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The levels of *Toxoplasma gondii* profilin and adiponectin in obese patients complicated with or without metabolic syndrome as compared to non-obese patients

Agustin Iskandar[1], Muhammad Rasjad Indra[2], Satuman[2], Novi Khila Firani[3], Titin Andri Wihastuti[4*]

[1]*Department of Parasitology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

[2]*Department of Physiology, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

[3]*Department of Biochemistry, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

[4]*Department of Nursing, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

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ABSTRACT

Objective: To find out the levels of *Toxoplasma gondii* (*T. gondii*) profilin and adiponectin in obese patients complicated with or without metabolic syndrome as compared to non-obese patients.
Methods: This study was an observational analytic study using cross sectional design. After interview, the subjects were performed with a anthropometric test and then a metabolic syndrome panel. The levels of profilin and adiponectin were detected by using ELISA method.
Results: There was a significant difference of *T. gondii* profilin between the obese complicated with metabolic syndrome group and the non-obese group (P = 0.00; α = 0.05), as well as the metabolically healthy obese, in which the level of profilin was significantly higher in the group as compared to the non-obese group (P = 0.001; α = 0.05). Adiponectin level of the obese complicated with metabolic syndrome group was significantly lower as compared to the metabolically healthy obese and non-obese group (P = 0.001; α = 0.05).
Conclusions: The level of *T. gondii* profilin in obese patients was higher than that in the non-obese, whereas the level of adiponectin in obese patients complicated with metabolic syndrome was lower than that in the obese without metabolic syndrome and non-obese patients.

1. Introduction

Metabolic syndrome is a cluster of clinical cardiovascular risk factors including obesity, dyslipidemia and hypertension. This syndrome has a relationship with the pathologic mechanism of cardiovascular diseases. Obesity, a risk factor of metabolic syndrome, generally was abdominal or visceral obesity. The obesity prevalence around the world and its relationship with the metabolic syndrome increase rapidly. In the United States, the prevalence of overweight and obesity combined (body mass index (BMI) ⩾ 25) was 71.1% (95% *CI*: 68.0%–74.2%) among men and 65.5% (95% *CI*: 61.8%–69.3%) among women, and the prevalence of obesity (BMI ⩾ 30) was 33.3% (95% *CI*: 30.5%–36.2%) among men and 35.8% (95% *CI*: 32.3%–39.4%) among women[1]. The number of obesity increases rapidly year by year. It was reported that over 300 million of adults have obesity. In the United Stated, there are 280 000 people passing away every year[Please confirm my revision] as a result of obesity in which it becomes the trigger for some diseases such as heart attack, arthritis, diabetes mellitus type 2 and hypertension[2].

Apicomplexan parasites spur actin-dependent gliding movements, which are very important to invade the host cell. Profilin is a key contributor in actin polymerization. *Toxoplasma gondii* (*T. gondii*) has profilin-like protein which will be recognized by toll-like receptor (TLR-11) of the natural immune system and followed by inflammation of the host cell. The damage to the host cell is known correlated to a gene encoding profilin in *T. gondii* parasites. When profilin does not play a role in the cell growth, this protein will stimulate gliding motility to invade the host cell and cause virulence in mice. Besides, the parasites

*Corresponding author: Titin Andri Wihastuti, Department of Nursing, Faculty of Medicine, Brawijaya University, Malang 65112, East Java, Indonesia.
Tel: +62 81805101827
Fax: +62 341 564755
E-mail: wihastuti.fk@ub.ac.id

The study protocol was performed according to the Helsinki declaration and approved by Health Research Ethics Comittee of Medical Faculty of Brawijaya University with the number of 54/EC/KEPK/ 02/ 2012, and the informed written consent was obtained from the patients.

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which do not have profilin are not able to induce TLR-11 to produce interleukin (IL)-12 (cytokine defense of the host cell) both *in vivo* and *in vitro*. Thus, profilin is an important element of the two aspects of *T. gondii* infection. Profilin plays a role in motility when the ligand of the natural immune system is recognized by the microbial host cell[3].

