

Positive correlation between retinol binding protein 4 (RBP4) and triglyceride level in central obesity

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Abstract. Obesity has become an epidemic in both developed and developing countries. Central obesity considered a risk factor that is closely related to several chronic diseases. Central obesity is associated with elevated triglyceride levels and associated with RBP4 which can lead to insulin resistance. Increased level of RBP4 can cause lipid metabolism disorders and can become a marker for insulin resistance and metabolic syndrome. This study aims to find the correlation of RBP4 with triglycerides and Apo B100 in central obesity. It was a cross-sectional study on 46 subjects with central obesity, aged 20-50 years old. Blood samples were taken in cubital vein and examined for RBP4 and triglyceride levels. Data analysis was performed using Spearman correlation test. The results showed that gender frequency distribution showed little difference between men and women, i.e., men 43.5% and women 56.5%. RBP4 level was positively correlated with triglyceride ($r = 0.48$) and statistically significant ($p = 0.001$). The rbp4 level was positively correlated with triglyceride, indicating the role of RBP4 on high triglyceride level in central obesity.

1. Introduction

Obesity has become an epidemic in both developed and developing countries.[1] Obesity is very closely related to metabolic syndrome, technology development and increased economic status in Western countries in the last century, leading to lifestyle changes that eventually increased the incidence of metabolic syndrome that threatens developing countries.[2,3] In many East Asian countries such as Indonesia, the nutritional problem is a double burden, on the one hand, there are malnutrition and body weight below normal, on the other hand, there are epidemic attacks of obesity, diabetes, and other diseases related to excessive nutrients.[4]

Obesity is a disorder of the weight regulation system which is characterized by the accumulation of excess body fat [5], food imbalance and low physical activity developed obesity; other factors are vitamin D deficiency.[6-8] Based on the fat distribution, central obesity and general obesity are classifications of obesity.[9]

In the past, adipose tissue was thought to be the reservoir of energy, but now adipose tissue is considered as an active endocrine organ that releases numerous active mediators (adipokine) that are useful for homeostasis of fat and glucose metabolism, blood pressure, inflammation, and



atherosclerosis. One of the active mediators that are released is Retinol Binding Protein 4 (RBP4).[10] RBP4 is a protein stored in the liver and also in the adipocyte tissue of about 20% compared to the liver and released into the circulation.[11]

Retinol-binding protein 4 (RBP4) is a transport protein for vitamin A (retinol) secreted by the adipose and liver tissue.[12] Retinol Binding Protein 4 can work through the mechanism of retinol dependent or retinol independent.[13] In previous studies, RBP4 was a marker for insulin resistance and metabolic syndrome.[14] Retinol-binding protein 4 (RBP4) stimulates the expression of Sterol regulatory element binding protein 1 (SREBP-1) gene in the fatty acid/triglyceride (TG) biosynthesis pathway.[15] Some studies suggest that there is a relationship between the increased circulation of RBP4 with adiposity [16-19], proving that there is an increased level of RBP4 in metabolic syndrome.

At the moment, studies on the correlation of RBP4 with triglyceride level in central obesity in Indonesia are still underway, so that the researchers intend to know further about the correlation of RBP4 with the level of triglycerides and Apo B100 in central obesity in Medan, North Sumatra Province, Indonesia. Therefore, there is a need to perform research to analyze the correlation of RBP4 with triglyceride level in central obesity.

2. Method

2.1. Subjects

This study was a cross-sectional study that aims to see the correlation of RBP4 with triglyceride level in central obesity. Research subject recruitment was at the educational institution of 'Siti Hajar' College of Health Sciences and Faculty of the Medicine-Islamic University of North Sumatera. Data collection was from November 2015 to January 2016.

The study population was male and female, aged 20-50 years old, selected by purposive sampling technique and in writing stated willingness to participate in the research, and had signed the subject approval sheet. The inclusion criteria were subjects with central obesity with waist circumference ≥ 90 cm for men and ≥ 80 cm for women. Exclusion criteria were pregnant and lactating based on anamnesis and urine test results if presumed to be pregnant. All participants were in writing informed consent forms approved by the Ethics Committee of Medical Faculty and Haji Adam Malik Hospital, Universitas Sumatera Utara, Indonesia, Number: 557/KOMET/FK USU/2015.

2.2. Measurement of Waist Circumference

Waist circumference was in centimeters by using a measuring tape. The waist circumference was in the abdomen in the middle between the lower coastal area and the iliac crest (the crest of the pelvic bone), where the patient stands with feet 25-30 cm apart. It was at the end of expiration. The tool used is a non-electric meter with the brand Butterfly.

2.3. Laboratory Analysis

RBP4 examination conducted after the patient fasted for approximately 10-12 hours. Sampling was done by taking venous blood with the amount of 3 ml and put into the SST tube. The tube was labeled (lab number, patient name, and sample type). Flip the tube slowly six times until homogeneous. The serum is stored in temperature $(-15) - (-25) ^\circ\text{C}$ until examination (3 months). RBP4 examination used the Enzyme-Linked Immunosorbent Assay (ELISA) tool, while the triglyceride examination used the equipment.

2.4. Statistical Analysis

Data were analyzed using version 11.5 of the IBM-SPSS statistical program (IBM Corp., Chicago, IL). Categorical variables were as percentages. Continuous, normally distributed variables were as mean \pm SD; and non-normally distributed continuous variables were as median (minimum-maximum). If the data is normally distributed, Pearson correlation analysis will be used, however, if it is not normally distributed then the Spearman correlation analysis is used.

