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Editorial Adress:
d/a Laboratorium Patologi Klinik RSUD Dr. Soetomo, Gedung Diagnostik Center Lt. IV
Jl. Mayjend. Prof. Dr Moestopo 6–8 Surabaya, Indonesia
Telp/Fax. (031) 5042113, 085-733220600 E-mail: majalah.ijcp@yahoo.com, jurnal.ijcp@gmail.com
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RESEARCH

CORRELATION BETWEEN VISCERAL ADIPOSE TISSUE-DERIVED SERPIN WITH FASTING BLOOD GLUCOSE LEVEL IN OBESITY

(Hubungan Kadar Visceral Adipose Tissue-Derived Serpin dengan Kadar Glukosa Darah Puasa pada Kegemukan)

Novi Khila Firani[1,2,3], Agustin Iskandar[1,4], Anik Widijanti[1], Nonong Eriani[5]

ABSTRAK

Abnormalitas jaringan lemak pada kegemukan berhubungan dengan timbulnya berbagai masalah kesehatan, antara lain terjadinya resistensi insulin. Adipositokin merupakan protein yang dihasilkan jaringan lemak, salah satunya adalah Visceral Adipose Tissue-Derived Serpin (Vaspin). Beberapa penelitian menunjukkan vaspin berhubungan dengan kepekaan insulin. Belum diketahui apakah dalam setiap peningkatan derajat kegemukan terdapat perubahan hasilan vaspin, yang berhubungan dengan perubahan kadar glukosa darah, sebagai manifestasi gangguan kepekaan insulin. Rancangan penelitian adalah potong silang, dengan jumlah sampel 60 orang usia dewasa, yang terbagi berdasarkan patokan WPRO (2000), yaitu 10 orang non-kegemukan, 10 orang kegemukan I dan 40 orang tergolong kegemukan II. Pemeriksaan kadar glukosa darah puasa menggunakan metode heksokinase. Pemeriksaan kadar vaspin menggunakan metode sandwich ELISA. Telitian menunjukkan kadar vaspin di kelompok kegemukan II dan I lebih tinggi dibandingkan kadar vaspin pada non-kegemukan (p=0,00). Kadar vaspin di kegemukan II dan I tidak berbeda bermakna. Kadar glukosa darah puasa di kelompok kegemukan II dan I lebih tinggi dibandingkan kadar glukosa darah puasa di non-kegemukan (p=0,017), namun kadar glukosa darah sebagian besar subjek penelitian masih dalam taraf normal. Hasil uji kenasaban Spearman menunjukkan ada kenasaban positif yang bermakna kadar vaspin terhadap kadar glukosa darah, namun kekuatannya rendah (r=0,384, p=0,001). Terdapat hubungan yang bermakna kadar vaspin dengan kadar glukosa darah puasa di kegemukan. Perlu dilakukan kajian lebih lanjut menggunakan subjek penelitian kegemukan dengan mengukur indeks kepekaan insulin, untuk memperjelas hubungan antara vaspin, sebagai adipositokin yang berperan dalam kepekaan insulin, terhadap kadar glukosa darah.

***Kata kunci**: Kegemukan, visceral adipose tissue-derived serpin, glukosa darah puasa*

ABSTRACT

Adipose tissue abnormality in obesity is associated with health problems, one of which is insulin resistance. Visceral adipose tissue-derived serpin (Vaspin) is one of the adipocytokines, produced by adipose tissue. Previous studies revealed the association of vaspin with insulin sensitivity. It is not known yet whether the increasing degree of obesity will change vaspin production that will lead to blood glucose level alteration, manifesting as an insulin sensitivity disorder. This used a cross-sectional study design. Sixty (60) adults were recruited, divided according to WPRO criteria (2000), 10 people as the non-obese group, 10 people as obese I and 40 people as obese II. Hexokinase method was used to examine fasting blood glucose level. Vaspin concentration was analyzed using sandwich ELISA method. The result showed that vaspin level in obese II and obese I groups was higher than non-obese (p=0.00). Vaspin level in obese II group was not significantly different with obese I. Fasting blood glucose levels in obese II and obese I groups was higher than non-obese (p=0.017), but most of the blood glucose concentration was at a normal level. Spearman correlation test showed a significant positive correlation between vaspin level and blood glucose level, but the strength was poor (r = 0.384, p=0.001). There was a significant correlation between vaspin and fasting blood glucose level in obesity. Further study is needed employing obese people with measuring insulin sensitivity index, to elucidate the relationship between vaspin, as adipocytokine that plays a role in insulin sensitivity, and blood glucose level.

