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Association between insulin, glucagon, high sensitive c-reactive protein, insulin resistance with visceral adipose tissue-derived serine protease inhibitor (VASPIN) in obese population

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ABSTRACT

Background: Obesity is an inflammatory condition that results in an increase in high sensitive C-Reactive Protein (hs-CRP), a risk for insulin resistance (IR). Vaspin has the effect of increasing insulin receptor sensitivity and on the other hand is a compensatory response to insulin resistance, one of which is measured by homeostasis models assessment-insulin resistance (HOMA-IR). This study was an analytical cross-sectional observational design, to determine the association between insulin, glucagon, CRP, and HOMA-IR levels with serum vaspin in obesity.
Method: This study is an analytical study with a cross-sectional design. Criteria of inclusion were subjects in the range 18-60 years Denpasar citizen with BMI above 25 kg/m^2 and willing to be involved in this study, while exclusion criteria were with either chronic conditions or acute inflammation such as infection, cancer, tumor, or chronic disease which is altered and threatening life. Correlation tests using spearman analysis.
Results: From 131 obese subjects in this study, there were 72 (55%) men and 59 (45%) women. Median age: 33 (22-57) years, fasting insulin: 8.90 (2.20-74.50) mIU/mL, glucagon: 48.10 (7.58-293.90) pq/mL, hs-CRP: 1.90 (0.20-9.50) mg/L, HOMA-IR: 2 (0.50-23.15), and vaspin levels: 0.16 (0.04-1.46) ng/mL. There was a significant positive correlation between HOMA-IR, insulin and glucagon with vaspin ($r = 0.20$, $p = 0.020$; $r = 0.20$, $p = 0.022$; and $r = 0.28$, $p = 0.001$), and a significant negative correlation between hs-CRP and vaspin ($r = -0.24$, $p = 0.005$) in obesity. In the path analysis, there was a direct and total negative effect of hs-CRP on vaspin (coefficient = -0.19; $p = 0.027$ and coefficient = -0.02; $p = 0.038$).
Conclusion: This study shows a positive association between insulin, glucagon, and HOMA-IR with serum vaspin, while CRP is negatively associated with serum vaspin in obesity.

Keywords: insulin, glucagon, CRP, HOMA-IR, vaspin, obesity.
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INTRODUCTION

As known, obesity increases the risk of cardiometabolic diseases, such as type 2 diabetes mellitus (T2DM), cancer, and cardiovascular disease (CVD).[1] Visceral adipose tissue-derived serine protease inhibitor (vaspin) is a new type of adipokine specifically expressed by the Otsuka Long-Evans Tokushima Fatty rat (OLETF), belonging to the serine protease inhibitor class. Vaspin has the effect of increasing insulin receptor sensitivity, on the other hand as a compensatory response to insulin resistance.[2]

Elevated serum vaspin levels are associated with obesity, impaired insulin sensitivity, and fitness. Serum vaspin was also significantly correlated with leptin levels and body fat mass. Meta-analysis studies showed that serum vaspin levels were higher in obese subjects than in non-obese controls, and significantly lower in long-diagnosed T2DM than newly diagnosed T2DM.[3] Serum vaspin was reported to be significantly lower in men with metabolic syndrome compared without.[4] Serum vaspin levels were found higher in diabetic female patients compared to the normal glucose tolerance (NGT) group.[5] The hypothesis explained vaspin influences the insulin signalling pathway and inflammation-related pathways to improve insulin resistance (IR) in peripheral tissues remains unknown and controversial. This study aims to determine the association between insulin, glucagon, inflammation showed by the level of hs-CRP, and HOMA-IR with vaspin in obesity subjects.

METHOD

This study is an analytical study with a cross-sectional design. This study was held in Prof. I.G.N.G. Ngoerah hospital in 2020

by consecutive sampling. Samples were collected from the diabetic centre clinic Prof. I.G.N.G. Ngoerah Hospital Denpasar and Prodia laboratory.

