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Current Issue



Association of Adipoq +45 T>G Gene Polymorphism with Insulin Resistance and Icam-1 Value in Obese Adolescents

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Abstract

Background: Obesity is defined as the accumulation of excess body fat tissue which harms health, such as cardiovascular disease, metabolic syndrome, diabetes mellitus type 2 and dyslipidemia. Lower adiponectin levels are strongly associated with overweight, obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular risk factors in adulthood. ADIPOQ +45 T>G gene polymorphism is one of a genetic variations that affect plasma adiponectin levels.

Methods: A cross-sectional study of 180 obese adolescents aged 13-18 years in Surabaya and Sidoarjo. Obesity is defined as body mass index (BMI)>p95 on the BMI for age curve according to Centers for Disease Control and Prevention (CDC) 2000. Blood samples were taken for ICAM-1 examination using the ELISA method, HOMA-IR with fasting insulin and glucose calculations, and ADIPOQ + 5 T>G gene polymorphisms by PCR-RFLP. Data were analyzed using the Chi-square, Mann-Whitney, and T-test using SPSS version 21, p-value<0.05 was considered significant.

Results: There was a significant difference in the mean BMI by age group (p <0.001). There was no statistically significant difference between the median BMI in the three groups (wild, mutant homozygote, and mutant heterozygote) of genotype distributions. ADIPOQ +45 T>G gene polymorphisms occur with the same frequency in both males and females. There was no association between the genotype distribution of the ADIPOQ +45 T>G gene polymorphism with insulin resistance and ICAM-1 value, but the ICAM-1 value was found to be higher in the ≥ 15 years age group. There was no association between insulin resistance and ICAM-1 values.

Conclusion: There was no association between ADIPOQ + 45 T>G gene polymorphisms with insulin resistance and ICAM-1 values in obese adolescents. There was no association between insulin resistance and ICAM-1 values.

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Keywords: obese adolescents, ADIPOQ +45 T>G gene polymorphism, HOMA-IR, insulin resistance, ICAM-1

Introduction

Obesity is defined as the accumulation of excess body fat tissue which harms health. This condition is more experienced by adolescents and adults. The lifestyle of today's teenagers often skip breakfast and

prefer to consume fast food. and tends to a sedentary lifestyle, making adolescents at risk for obesity.¹ Being overweight and obese are risk factors for several life-long complications, such as cardiovascular disease, metabolic syndrome, type 2 diabetes mellitus, and dyslipidemia.² Obese adolescents tend to be obese adults, the condition of obesity will have an impact on metabolism.³

Several studies explaining the role of genetics in the pathogenesis of obesity have been conducted. Lower adiponectin levels are strongly associated with overweight, obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular risk factors in adulthood.⁴ In children and adolescents, hypoadiponectinemia has been shown to predict obesity, metabolic syndrome, hypertension, insulin resistance, and visceral fat accumulation.⁵ Obese adolescents will experience insulin resistance. Research on obese adolescents aged 13-18 years found 78% of children with insulin resistance.⁶ Four polymorphisms in ADIPOQ have been studied, two located in the promoter region of the gene (-11391 G>A and -11377 C>G), one in exon 2 (+45 T>G), and one in intron 2 (+276 G>T).⁷ In humans, hypoadiponectinemia is associated with lower vasodilator response in diabetic patients, and adiponectin administration increases nitric oxide (NO) production in aortic endothelial cells.⁸ Initial stimulation of inflammation, such as a diet high in saturated fatty acids, hypercholesterolemia, obesity, insulin resistance, hypertension, and smoking stimulates adhesion molecules such as P-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM- 1) so that monocytes and lymphocytes that are in the bloodstream can stick to the endothelial surface which is an early sign of endothelial dysfunction that can cause cardiovascular abnormalities.⁹

In Indonesia, gene research still has several challenges. The ADIPOQ gene polymorphism in obese adolescents has never been studied. The finding of the relationship between gene polymorphisms and insulin resistance and tilapia ICAM-1 can be used as prevention and management in obese adolescents. This prompted researchers to analyze the relationship between ADIPOQ

+45 T>G gene polymorphisms with insulin resistance and ICAM-1 values in obese adolescents.