Key words: Obesity, visceral adipose tissue-derived serin protease inhibitor, fasting blood glucose

[1] Department of Clinical Pathology, Faculty of Medicine, Brawijaya University, Malang, Indonesia. E-mail: novikhila@yahoo.com
[2] Department of Biochemistry-Biomolecular, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[3] Department of Oral Biology, Faculty of Dentistry, Brawijaya University, Malang, Indonesia
[4] Department of Clinical Parasitology, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[5] Clinical Pathology Laboratory, dr Fauziah Hospital, Bireuen, Aceh, Indonesia

INTRODUCTION

The incidence of obesity in the world in recent decades has increased. Based on the results of Low's research it showed that in Japan the incidence of obesity was 23.2% and in the United States was 66.3%.[1] In Indonesia, a health research in 2007 found that 10.3% of the population of people over 15 years old were obese and the percentage of females was higher than males, ie, 23.8% were females, while males were 13.9%.[2]

Obesity is a state of large amounts of adipose tissue accumulation in the body. The definition of obesity according to World Health Organization (WHO) is the Body Mass Index (BMI) ≥ 30 kg/m^2. In the Asia Pacific region, according to the criteria of the WHO Western Pacific Region (WPRO), the definition of obesity is BMI ≥ 25 kg/m^2.[3] It is estimated that approximately 70-80% of obese people will undergo abnormal metabolism.[4] The cause of obesity involves many factors, including the nutritional factor and genetic abnormality. In addition, behavioral, lifestyle and environment factors also affect the increase of obesity.[5]

Adipose tissue is an endocrine organ that can produce several active proteins, called adipocytokines. One of adipocytokines produced by visceral adipose tissue is visceral adipose tissue-derived serpin (Vaspin). The previous study said that vaspin can be found in the blood of healthy people, with a level in females 2.5 times higher than males. Vaspin level in the blood is thought to be influenced by the glucose metabolism. Several previous studies mentioned that a low serum level of vaspin was an independent risk factor for the occurrence of diabetes mellitus in obese people. Treatment of vaspin in experimental obese animals showed that vaspin could improve glucose tolerance and insulin sensitivity.[6-8] Another researcher found that vaspin gene alteration was responsible for providing the compensated effect on the metabolic abnormalities associated with obesity. This statement based on a research in mice vaspin-transgenic could be protected from food-induced obesity, glucose tolerance impairment and fatty liver, whereas mice vaspin-deficient suffered from glucose intolerance.[9] The role of vaspin in blood glucose regulation disorders in humans is not well understood until now. It is not known yet whether any increase in the degree of obesity will change the vaspin production and correspond with the blood glucose levels alteration, as a manifestation of insulin sensitivity disorder.

METHODS

This research was a cross-sectional study and used consecutive sampling method for subjects recruitment, from January until April. The participants were adult females (age ≥25 years old). Informed written consent was obtained from the participants and the research protocol was done according to the Helsinki declaration and approved by Faculty of Medicine Brawijaya University Health Research Ethics Committee (certificate no. 54/EC/KEPK/02/2016).

Sixty participants enrolled in this study were divided into 3 groups based on the criteria of the Western Pacific Region (2000), 40 people with BMI ≥ 30 as obese group II, 10 people with BMI of 25–29.9 as the obese group I and 10 people with BMI <25 as a non-obese group. Criteria for inclusion of subjects used in this study were adult (> 25 years old), females and willing to follow this research voluntarily and undersigned an informed consent, after given a full explanation about this research. Exclusion criteria were pregnancy, breastfeeding females, use of hormonal contraceptives and having a history of chronic liver disease.