3. Results and Discussion

This study reported that the age frequency distribution showed age ≤ 32 years and >32 were the same in amount, which was 50%. The distribution of gender frequency does not differ much between male and female, which was 43% and 56.5% respectively, as presented in **Table 1**.

Table 1. Demographic data of the subject based on age and gender.

Characteristics	n	%
Age (years)		
≤ 30	23	50.0
>30	23	50.0
Gender		
Male	20	43.5
Female	26	56.5

Normality test result showed the data for waist circumference was not normally distributed, with a mean of 99.48 cm, minimum 81 cm, and maximum 134 cm. Normality test result showed data for Apo B100 was normally distributed, with a mean of 103.63 mg/dL, with the minimum level of 68 mg/dL and the maximum of 137 mg/dL, as presented in **Table 2**. From the above data, it indicates a moderate positive correlation between RBP4 and Triglycerides ($r = 0.478$) and statistically significant ($p < 0.005$), also there was a weak positive correlation between RBP4 with Apo B100 ($r = 0.149$) and not statistically significant ($p > 0.005$).

Age is related to changes in body composition. After the age of 20-30 years, Fat-Free Mass (FFM) progressively decreases, where Fat Mass increases. FFM (especially muscle tissue) decreases by 40% starting from 20-70 years.[20-21] An increase in the muscle fat mass that occurs with increasing age can increase energy intake and decrease energy expenditure, where the relationship between energy intake and energy expenditure is an important factor of body fat tissue.[22] Increase in age is also associated with the division between body fat and FFM. With increasing age, there is a large increase in intra-abdominal fat than in subcutaneous or total body fat, and there is a large decrease in peripheral FFM than in central FFM due to skeletal muscle loss.[23]

Table 2. Characteristic data of the subjects based on waist circumference, RBP4, triglyceride level.

Characteristics	Mean	Median	Standard deviation	Minimum	Maximum
Waist circumference (cm)	99.48	99.50	12.32	81.0	134.0
RBP4	30.49	30.03	8.4	16.95	59.08
Triglyceride	132.52	115.0	57.34	55.0	287.0

This study showed that there was not much difference in the frequency distribution of gender between male and female, 43.5% and 56.5% respectively. However, women had a higher percentage of body fat compared to men. There is a difference between the fatty acid metabolism site in men and women. Firstly, catecholamines that mediate the release of free fatty acids are lower in women than in men, where free fatty acids are released from the upper body depots to be comparable. Secondly, free fatty acids released from upper body subcutaneous fats are higher in men than in women, which means there is the high resistance of antilipolytic effects of the food digestive process in the upper body fat storage in men. Thirdly, there is a suggestion that basal fat oxidation (regulating Fat-Free Mass) is lower in women than in men. Therefore women have higher fat stores. Lastly, postprandial fat stores will be higher in the subcutaneous adipose tissue in women than in men.[24]

The upper limit of RBP4 level for normal people has not been established, but the reported RBP4 minimal concentration is 15-30 $\mu\text{g} / \text{mL}$ in healthy individuals without vitamin A deficiency.[25-26]

In 2007, Graham reported the mean of the highest RBP4 concentrations in people with normal glucose tolerance. Using several methods which are sandwich ELISA with the amount of 34 µg/mL (SB: 9), competitive Enzyme-linked immunoassays (EIAs): EIA no.1 of 25 µg / mL (SB: 4) and EIAs no.2 of 34 µg / mL (SB: 5).

Retinol-binding protein 4 (RBP4) relates to visceral fat, and RBP4 can describe the relationship between visceral obesity and cardiovascular diseases, expressed in the positive correlation with triglyceride as presented in **Table 3**. Adipocytes produce and secrete various active mediators (adipocytokine) such as leptin, tumor necrosis factor- α (TNF- α), plasminogen activator inhibitor-1, adiponectin and resistin, and retinol binding protein 4 (RBP4). [14,27,28]

Table 3. Correlation analysis between RBP4 and triglyceride level.

RBP4		Triglyceride
	r	0.478
	p	0.001
	n	46

Using Spearman Correlation test

Adipose tissue and RBP4 secrete retinol Binding Protein 4 (RBP4) is exclusively in mature adipocytes. [30] Although RBP4 is expressed primarily in mature adipocytes, there is a difference in gene expression between two fat stores; this may be affected by higher absorption by macrophages in the viscera compared to subcutaneous. [31] As a result, it is expected that the higher downregulation of RBP4 in visceral fat is due to signals derived from macrophages such as TNF- α . [32]

Obesity-related to the increased prevalence of dyslipidemia. Dyslipidemia is the number of abnormal lipids such as cholesterol and triglycerides, in the blood and is considered a risk factor for cardiovascular disease. Obesity-related to dyslipidemia mainly characterized by an increase in plasma free fatty acid (FFA) and elevated triglycerides, decreased high-density lipoprotein (HDL) and increased low-density lipoprotein (LDL). [33] An increase in free fatty acid (FFA) released from adipose tissue through lipolysis can increase FFA to the liver tissue. Increased FFA will increase triglycerides and Very-Low-Density Lipoprotein (VLDL) in the liver as well as inhibiting lipoprotein in adipose tissue and skeletal muscle, thereby causing hypertriglyceridemia. Triglycerides present in the VLDL are subsequently exchanged for cholesterol ester derived from LDL and HDL, and produce triglyceride-rich LDL and HDL. [34] Limitation of the research is the distribution of RBP4 had a large variant; this caused researchers did not consider exercise habit factor because exercise can reduce the level of RBP4.

4. Conclusions

There is a moderate positive correlation between RBP4 and triglycerides in central obesity ($r = 0.478$), and it is statistically significant ($p < 0.05$).

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