The sample involved in this study were subjects who fulfilled inclusion and exclusion criteria. Criteria of inclusion were subjects in the range 18-60 years Denpasar citizen with BMI above 25 kg/m^2 and willing to be involved in this study, while exclusion criteria were with either chronic conditions or acute inflammation such as infection, cancer, tumor, or chronic disease which is altered and threatening life. Totally 131 samples was collected.

Body mass index (BMI) is a measure of fatness performed on adults whose physical developments were relatively stable. BMI=weight (kg)/[height(m)]^2. The collected blood specimens from the patients were placed in a refrigerator for about an hour at 4°C with an angle of 45°–60°. Serum specimens were obtained by low-speed centrifugation at 3000rpm/min for 5min and stored at –20°C. The vaspin level was detected using an enzyme-linked immunosorbent assay (ELISA) kit Human/Mouse/rat SERPINA12/Vaspin (Competitive EIA) kit" -LifeSpan BioSciences, Inc.

This research has been obtained and approved. The letter of ethical negligence of the research ethics commission Udayana University Faculty of Medicine/Prof. I.G.N.G. Ngoerah Hospital Denpasar and a formal letter of the permission obtained from the education & research division Prof. I.G.N.G. Ngoerah Hospital Denpasar. All the subject information collected from the medical record will be kept confidential. The study was approved by the local Institutional Ethics Committee number 2990/UN14.2.2VII.14/LP/2019

This study used univariate and bivariate analysis. Numeric data such as age were tested using the normality test (Kolmogorov-Smirnov). Numeric data were presented using means and standard deviations (SD) and interquartile range (IQR). Normality and frequency test was used as univariate analysis to describe the characteristic and proportion of the sample, and determine the type of test used in bivariate analysis. Correlation tests using spearman and path analysis were used to describe the association between insulin, glucagon, hs-CRP, and HOMA-IR levels with serum vaspin in the obese population.

RESULTS

The characteristic of this study is described in Table 1; the median age is 33 years old (22-57). The ratio gender is nearly 1: 1 female and male in the sample 72 (55%) males and 59 (45%) females.

Spearman analysis showed that serum vaspin positively correlated with HOMA-IR, insulin, glucagon, and a negative correlation with hs-CRP.

The multivariate analysis result shows a direct and total negative effect of hs-CRP on vaspin (coefficient = -0.19; p = 0.027 and coefficient = -0.02; p = 0.038), while the others are not statistically significant.

The relationship pattern of several independent variables with vaspin in obesity subject detailed as below (figure 1).

DISCUSSION

Vaspin has shown beneficial effects in normal plasma glucose levels and regulates the expression of genes involved in the pathogenesis of insulin resistance, such as resisting leptin, TNF, glucose transporter 4, and adiponectin. Vaspin is an insulin sensitizer with anti-inflammatory effects and may act as a compensatory mechanism targeting white adipose tissue (WAT), activated when insulin sensitivity decreases due to insulin resistance.[2,6] Nakatsuka *et al.*, found that vaspin is a novel ligand for the GRP-78/MTJ-1 complex cell surface, and the subsequent signalling on endoplasmic reticulum (ER) stress-induced metabolic dysfunction.[7] Study conducted by Heiker *et al.*, states increasing serum vaspin is related to human's obesity and insulin resistance.[8] In obese subject with IR condition having risk to increase insulin secretion (hyperinsulinemia) and as equal with the secretion of glucagon, insulin and glucagon associated with vaspin. In an animal study, vaspin may improve the

Table 1. Participant Characteristics at Enrolment (n=131).

Variable	Mean	Standard Deviation	Median	Interquartile range	Min	Max
Age (years)	36.05	8.43	33	12	22	57
Triglyceride (mg/dL)	146.31	95.93	122	79	38	506
HDL (mg/dL)	44.72	8.83	44	12	18	73
SBP (mmHg)	119.01	10.73	120	10	100	160
DBP (mmHg)	77.939	6.98	80	10	60	100
FPG (mg/dL)	96.13	14.39	94	9	76	213
WC (cm)	99.02	8.76	98	12	81	127
Height (cm)	164.04	8.32	165	13	146	184
Weight (kg)	82.64	13.85	79.1	18	60	130
BMI (kg/m^2)	30.61	3.78	30.04	5	24	46
Insulin (mIU/mL)	10.39	7.91	8.9	7.9	2.2	74.5
HOMA-IR	2.49	2.30	2	1.75	0.5	23.15
CRP (mg/L)	2.58	2.08	1.9	2.7	0.2	9.5
Glucagon (pq/mL)	60.36	46.12	48.1	44.89	7.58	293.9
Vaspin (ng/mL)	0.24	0.26	0.16	0.16	0.04	1.46