Emerging researches on etiology of obesity has investigated the potential role of environmental infections, a concept referred to as "infectobesity", as well as gut microbiota in obesity pathogenesis. An understudied pathogen of the potential interest in obesity researches is the protozoan parasite *T. gondii*. Experimentally, in rats, *T. gondii* infection was associated with significant weight gain after 30 days of inoculation followed by weight loss over the next 60 days. The authors hypothesized that weight gain may have been due to direct central effects, *i.e.*, behavioral changes, such as increased food intake associated with *T. gondii* cysts in the brain and/or indicated central effects, *e.g.*, altered hypothalamic function (*e.g.*, appetite regulation) caused by peripheral tissue inflammation. Weight related effects of *T. gondii* infection may be influenced by strains. In another animal study, two different strains of *T. gondii* had opposite effects on body weight[4].

Reeves *et al.*, reported that individuals who were positive for *T. gondii* immunoglobulin G had approximately twice the odds of being obese as compared to seronegative individuals, but they were unable to determine if there is a causal relationship between *T. gondii* seropositivity and obesity[5]. However, how the changes in the quality of adipocytes in adipose tissue after exposure to the pathogen is still very little known. Besides, the prevalence of infectious diseases in developing countries is still high and it is mentioned that there is a relationship between infection and obesity. The study conducted by Susanto *et al.*[6] resulted that the exposure of *T. gondii* profilin on subcutaneous fat cell cultures can raise the levels of IL-6 and tumor necrosis factor-alpha (TNF-α) as well as lower the level of TLR-11, and the increase of the levels of IL-6 and TNF-α in the subcutaneous fat cells indicates the occurrence of adiposopathy and metabolic syndrome caused by *T. gondii* profilin infection.

The study conducted by Iskandar *et al.*[7] states that there is a significant difference between the level of profilin of the obese and healthy individuals. The study also mentions that the increase of profilin level in obese individuals is associated with the increase of IL-6 and IL-12 as well as inflammatory cytokines in obese individuals. Although the correlation is weak, it suggests that in obese individuals there are increases of the level of profilin triggering increases in inflammatory cytokines such as IL-6 and IL-12, which is an early marker of adipocyte dysfunction in obese individuals.

Adiponectin is an anti-inflammatory cytokine which is secreted exclusively by adipocytes in large quantities in plasma. Adiponectin, which is mainly produced in white adipose tissue, characteristically differs from most adipokines as it is negatively correlated with obesity. In healthy people, adiponectin plays a role in preventing the development of vascular changes and the failure of glucose and fat metabolism induced by various factors such as chemicals, excessive eating and so on. The physiological role of adiponectin has not yet been fully elucidated, but it is believed that it has the ability to reduce glucose, triglycerides, and free fatty acids and it plays a major role in the pathogenesis of metabolic syndrome. Adiponectin has a role in increasing the sensitivity of insulin receptors, *i.e.* as anti-inflammatory and anti-atherogenic. On the state of obesity and insulin resistance, the level of adiponectin decreases. While in the state of weight loss, there is an increase of the level of adiponectin. The increase and decrease of the level of adiponectin are influenced by various factors such as TNF-α and IL-6[8].

This study aimed to investigate the basic mechanism of metabolic syndrome pathogenesis, the role of *T. gondii* parasite infection, as well as determine the relationship between profilin increase and adiponectin as anti-inflammatory adipocytokine in obese individuals with or without metabolic syndrome.

2. Materials and methods

2.1. Ethical clearence and informed concent

The study protocol was performed according to the Helsinki declaration and approved by Health Research Ethics Comittee of Medical Faculty of Brawijaya University with the number of 54/ EC/KEPK/ 02/ 2012, and the informed written consent was obtained from the patients.

2.2. Study protocal

The research was conducted from May 2012 to September 2012. Sampling was collected consecutively, *i.e.* the obese patients who came to the central laboratory of Saiful Anwar Hospital Malang, from May to September 2012.

A patient is called an obese one if his/her BMI is greater than or equal to 27 in accordance with the criteria of World Health Organization-Western Pacific Region which are suitable for people of Asia, including Indonesia. The criteria for metabolic syndrome is taken from the National Cholesterol Education Program Adult Treatment Panel in 2001 in which there are at least three main features: the abdominal circumference is over 102 cm in men and more than 88 cm in women; the triglyceride blood level is over 150 mg/dL; the high-density lipoprotein (HDL) cholesterol is lower than 40 mg/dL in men and 50 mg/dL in women; the blood pressure is above 130/85 mmHg; and the fasting blood sugar is over 110 mg/dL.

The anthropometric examination, blood glucose, HDL cholesterol, total cholesterol and triglycerides were done in the central laboratory of Saiful Anwar Hospital Malang by using enzymatic method.

