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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2016.11.017>The level of chemerin and adipocyte fatty acid binding protein in *Toxoplasma gondii* seropositive obese individualsAgustin Iskandar^{1,2*}, Karomah Sriwedari², Indah Adhita Wulanda², Muhammad Rasjad Indra³, Hartojo², Novi Khila Firani⁴, Ery Olivianto⁵¹Department of Parasitology, Medical Faculty of Universitas Brawijaya, Malang, East Java, Indonesia²Department of Clinical Pathology, Medical Faculty of Universitas Brawijaya, Malang, East Java, Indonesia³Department of Physiology, Medical Faculty of Universitas Brawijaya, Malang, East Java, Indonesia⁴Department of Biochemistry, Medical Faculty of Universitas Brawijaya, Malang, East Java, Indonesia⁵Department of Child Health, Medical Faculty of Universitas Brawijaya, Malang, East Java, Indonesia

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ABSTRACT

Objective: To know the difference between chemerin and adipocyte fatty acid-binding protein (AFABP) levels in obese individuals with positive *Toxoplasma gondii* (*T. gondii*) immunoglobulin G (IgG) compared with negative *T. gondii* IgG.**Methods:** This study is a cross-sectional study by using consecutive sampling methods conducted from January to April 2013. The subjects were 57 obese individuals who were divided into obese group of positive and negative *T. gondii* IgG. The level of chemerin, AFABP and *T. gondii* IgG was done by ELISA. The data were analyzed by independent *t* test.**Results:** The results showed that the level of chemerin of positive *T. gondii* IgG group was significantly higher than the negative *T. gondii* IgG group [(70.0 ± 16.5) vs. (64.4 ± 16.1) pg/mL; *P* = 0.003], but there was not significant AFABP difference between seropositive and negative IgG groups [(83.6 ± 41.9) vs. (74.2 ± 36.7) pg/mL; *P* = 0.598].**Conclusions:** It can be concluded that the level of chemerin of seropositive *T. gondii* IgG was higher than that in the negative *T. gondii* IgG group.

1. Introduction

Obesity is a serious problem due to its association with various diseases such as diabetes mellitus, atherosclerosis, and cancer [1]. The prevalence of obesity has increased worldwide in recent decades. The prevalence of obesity in United State is

around 20%–25%, while in Europe it is 10%–25%. While 29.5% people in China have overweight, and the prevalence of obesity is approximately 4.3% [2]. In Indonesia, the national prevalence of obesity aged over 15 years is 10.3% in 2011 and the prevalence in women and men aged > 18 years in 2013 is 32.9% and 19.7%, respectively [3].

The pathophysiological mechanisms of obesity involve many factors including nutrition, environment, and genetics. Increased prevalence of obesity has led to some hypothetical explanations because lifestyle and diet modification do not fully explain the phenomenon. Some studies suggest that obesity is triggered by the presence of chronic inflammation which probably is a response to particular infection. The studies lead to a consideration that infection has causal relationship with obesity [4,5].

Toxoplasma gondii (*T. gondii*) is an intracellular pathogenic parasite that has ability to infect nucleated mammalian cells by using host macrophages as carrier vehicle [6]. Although severe clinical manifestation such as cerebral toxoplasmosis could

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The study protocol was performed according to the Helsinki declaration and approved by Health Research Ethics Committee of Medical Faculty Brawijaya University. Informed written consent was obtained from the patients.

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occur, *T. gondii* infection is generally asymptomatic or shows atypical symptoms that make it difficult to identify. The prevalence of *T. gondii* infection in Indonesia ranged from 6% to 68% [7]. *T. gondii* allegedly associates with obesity because it can cause chronic inflammation in adipocyte.

