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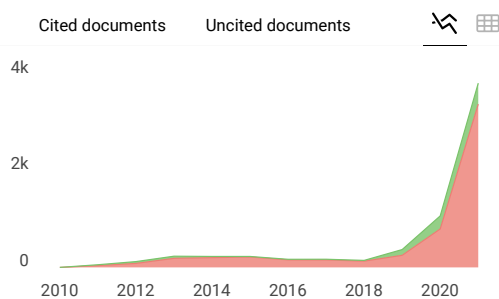
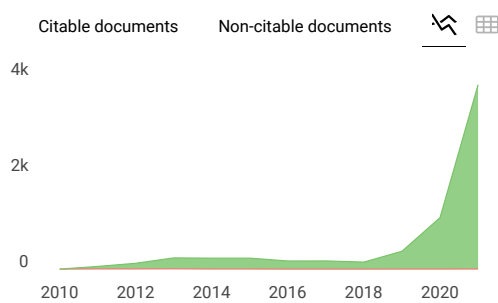
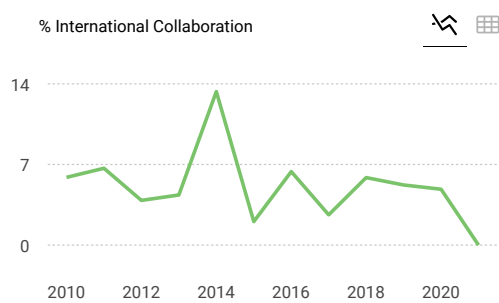
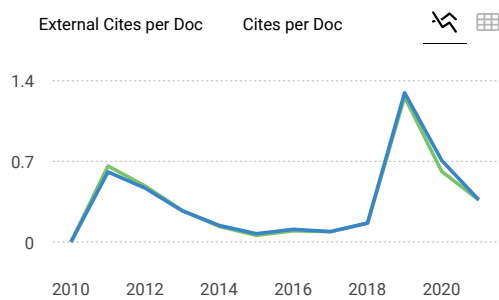
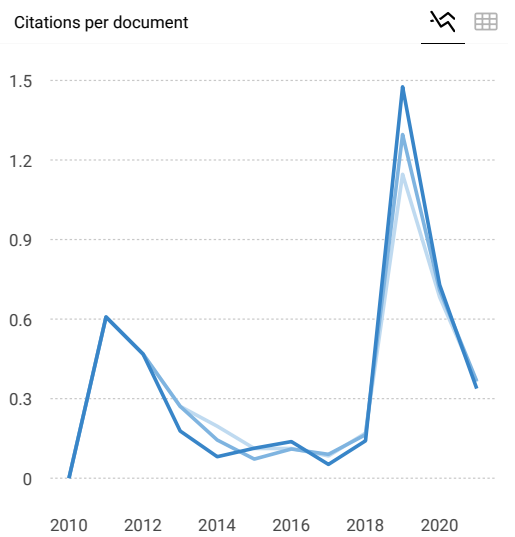
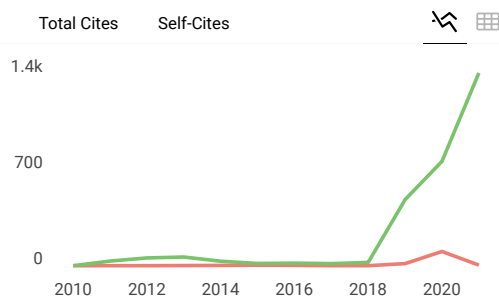
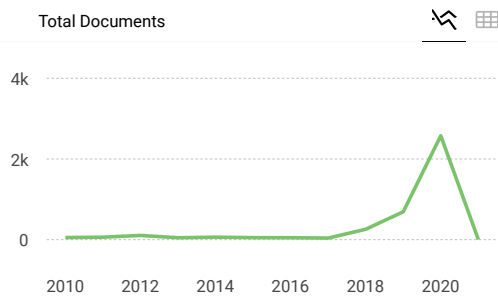
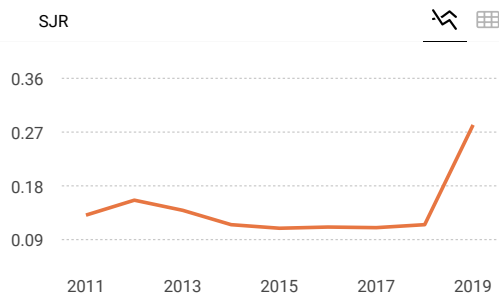
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**Research Article**