The level of *T. gondii* profilin and adiponectin were measured in the Molecular Physiology Laboratory of Medical Faculty of Brawijaya University by using ELISA method.

2.3. Processing and data analysis

The entire technical data processing were analyzed in computer software with Lavene's test and then followed by *t*-test and correlation test using software statistical product and solution service 17 PS (15 PS SPSS) ($P < 0.05$).

3. Results

3.1. Sample characteristics

The majority of the samples were from female (56 patients, 83.3%), while the male samples were only 11 patients (16.7%). The characteristics of the subjects can be seen in Table 1.

Table 1

The characteristics of the subjects.

Variable	Obese with MS	Obese without MS	Non-obese	*P*
BMI	32.77	31.79	21.81	0.00
Waist circumference (cm)	101.27	100.90	83.40	0.00*
Systolic pressure (mmHg)	145.00	125.00	110.00	0.00*
Diastolic pressure (mmHg)	93.00	85.00	72.00	0.00
HDL cholesterol (mg/dL)	40.00	41.00	55.00	0.00
Triglyceride (mg/dL)	221.00	126.00	99.00	0.00*
Fasting blood glucose (mg/dL)	111.00	76.00	79.00	0.118*

Data are means of the measurements of the 67 patients; MS: Metabolic syndrome; *: Kruskal Wallis test.

The results (as shown in Table 1) showed that the BMI, waist circumference, systolic pressure, diastolic pressure, trigliceride and fasting blood glucose in the obese patients complicated with metabolic syndrome were higher as compared to obese without metabolic syndrome and non-obese subjects. In contrast with those, the HDL cholesterol in the obese complicated with metabolic syndrome was lower than those without metabolic syndrome and the non-obese. The ANOVA or Kruskal Wallis test showed the significant differences on all the variables except the fasting blood glucose.

3.2. The level of profilin in obese individuals with or without metabolic syndrome

The results of the study on 67 obese subjects showed that the average level of profilin was significantly different as compared to the non-obese. The mean of the profilin level in obese individuals with metabolic syndrome was 2.5 ng/mL, while in obese individuals without metabolic syndrome was 2.1 ng/mL and in non-obese subjects was 1.5 ng/mL.

The results of statistical analysis by using SPSS 17.0 showed that data distribution was not normal (Kosmogorov-Sminov test, P = 0.00), so we used Kruskal Wallis test. The result of Kruskal Wallis test then followed by Mann-Whitney U test showed that there was significant difference of the *T. gondii* profilin level between the metabolic syndrome obese group and the non-obese group, and the level of profilin in this group was significantly higher as compared to non-obese group (P = 0.00; α = 0.05). Based on the statistical analysis, it was found that there was no significant difference in the level of *T. gondii* profilin between the obese subjects complicated with metabolic syndrome and those without metabolic syndrome (P = 0.107; α = 0.05).

3.3. The level of adiponectin in obese individuals with or without metabolic syndrome

The results of the study showed that the mean of adiponectin in obese patients complicated with metabolic syndrome was lower than that in those without metabolic syndrome (21.07 μg/L *vs.* 42.25 μg/L) and so is the non-obese patients (48.05 μg/L).

The Kruskal Wallis test followed by Mann-Whitney U test showed that there was significant difference in the level of adiponectin between the obese subjects complicated with metabolic syndrome and those without metabolic syndrome (P = 0.00; α = 0.05). The level of adiponectin in obese subjects with metabolic syndrome was significantly lower than that of the non-obese subjects (P = 0.00; α = 0.05). The statistical analysis also showed that there was no significant difference in the level of adiponectin between the obese group without metabolic syndrome and the non-obese group (P = 0.355; α = 0.05).

The Spearman correlation test was used to determine whether there was a significant correlation between the levels of adiponectin and profilin. It showed that there was a significant correlation between the level of profilin and the level of adiponectin (P = 0.005, r = -0.36). The Spearman correlation test result also showed the correlation coefficient value (-0.36) meant that 36% of the level of adiponectin was affected by the level of profilin. The negative correlation coefficient meant that the higher level of profilin was, the lower level of adiponectin became.

4. Discussion

The majority of the samples were from female (56 patients, 83.3%), while the male samples were only 11 patients (16.7%). This is likely due to the high prevalence of obesity in women. The Riskesdas report in 2013 stated that the prevalence of obesity in women in age up to 18 years is 32.9% whereas the prevalence in men also in age up to 18 years is 19.7%. For the Malang City, the Riskesdas survey also showed that as many as 17.8% of the population have obesity and 68% of them are women[9].