Adipocyte tissues produce and secrete adipokines, such as chemerin and adipocyte fatty acid-binding protein (AFABP). Chemerin is a chemoattractant protein functioning as a ligand for G-protein receptor of chemokine-like receptor 1 (CMKLR1) and has a role in adaptive immunity [8]. Chemerin is secreted as an 18-kDa inactive protein and undergoes extracellular proteolysis at the C-terminal serine protease to generate 16-kDa active chemerin [9,10]. The concentration of active chemerin in human plasma and serum is 3.0 nmol/L and 4.4 nmol/L, respectively. CMKLR1 expression and chemerin secretion play role in the regulation of adipogenesis [10,11].

The study conducted by Roman *et al.* showed that levels of chemerin in circulation were strongly associated with some characteristics of metabolic syndrome (triglycerides, blood pressure, body fat content, and insulin resistance) in individuals with normal glucose tolerance [8]. The study suggests that chemerin may play role in the pathophysiology of obesity and metabolic syndrome.

Chemerin is expressed by several tissues, including liver, pancreas, and lungs, whereas CMKLR1 expression is predominantly found in the cells involved in immune system such as neutrophils, activated macrophages, and dendritic cells. Chemerin and CMKLR1 are also expressed predominantly by mature adipocytes [10].

AFABP, also known as adipocyte protein 2 and fatty acid binding protein 4, is one of the most abundant proteins in mature adipocytes, accounting for ~6% of total cellular protein content. AFABP is also expressed in macrophages and lymphocytes. Although AFABP was originally identified as a cytoplasmic protein, data from both humans and rodents suggest that it is also secreted by adipose tissue into the bloodstream [12].

AFABP has recently been identified as a biomarker for the development of metabolic syndrome based on insulin resistance. The published results suggest that AFABP might be the central regulator of insulin resistance, lipid metabolism and inflammation. In obese subjects, enlarged adipose tissue is infiltrated by activated macrophages and several other types of inflammatory cells, leading to increased production of proinflammatory adipokines, including AFABP and others, such as tumor necrosis factor- α , interleukin-6, monocyte chemoattractant protein-1, resistin, leptin, lipocalin-2 and plasminogen activator inhibitor-1 [13].

It is clearly understood recently that obesity is associated with a chronic systemic inflammation. Moreover, adipocyte tissues contain numerous lymphocytes located in the stromal vascular fraction, as well as macrophages, natural killer cells, and T cells. In obese individuals, molecular and cellular changes of adipocyte tissues affect metabolism and systemic inflammation [14].

However, the association of chemerin and AFABP with *T. gondii* infection in obese individuals has not been reported. It is necessary to investigate chemerin and AFABP levels in seropositive *T. gondii* obese individuals compared to those seronegative individuals.

2. Materials and methods

The study protocol was performed according to the Helsinki declaration and approved by Health Research Ethics Committee

of Medical Faculty of Universitas Brawijaya. Informed written consent was obtained from the patients.

This study was an observational analytic study performed from January to April 2013. Consecutive sampling method was applied to meet 60 subjects.

Subjects were enrolled if they aged over 21 years, with body mass index (BMI) > 25 kg/m², waist measure > 90 cm (male) and > 80 cm (female). Pregnant and breast feeding women were excluded, as well as subjects using hormonal contraception.

The 57 obese individuals who met the inclusion and exclusion criteria had their venous blood samples taken. *T. gondii* immunoglobulin G (IgG) level was examined using ELISA method. Furthermore, the ELISA method was read by Bio-Rad[®] Microplate Reader Model 550. Cut-off value of positive *T. gondii* IgG was > 35 IU/mL.

3. Results

3.1. Subject characteristics

Fifty-seven obese individuals were enrolled during the study. The subjects were groups of positive and negative *T. gondii* IgG obese individuals. Ninety-one percent of subjects were female. In this study, 88% subjects had positive *T. gondii* IgG. The characteristics of subjects are shown in Table 1. There were no significant differences in age, height, weight, BMI, and waist circumference between the groups of positive and negative *T. gondii* IgG.

Table 1

Characteristics of obese individual subjects.