# IL-18, Adiponectin and Metabolic Parameters in Obese Adolescents

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**ABSTRACT**

**Background:** The prevalence of obesity in adolescents in developing countries is increasing. Obesity causes an imbalance of cytokines, which causes inflammation that triggers metabolic disorders. Early biomarkers associated with metabolic complications in obese adolescents need to be assessed, but this is limited in developing countries.

**Purpose:** To analyze the correlation between IL-18 and adiponectin, fasting blood glucose, insulin and lipid profile levels in obese adolescents

**Methods:** This was a cross-sectional study conducted on 59 obese adolescents. Obesity was established if the body mass index (BMI) was higher than the 95<sup>th</sup> percentile according to age and gender, based on the Centers for Disease Control growth charts. Blood tests performed on the subjects included IL-18, adiponectin, lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), insulin and fasting blood glucose levels. Both IL-18 and adiponectin were measured using enzyme-linked immunosorbent assay. Data were analysed with correlation tests and regression using SPSS software

**Results:** In this study, the mean IL-18 level was  $0.777 \pm 0.825$ . IL-18 was not correlated with adiponectin, blood glucose, insulin or lipid profiles ( $p > 0.05$ ) but it was correlated with HDL-cholesterol levels ( $p = 0.036$ ;  $r = -0.274$ ).

**Conclusion:** IL-18 was negatively correlated with HDL-cholesterol levels, with a regression model of  $HDL = 64.367 - 25.649(IL-18)$ . IL-18 can thus be used as a potential marker for predicting metabolic complications associated with obesity in obese adolescents.

**Keywords:** Obesity; Adolescents; IL-18; Adiponectin; Metabolic parameters.

**INTRODUCTION**

Obesity is a global health problem in developing countries.(Popkin et al., 2012) The prevalence of obesity in adolescents is increasing in Indonesia (Rachmi et al., 2017) and for the whole of Asia it is 8.6% (Mazidi et al., 2018). Previous studies in Asia stated that most adolescents displayed sedentary behaviour and unhealthy food consumption (Allafi et al., 2014), both of which increase the risk of obesity in adolescents.(Rachmi et al., 2017)

In obesity there is chronic low-grade inflammation due to an imbalance of pro-inflammatory and anti-inflammatory cytokines. It is the increase in the size of fat cells and free fatty acids that causes systemic inflammation (Amin et al., 2019; Asghar & Sheikh, 2017). Studies in humans showed the relationship of cytokines to the risk of metabolic disorders, with cytokines such as IL-10, visfatin, IL-6, TNF- $\alpha$  and IL-18 acting as predictors or possibly therapeutic targets (Himani et al., 2019; Liu et al., 2016). In contrast, the study of IL-18 in experimental animals showed a protective effect

on obesity and metabolic syndrome by the AMP-activated protein kinase (AMPK) pathway, which balances fat accumulation and decreases central adiposity.(Murphy et al., 2016; Lindegaard et al., 2013). However, studies in humans show that IL-18 is one of the pro-inflammatory cytokines that is increased in obesity and associated with metabolic syndrome (Trøseid et al., 2010). The higher the concentration of IL-18, the higher the risk of metabolic syndrome and the higher the number of metabolic disorders that occur (Hung et al., 2005). Previous studies suggested that increased IL-18 expression is associated with insulin resistance, even in individuals without a history of diabetes (Ahmad et al., 2017). However, the majority of IL-18 studies with metabolic disorders were performed in adults (Hung et al., 2005). Studies of IL-18 related to metabolic complications in obese adolescents are still limited.