For the metabolic syndrome panel, the result showed that all of the metabolic syndrome panel increased in the obese complicated with metabolic syndrome, except for HDL. It can be explained by the fact that the increased fasting plasma triglycerides, high low density lipoprotein cholesterol, low HDL cholesterol, elevated blood glucose and insulin levels and high blood pressure are risk factors of cardiovascular diseases which can be developed in obesity. All these lipid abnormalities are typical features of the metabolic syndrome and may be associated to a pro-inflammatory gradient which in part may originate in the adipose tissue itself and directly affect the endothelium. An important link between obesity, the metabolic syndrome and dyslipidemia seems to be the development of insulin resistance in peripheral tissues leading to an enhanced hepatic flux of fatty acids from dietary sources, intravascular lipolysis and from adipose tissue resistant to the antilipolytic effects of insulin[10].

The results also showed that the *T. gondii* profilin level in obese subjects complicated with metabolic syndrome was significantly higher as compared to the non-obese subjects, but there was no significant difference in the level of *T. gondii* profilin between the obese subjects complicated with metabolic syndrome and those without metabolic syndrome. This might be due to the excessive fat deposits shown in the obese subjects. The occurrence of infection by *T. gondii* would increase the expression of profilin required to the invasion of parasites on the host cells, including fat cells. The

bond of profilin-like protein with TLR-11 would further increase the expression of pro-inflammatory cytokines leading to increase in the inflammation in adipocytes and causing adiposopathy and obesity. There was no significant differences between metabolic syndrome subject and non-metabolic syndrome subject allegedly caused by a role of profilin in the pathogenesis of metabolic syndrome.

The pathogenesis of metabolic syndrome has still developed until now. One factor believed to play a role is insulin resistance[10]. There are many factors other than insulin playing a role in the pathogenesis of metabolic syndrome. The results of this study indicate that it still needs further studies about profilin involvement in the pathogenesis of metabolic syndrome.

Adiponectin (also called ACRP30, AdipoQ, apM1 and GBP28) is a hormone peptide with 247 amino acids found in 1995. Adiponectin is induced in the early differentiation of fat cells (adipocytes), consisting of a collagen with N terminal and globular domain with terminal C, and has a structure which is homologous with C1q complement factor subunit[8,11]. Contrary to other hormones derived from the adipose tissue, adiponectin circulates with relatively high concentrations in the bloodstream accounting for 0.05% of the total serum protein and is inversely related to obesity, insulin resistance, type 2 diabetes mellitus and cardiovascular diseases. The plasma concentration of adiponectin has been shown to correlate strongly with insulin sensitivity, which suggests that a low concentration is related to insulin resistance[12]. In our study, obese individuals complicated with metabolic syndrome have higher adiponectin levels as compared to those without metabolic synrome. It can be said that a high level of adiponectin was therefore a notable protective factor for the development of metabolic syndrome among obese individuals.

The results of this study support the previous studies that adiponectin is inversely related to obesity and insulin resistance. In non-obese subjects, it was found that the level of adiponectin was significantly higher as compared to those obese subjects complicated with or without metabolic syndrome.

The Spearman correlation test showed that the higher the level of profilin was, the lower the level of adiponectin became. This is consistent with the previous studies showing that adiponectin is inversely related to the degree of obesity. Adiponectin is said to be anti-inflammatory adipocytokine providing protection against either infections or adipocytes from chronic inflammation. The high levels of *T. gondii* profilin in this study showed that *T. gondii* profilin causing inflammation in adipocytes resulted in the decrease of the level of adiponectin.

Similarly, in case of metabolic syndrome, the higher the level of profilin is, the higher the degree of obesity is and the lower the level of adiponectin becomes. Thus, it could be said that the level of adiponectin which was low in obese subjects complicated with metabolic syndrome was caused by the high level of *T. gondii* profilin. Further research is needed to determine other factors affecting the low level of adiponectin and high level of profilin in obese individuals with or without metabolic syndrome.

There is a significant difference in the level of *T. gondii* profilin between obese individuals complicated with metabolic syndrome and the non-obese individuals. There is a significant difference in the level of adiponectin between obese individuals complicated with metabolic syndrome and obese individuals without metabolic syndrome. There is also a significant relationship between the increasing level of *T. gondii* profilin and the decreasing level of adiponectin in obese individuals.

Conflict of interest statement

We declare that we have no conflict of interest.

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