The participant's venous blood samples were taken after fasting for 8–10 hours. Venous blood was taken as much as three (3) mL, collected in a non-EDTA vacutainer and then centrifuged at 3000 rpm for 10 minutes. Serum was taken for the measurement of fasting blood glucose and vaspin level.

Measurements of fasting blood glucose level used hexokinase method (Roche diagnostic, Indianapolis) with Cobas C6000 analyzer. Measurements of Vaspin level was using sandwich Enzyme-Linked Immunosorbent Aassay (ELISA) method (AdipoBioscience human vaspin kit, Catalog No. SK 00560-01).

The data were presented in mean and standard deviations (mean ± SD). Analysis of the data used ANOVA and the Spearman correlation test to determine the relationship between vaspin level and fasting blood glucose levels, with a value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSIONS

Sixty (60) females were obtained as participants of this research. The research used only female subjects based on the prevalence study previously, the incidence of obesity was higher in females than males, in addition to gain homogeneity of research subjects, so

Table 1. Characteristics of research subjects

Body Mass Index (BMI)	Category	Number (people)	Mean ± SD of age (y.o)	Mean ± SD of fasting blood glucose level (mg/dL)	Mean ± SD of vaspin level (ng/mL)
BMI ≥ 30	Obese II	40	47.7±10.5	93.03±35.70*	90.91±18.68*
BMI 25-29,9	Obese I	10	42.8±11.2	84,20±20.01*	95.81±11.39*
BMI < 25	Non-obese	10	25.37±1.7	61.00 ±7.08	60.06±7.98

Note: * p<0.05 compared to non-obese group

the variation of the data was not high. Characteristics of research subjects can be seen in Table 1.

The results showed that the mean age in the obese group II was 47.7 ± 10.5 years old, the obese group I was 42.8 ± 11.2 years old and in a non-obese group the average age was younger than obese group I and II with the mean was 25.37 ± 1.7 years old. These results indicated that aging is related to the increasing of obesity incidence. The data corresponded with the results from a survey of Indonesia's obesity and overweight prevalence in 2004[10] and was in accordance with the results of a research in the United States,[11] which stated that the prevalence of obesity was increased with the enhancement of age.

The enhancement of obesity incidence is in line with the aging process, which is associated with substantial changes in body composition. After 30 years old, there is a progressive decrease in body fat-free mass, otherwise there is an increase in body fat mass. The relationship between energy intake and energy expenditure is an important determinant factor of body fat mass. The enhancement of total fat mass that occurs in aging is due to increased energy intake, decrease energy expenditure, or both.[12] The reduction of energy expenditure is due to decrease of basal metabolic rate[13] and decrease in physical activity along with aging.[14] A hormonal change also played a role in the increasing accumulation of fat in aging. In the aging process there is a decline in growth hormone secretion and development of leptin resistance. A decline in growth hormone secretion will lead to an increase in fat mass. Resistance to the leptin causes a decrease in the body's ability to reduce appetite.[15,16]

The mean fasting blood glucose levels are presented in Table 1. Based on these data, our analysis found a significant difference (p=0.017) between the mean fasting blood glucose levels in group obese II (BMI ≥30) and group obese I (BMI 25-29.9), with non-obese group (BMI <25). Fasting blood glucose levels in non-obese group was lower than fasting blood glucose in obese group I and II, but there was no significant difference of fasting blood glucose levels in the obese group I and II. The results indicated that an increasing in fat mass in obesity can lead to enhancement of fasting blood glucose level. A previous study had found a strong correlation between increased BMI with the occurrence of diabetes mellitus. This finding can be explained that obesity may induce insulin resistance due to decrease in insulin receptor sensitivity along with weight gain.[17] There was no significant difference between fasting blood glucose levels and the degree of obesity, where both of obese groups were equally increased in fasting blood glucose levels. The weakness of this research was that the researcher did not measure insulin sensitivity index or insulin resistance index in the participants. Other researchers mentioned that the increase of blood glucose levels in obesity can also be caused by an increase in the resistin secretion. Resistin is a hormone produced by fat tissues which undergo an inflammation process. Resistin has an opposite effect on insulin in adipose tissue.[18] Based on our study, most of the results of blood glucose levels measurement in obese group showed a normal level. Further studies with index of insulin sensitivity, index of insulin resistance and resistin level measurement are needed to explain the mechanism of blood glucose increase in obesity.

