist to hip ratio (WHR), waist to height ratio (WHtR), are suggested to be measured in further study.

Conclusion

There are difference levels of hepcidin and IL-6 between obese and non obese group with type 2 DM. Hepcidin and IL-6 in type 2 DM with obese higher than non obese but still in normal reference range. Further studies are needed with considering sex and using body anthropometry measurement such as WC, WHR, and WHtR.

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Authors Contribution

IKS and MH were involved in research planning, supervising the research, analyzing data and reviewing manuscript. NSW and MR were involved supervising the research and reviewing manuscript. MM was involved in research planning, measurements, data analysis, literature searching and compiling the manuscript. All authors discussed and giving critical revision and approved final manuscript. None of the authors have conflict of interest.

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