Characteristics	Positive <i>T. gondii</i> IgG (n = 50)	Negative <i>T. gondii</i> IgG (n = 7)
Age (year)	47.5 ± 11.4	43.4 ± 11.1
Height (cm)	155.7 ± 6.5	156.5 ± 5.4
Body weight (kg)	76.3 ± 8.4	81.3 ± 6.0
BMI (kg/m ²)	32.2 ± 3.3	31.0 ± 3.6
Waist circumference (cm)	102.6 ± 6.7	102.7 ± 7.3

3.2. Chemerin levels in obese individuals

The average level of chemerin of seropositive *T. gondii* IgG group was higher than that in seronegative *T. gondii* IgG group [(70.0 ± 16.5) vs. (64.4 ± 16.1) ng/mL; *P* = 0.003].

3.3. AFABP levels in obese individuals

The average level of AFABP in seropositive *T. gondii* IgG group was not significantly higher than that in seronegative group [(83.86 ± 41.90) vs. (74.17 ± 36.72); *P* = 0.598].

4. Discussion

We found that female obese subjects were more common than male obese subjects. This is relevant to Essential National Health Research in 2013 which revealed the prevalence of obesity in women is higher than that in men.

Seropositive *T. gondii* in this study was found in 88% subjects which suggest that they have been infected by this parasite. Previous studies had associated obesity with *T. gondii* infection. *T. gondii* is said to be one of the microorganisms that can cause

chronic inflammation in adipocyte and it will lead to obesity [6]. The study conducted by Arroyo-Olarte *et al.* suggested that *T. gondii* can affect lipid metabolism by reducing muscle lipase lipoprotein and changing the activity of lipoprotein lipase tissue during chronic toxoplasmosis to enhance distribution of triglycerides into adipose tissue [15]. The results of study in 999 adult subjects conducted by Reeves *et al.* in 2013 showed that obese individuals have higher level of *T. gondii* IgG compared to non-obese ones. They also found that seropositive *T. gondii* individuals have doubled risk to have obesity compared to seronegative *T. gondii* individuals [16].

Chemerin is a chemoattractant protein secreted by adipocyte and as an adipokine, it is associated with a number of chronic infections and inflammation. This study showed that chemerin levels of seropositive *T. gondii* IgG group were higher than those in seronegative *T. gondii* IgG group. The study by Catalán *et al.* showed that chemerin expressed on infection condition included psoriatic lesions and several effector cells of the immune system, including dendritic cells, monocytes, macrophages, and natural killer cells which are involved in the pathogenesis of psoriasis [10]. Chemerin expression is believed to be an early marker in the progression of the lesion development. Chemerin and CMKLR1 also appear to play an important role in several autoimmune disease by mechanisms that are still in the study.

The study conducted by Jin *et al.* demonstrated that the chemerin levels in the group of severe pneumonia are higher than those in mild pneumonia and control groups. It also revealed that the increase of chemerin levels is related to severity after infection by respiratory syncytial virus. Increased level of chemerin in infection or chronic inflammation is expected to be developed as an infection marker for obese individuals [17].

AFABP, an adipokine which is highly expressed in adipose tissue, has recently been identified as a biomarker for the development of metabolic syndrome based on insulin resistance. In obese subjects, enlarged adipose tissue is infiltrated with activated macrophages and several other types of inflammatory cells, leading to augmented production of proinflammatory adipokines, including tumor necrosis factor- α , interleukin-6, monocyte chemoattractant protein-1, resistin, leptin, lipocalin-2, AFABP, and plasminogen activator inhibitor-1 [13].

Reeves *et al.* reported that individuals who were positive for *T. gondii* IgG had approximately twice the odds of being obese compared to seronegative individuals, but they were unable to determine if there is a causal relationship between *T. gondii* seropositivity and obesity [16]. So it could be understood that obese individuals with seropositive IgG have higher AFABP levels than those seronegative individuals, although these statements still need further research. Obese subjects which have higher IgG *T. gondii* levels showed a more chronic inflammation in the body, including adipocyte. These facts also followed by increase of pro-inflammatory adipokines including AFABP.

Conflict of interest statement

We declare that we have no conflict of interest.

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