The results of serum levels vaspin measurements were presented in Table 1. Based on this analysis, a significant difference (p=0.00) was towards between the average levels of vaspin in the obese group II (BMI ≥ 30) and obese I group (BMI 25–29.9), compared with vaspin level in the non-obese group (BMI <25). Serum vaspin levels in non-obese group were lower than the vaspin levels in obese groups I and II. There was no significant difference between the vaspin level in the obese group I and II. The study showed that the levels of vaspin in the obese people were higher than non-obese and the serum vaspin levels were not affected by the degree of obesity. The correlation test results showed there was a significant positive relationship between the level of vaspin with fasting blood glucose levels, but the strength of correlation was poor (r=0.384; p=0.001).

Vaspin or Visceral adipose tissue-derived serpin is one of the adipocytokines, produced by visceral fat tissue, which is recently found.[19] Vaspin is a family member of the serine protease inhibitor (serpin). Serpin has several functions, including regulating protein breakdown or proteolysis by inhibition and affecting various biochemical pathways within the cell. Regulation of protease enzyme activity plays an important role in maintaining homeostasis in the body. It is known that the serpin has many functions, including regulating cell differentiation, the complement cascade, regulation of intracellular proteolysis and modulation of immune response.[20]

A previous research found that high serum levels vaspin and increase of vaspin mRNA expression in adipose tissue was associated with obesity, insulin resistance and type 2 diabetes mellitus in humans. However, the mechanism of vaspin secretion in correlation with the impairment of glucose metabolism and insulin sensitivity until now is still unclear, as well as the molecular target of vaspin. In addition to the adipose tissue, vaspin is also expressed in the skin, hypothalamus, pancreas and stomach. Vaspin treatment on obese mice could improve glucose tolerance, insulin sensitivity and decrease appetite.[8]

Vaspin has the effect of increasing insulin sensitivity in obese conditions. It is presumed that while there is an increase in fat mass, adipocytes will produce high proinflammatory cytokines causing insulin resistance, with the result an increase in blood glucose levels. Vaspin will act as an inhibitor of protease enzyme in the hormone of blood glucose regulation. The enhancement of vaspin synthesis is a mechanism to inhibit the protease enzyme that reduces the action of insulin. This statement proven by the treatment with recombinant vaspin in rats, could suppress the expression of multiple genes of adipocytokines such as leptin, resistin and Tumor Necrosis Factor (TNF)-α, adipocytokines which cause insulin resistance in peripheral tissues. Treatment with vaspin also increases the expression of glucose transporter 4 (Glut-4) and adiponectin, which improves insulin resistance.[21] Therefore, from this study it was presumed that the condition of obesity, which shows fat tissue enhancement, will increase the risk of insulin resistance and cause increase in blood glucose levels. The increase of blood glucose levels leads to the enhancement of vaspin secretion by adipose tissue, as a compensatory mechanism to improve insulin sensitivity. Unfortunately, although significant, the correlation test of our results about vaspin level with fasting blood glucose level in this study was poor and insulin sensitivity index had not been measured yet. Further study is needed to clarify.

CONCLUSIONS AND SUGGESTIONS

The results of these study showed that there was a significant positive correlation between vaspin levels and fasting blood glucose levels in obese conditions, but the correlation strength was poor. Further studies are needed by measuring the insulin sensitivity index, to clarify the relationship between vaspin, as adipocytokines play a role in insulin sensitivity, to blood glucose levels in obese people.

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