**MATERIAL AND METHODS****Study Population**

A cross-sectional study was conducted on obese adolescents who visited the Pediatric Nutrition and Metabolic Diseases department in Dr. Soetomo General Hospital, Surabaya. Subjects were chosen using the total sampling technique and were included in the study if they met the following inclusion criteria: obesity, aged 13–16 years and having parents/guardians who were agreeable to their participation in this study.

Obesity was established based on the Body Mass Index (BMI) / Age curve established by the Centers for Disease Control 2000 growth charts. Subjects were classified as obese if they had a BMI higher than the 95th percentile according to age and gender. Exclusion criteria in this study include smoking, consuming alcohol, taking drugs for dyslipidaemia and hyperglycaemia, suffering from chronic diseases, having infections or inflammatory diseases, suffering from endocrine disorders and receiving hormone therapy.

#### Anthropometry Measurement

Various anthropometric measurements were performed on the subjects, including weight and height. Weight was measured using a digital scale with an accuracy of 0.1 kg (Seca, Germany; No. 224-1714009) and height was measured using a stadiometer with an accuracy of 0.1 cm (Seca, Germany; No. 224-1714009). When measuring weight and height the subjects did not wear shoes or other accessories.

#### Biochemical laboratory examination

Blood examination, including that for IL-18 and adiponectin, was performed on all subjects. Sampling was carried out at 08.00–09.00 WIB (Western Indonesian Time) on the mediana cubital vein after the subjects had fasted for 12 hours. Subjects were sampled in a seated position in accordance with standard operational procedures. After the blood was drawn it was centrifuged to obtain serum according to standard laboratory techniques and stored at  $-70^{\circ}\text{C}$ .

Both the IL-18 and adiponectin measurements were performed by enzyme-linked immunosorbent assay. Measurement of blood glucose levels was carried out with glucose hexokinase FS reagent using a TMS Premium 24i analyser (DyaSys Diagnostic System GmbH,

Germany). Insulin was measured using a Cobas e411 (Roche Diagnostics GmbH, USA). Lipid profiles were measured with enzymatic kits for total cholesterol (Pureauto S Cho-N, Sekisui Medical, Japan), LDL-cholesterol (Cholestest<sup>®</sup> LDL, Sekisui Medical, Japan), HDL-cholesterol (Cholestest<sup>®</sup> HDL, Sekisui Medical, Japan) and triglycerides (Autosera S TG-N, Sekisui Medical, Japan) using a TMS Premium 24i analyzer. All blood examinations were carried out according to the manufacturers' instructions.

#### Statistical analysis

Quantitative variables, including IL-18, adiponectin, fasting blood glucose, insulin and lipid profiles, were described as the mean  $\pm$  standard deviation and minimum and maximum values. The normality of the variables was assessed using the Kolmogorov-Smirnov test. The correlation between IL-18 and adiponectin, fasting blood glucose and lipid profile levels in obese adolescents was analyzed using Pearson's correlation because the data were normally distributed. The correlation between IL-18 and insulin level was analyzed using Spearman's rho because the data had an abnormal distribution. Variables that have correlations were analyzed further by analysis of variance to define any influences between variables. A value of  $p < 0.05$  was considered to be significant. Data were analyzed using SPSS software version 21.0.

#### Ethics

This study was conducted after obtaining ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya. Before conducting the research, the procedure was fully explained to the parents. The study was conducted only after informed consent was signed by the parents/guardians. The confidentiality of the research subjects was maintained in this study.

## RESULT AND DISCUSSION

A total of 59 obese adolescents were involved in this study. Subjects were 13–16 years old, consisting of 27 (45.8%) females and 32 (54.2%) males. The laboratory parameters are explained in Table 1.