BMI: Body Mass Index ; CRP: *C-Reactive Protein;* DBP: Diastolic Blood Pressure; FPG: Fasting Plasma Glucose; HDL: *High-Density Lipoprotein;* HOMA-IR: *Homeostasis Model Assessment-Insulin Resistance*; SBP: Systolic Blood Pressure; Vaspin: *Visceral Adipose tissue-derived Serine Protease Inhibitor*; WC: Waist Circumference.

insulin resistance of rats by activating the IRS/PI3K/Akt/Glut signalling pathway and inhibiting the IκBα/NF-κB inflammatory pathway.[9] Vaspin seems to act as an anti-inflammatory to increase insulin sensitivity and improve IR in obesity.

Bivariate analysis of this study showed that obesity has a significant positive correlation between HOMA-IR, insulin, and glucagon with vaspin, and a significant negative correlation between hs-CRP with vaspin (r = -0.24; p = 0.005). In obesity, there is an increase in insulin as a compensatory response to IR and an increase in glucagon due to the resistance of pancreatic alpha cells induced by obesity and the secretion of the hormone somatostatin by the pancreatic delta cells in obesity. Obesity also inhibits paracrine effects on pancreatic alpha cells (paracrinopathy). Insulin and glucagon were positively associated with vaspin, and in turn the vaspin acts as an anti-inflammatory to ameliorate the IR, which was due to the negative association of hs-CRP with vaspin. As right with the previous study revealed vaspin has a positive correlation with insulin, HOMA-IR, hs-CRP statically significantly, which provides support to the mentioned compensatory mechanism of vaspin overexpression in human adipose tissue in IR condition.[10]

This study investigated the relationship of several variables with vaspin. Path analysis showed in obesity, there was a direct and negative total effect of hs-CRP with vaspin. It consistently shows that vaspin seems to act as an anti-inflammatory to ameliorate IR that occurs in obesity.

Other studies have found a relationship between various variables with vaspin. Feng *et al.,* stated that serum vaspin levels were significantly higher in obese subjects and type 2 diabetic patients, significantly lower in longer duration T2DM patients than in newly diagnosed T2DM patients.[3] Kim *et al.,* found that serum vaspin concentrations were significantly lower in men than women, and especially men with metabolic syndrome, negatively correlated with waist circumference, systolic and diastolic blood pressure, and serum triglyceride levels, however it is positively correlated with HDL cholesterol levels.[4] While Esteghamati *et al.,* found that vaspin levels were associated with metabolic syndromes such as triglyceride levels, high glucose levels and obesity.[11] Yang *et al.,* studied serum vaspin levels in patients with normal glucose regulation and in patients newly diagnosed with type 2 diabetes and explored the relationship between vaspin and body mass index, age, sex, lipid metabolism, and insulin sensitivity.[12] Mean serum vaspin levels were higher in obese patients than in non-obese patients in the DM and control groups. Association analysis showed that serum vaspin levels were significantly associated with body mass index, waist-to-hip ratio (WHR), fat percentage, triglycerides, and insulin sensitivity index.

This study has several limitations such as 1) several other variables that influence vaspin, such as genetics, other hormones, mitochondrial dysfunction and free radicals are not calculated and analyzed in this study ; 2) this study uses circulating or serum vaspin levels and may not capture mRNA expression in adipose tissue; and 3) another bias activity of receptors, cofactors, or other proteins was not examined in this study due to the limited-resource setting.

Table 2. Correlation between Insulin, Glukagon, Hs-CRP, HOMA-IR with Vaspin in Obese Subject.

Variable	r	P
Insulin	0,20	0,022*
Glucagon	0,28	0,001*
Hs-CRP	-0,24	0,005*
HOMA-IR	0,20	0,020*

*significant p<0,05

Hs-CRP: high sensitive *C-Reactive Protein;* HOMA-IR: *Homeostasis Model Assessment-Insulin Resistance.*

CONCLUSION

This study shows a positive association between insulin, glucagon, and HOMA-

Table 3. Association between other factors with vaspin in obese subject.

Variable	Direct		Indirectly association		Total effect	
	Coefficient	p	Coefficient	p	Coefficient	P
CRP	-0,19	0.027*	0,00	0,486	-0,02	0,038*
SBP			0,00	0,518	0,00	0,518
Triglyceride	0,05	0,642	0,00	0,461	0,00	0,482
HDL	0,03	0,775	-0,00	0,683	0,00	0,800
FPG	0,02	0,811	0,00	0,500	0,00	0,734
Insulin	0,07	0,469	0,00	0,705	0,00	0,450
Glucagon	0,09	0,280			0,00	0,282
Age			0,00	0,150	0,00	0,150
Sex			-0,04	0,209	-0,04	0,209
WC			0,00	0,712	0,00	0,712
BMI			-0,01	0,123	0,01	0,123

*significant p<0,05

BMI: Body Mass Index; CRP: *C-Reactive Protein;* FPG: Fasting Plasma Glucose; HOMA-IR: *Homeostasis Model Assessment-Insulin Resistance*; SBP: Systolic Blood Pressure; WC: Waist Circumference.

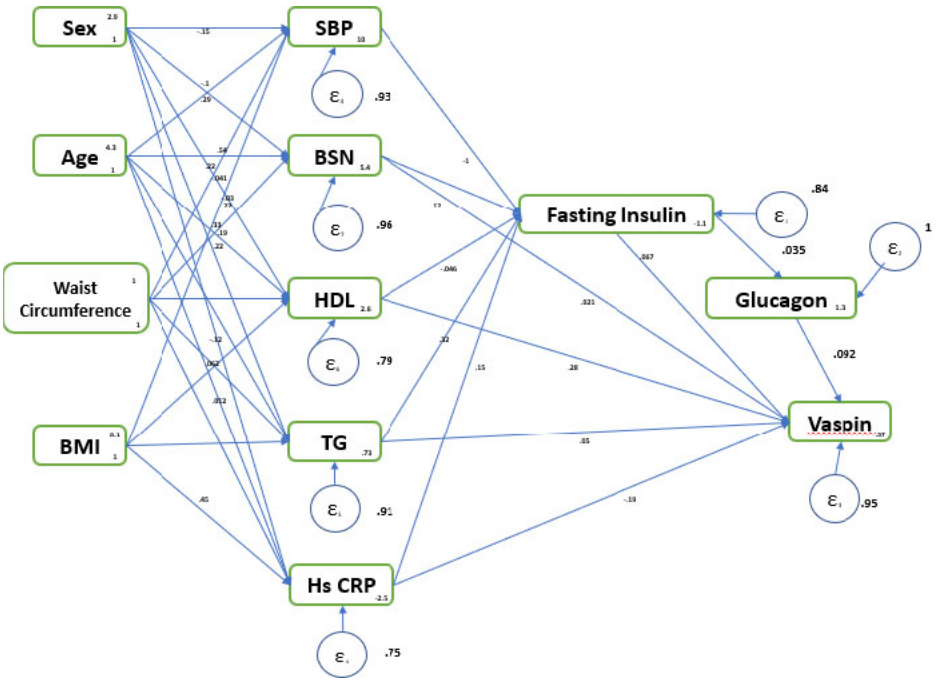

Figure 1. Path Analysis of another factor related to Vaspin.

IR with serum vaspin, while hs-CRP is negatively associated with serum vaspin in obesity.

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AUTHOR CONTRIBUTIONS

All named authors had full access to all the data in this study and took complete responsibility for the integrity of the data and the accuracy of the data analysis.

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None.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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