**Table 1: Laboratory measurements in obese adolescents**

Variable	Mean $\pm$ Standard Deviation	Min	Max
IL-18	0.777 $\pm$ 0.825	0.371	0.919
Adiponectin (ng/dl)	7838.804 $\pm$ 3808.187	2341.914	19129.056
Fasting blood glucose (mg/dl)	80.067 $\pm$ 7.554	67.000	109.000
Insulin (mU/ml)	21.703 $\pm$ 24.157	4.320	162.700
Total cholesterol (mg/dl)	176.135 $\pm$ 32.871	119.000	278.000

HDL-cholesterol (mg/dl)	44.423 ± 7.733	31.000	67.000
LDL-cholesterol (mg/dl)	117.813 ± 27.707	62.000	196.000
Triglyceride (mg/dl)	118.118 ± 63.744	30.000	343.000

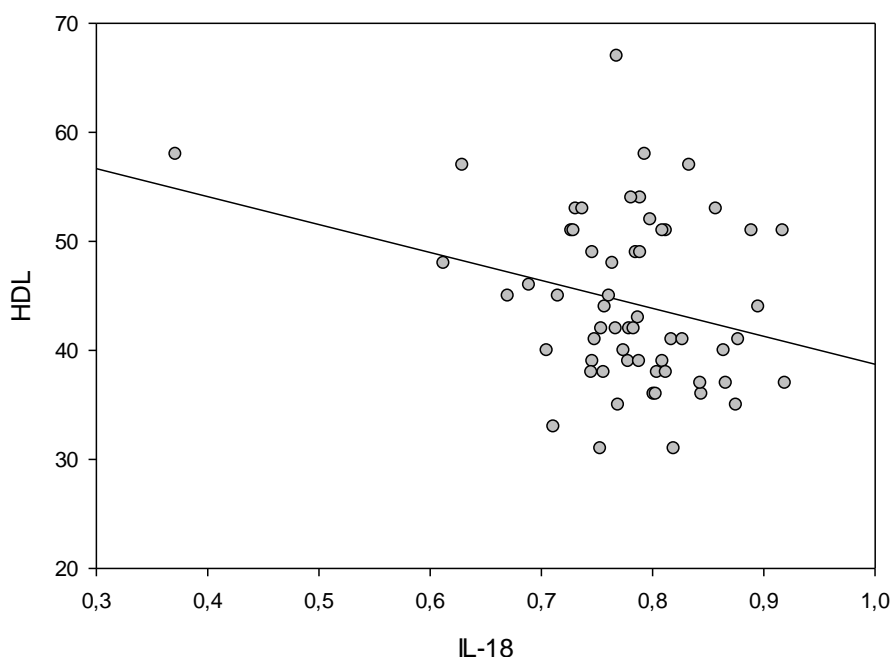
**Table 2: Correlation between variables**

Variable	IL-18	
	<i>p</i>	<i>r</i>
Adiponectin (ng/ml)	0.542	-0.081
Fasting blood glucose (mg/dl)	0.137	0.196
Insulin (mU/ml)	0.291	0.140
Total cholesterol (mg/dl)	0.443	-0.102
HDL-cholesterol (mg/dl)	0.036*	-0.274
LDL-cholesterol (mg/dl)	0.676	-0.056
Triglyceride (mg/dl)	0.312	0.134

\**p* < 0.05.

There was no correlation between IL-18 and adiponectin, fasting blood glucose, insulin and lipid profile levels (total cholesterol, LDL-cholesterol and triglyceride) in obese adolescents (*p* > 0.05) but there was a significant negative

correlation between IL-18 and HDL-cholesterol levels (*p* = 0.036; *r* = -0.274) (see Table 2 and Figure 1).



**Fig.1: Correlation between IL-18 and HDL-cholesterol**

Further statistical analysis using regression found that there was an influence of IL-18 on HDL-cholesterol (*F* = 4.164; *p* = 0.036), with a regression model of HDL = 64.367 - 25.649 (IL-18). In obesity there is chronic low-grade inflammation (Asghar & Sheikh, 2017). Inflammation that occurs in obesity causes metabolic complications such as metabolic syndrome, diabetes mellitus and dyslipidaemia (Asghar & Sheikh, 2017; Cooke et al., 2016). The prevalence of metabolic syndrome is still very

high in adolescents in developing countries (Mohsin et al., 2012). Loss of balance in cell metabolism due to obesity activates the immune system, resulting in the release of pro-inflammatory cytokines (Murphy et al., 2016). Early detection of metabolic disorders in obese adolescents with existing inflammatory parameters is therefore needed.

One of the cytokines that has been studied is IL-18, which is a member of the IL-1 family that requires enzymes or caspase-1 to become an

active biological form (Sedimbi et al., 2013). IL-18 is able to induce the production of interferon gamma, TNF and IL-1 (Sedimbi et al., 2013; Dinarello, 1999) The mean IL-18 level in this study was  $0.777 \pm 0.825$ . Previous studies suggested that IL-18 is increased in obesity and is associated with metabolic disorders in obesity (Trøseid et al., 2010).

In obesity, adiponectin levels decrease (Borges et al., 2017). Adiponectin is an adipocytokine that is protective against insulin resistance and is anti-atherogenic (Li et al., 2017). Insulin resistance is a predictor of metabolic syndrome in adolescent obesity (Vardi et al., 2007) and adiponectin is associated with metabolic syndrome (Li et al., 2017; Falahi et al., 2015) Adiponectin plays a role in insulin sensitization in fat tissue (Li et al., 2017) and decreased levels of adiponectin are found in diabetes mellitus (Al-Daghri et al., 2009). In this study the mean adiponectin level was  $7838.8 \pm 3808.19$  ng/ml (Table 1), which is lower than previous studies on obese subjects (Tamang et al., 2013).

Previous studies suggested that adiponectin levels are a protective factor against inflammation by blocking IL-18 cell death mediated via AMPK (Chandrasekar et al., 2008). In this study there was no relationship between IL-18 and adiponectin levels. Adiponectin, IL-18, TNF- $\alpha$ , IL-6 and C-reactive protein (CRP) levels are strongly correlated with type 2 diabetes mellitus (Liu et al., 2016). In this study no IL-18 relationship was found with fasting blood glucose, insulin or lipid profiles levels but there was a relationship with HDL-cholesterol level. An IL-18 level of  $\geq 336.4$  pg/ml is associated with hypertension and hypertriglyceridemia (de Oliveira et al., 2015). Ethnic differences can affect the distribution of body fat associated with inflammation in obesity (Misra & Vikram, 2007). Previous studies were mostly performed in adults (de Oliveira et al., 2015).

In this study, IL-18 has a negative correlation with HDL-cholesterol level: an increase in IL-18 level was followed by a decrease in HDL-cholesterol level. This is consistent with previous studies reporting that IL-18 is associated with the risk of cardiovascular disease, hypertension and dyslipidaemia (de Oliveira et al., 2015). IL-18 also increases chemokine and inflammatory exposure in the myocardium (Xiao et al., 2018). There were several limitations to this study: the number of subjects was limited; blood tests were only performed once, so measurements may be influenced by circadian rhythms and fluctuations and therefore cannot describe the relationship with certainty; and no control subjects with a normal BMI were recruited as a comparison.

The study of IL-18 and metabolic disorders in obese adolescents in developing countries needs further investigation. The influence of IL-18 on HDL-cholesterol levels may allow the opportunity for IL-18 to be used as a biomarker for early detection or as a therapeutic target in obese adolescents with metabolic disorders.

## CONCLUSION

IL-18 was correlated negatively with HDL-cholesterol level, with a regression model of HDL =  $64.367 - 25.649$  (IL-18). Further studies with more subjects and serial blood measurements are needed to assess the correlation of IL-18 with adiponectin, fasting blood glucose, insulin and lipid profiles levels in obese adolescents as early markers for detecting metabolic abnormalities.

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## ETHICAL CLEARANCE

This study was conducted after obtaining ethical approval from the Health Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya (No. 1360/KEPK/VII/2019).

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Universitas Airlangga, Surabaya

## CONFLICT OF INTEREST

The author(s) declares no conflict of interest.

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