Methods

Data Collection

A cross-sectional study was conducted on 180 obese adolescents in junior and senior high schools in Surabaya and Sidoarjo, East Java, Indonesia in May-September 2020. Subjects were recruited with a total population sampling method that eligible for the inclusion and exclusion criteria. The inclusion criteria in this study were adolescents aged 13-18 years with obesity, as well as both parents and subjects who agreed to participate in this study. Adolescents with a history of corticosteroid consumption for more than two months up to a period of 6 months before the study took place or subjects who were in a sick condition, alcohol and smoking consumption, have diabetes mellitus were excluded. Measurement of body weight is performed with digital weight scale Seca, Germany with a precision of 0.1kg. Height measurement was performed using stadiometers Seca, Germany with an accuracy of 0.1cm. The subjects had been fasting for 12 hours before the blood samples were taken (plasma insulin and plasma glucose). ICAM-1 examination was carried out in the laboratory of the Institute Tropical Diseases (ITD) Airlangga University with the Human intercellular adhesion molecule 1 Elisa kit from the Bioassay Technology Laboratory. Analysis of ADIPOQ +45T>G is using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method.

Definitions

Obesity is defined as BMI>p95 on the BMI for age curve according to CDC 2000. Insulin resistance was calculated using the Homeostatic model assessment for insulin resistance (HOMA-IR) with the formula $\text{insulin } (\mu\text{U/mL}) \times \text{glucose (mg/dL)} / 405$ and defined if $\text{HOMA-IR} \geq 3.4$.¹⁰ The cut-off reference value used the median ICAM-1 in obese children (284.4 ng / ml) for male and female sex.¹¹

Statistical Analysis

Some data were reported as mean, standard deviation (SD), and median. The association of gene polymorphism, insulin resistance, and ICAM-1 value were calculated using IBM-SPSS statistics version 21.0.

Result and Discussion

This study is an analytic observational study with a cross-sectional approach to obese adolescents in secondary schools in Surabaya and Sidoarjo. The total of subjects was 180 students.

Table 1 Age and Sex Distribution

Base Characteristics	n	%
Age		
13-<15	77	42,80
≥15-18	103	57,20
Sex on age 13-<15		
Male	44	57,10
Female	33	42,90
Total	77	
Sex on age ≥15-18		
Male	61	59,20
Female	42	40,80
Total	103	

The sample of this study was more in adolescents aged ≥15-18 years than in the 13- <15 years age group. The largest sample was found in males in both age groups. These findings were similar to studies on the prevalence of overweight and obesity in Argentine adolescents with a mean age of 15 reported 10.9% and 2.2%, respectively, with a higher prevalence in males than females.¹²

The reason of male adolescents are more obese than female is that adolescent male consume more calories than the recommended calorie intake per day, consume more sugary drinks, and spend more time watching television than girls. Teenage girls tend to feel fat, so they make more weight loss efforts through diet patterns.¹³

Table 2 Status Data of Weight, Height, IMT, Blood Sugar, Insulin, HOMA-IR, and ICAM-1 based on Ages

Variables	Age 13-<15 years			Age ≥15-18 years			p
	Mean	SD	Median (min-max)	Mean	SD	Median (min-max)	
Weight (kg)	78,11	12,37	77,90	89,67	13,26	88,60	*<0,05
Height(cm)	160,95	161,50	161,50	160,93	10,35	161,00	*0,98
BMI	30,17	3,58	29,80	34,48	4,45	33,56	**<0,05
Blood sugar	87,74	7,30	85	85,77	6,04	85	**0,09
Insulin	25,53	17,93	20,33	21,82	9,54	19,45	**0,71
HOMA-IR	5,52	3,88	4,32	5,52	3,88	4,32	**0,55
ICAM-1	600,75	474,47	568	662,53	459,05	663	**0,04

*T-test, **Mann-Whitney test

Table 2 shows a significant difference between body weight and BMI with the sample age group. The age group $\geq 15-18$ years is greater than 13- <15 years. There was a significant result of ICAM-1 score in the age group. The ICAM-1 score in the $\geq 15-18$ years group was greater. There was insignificant result between blood sugar, HOMA-IR, and ICAM-1 insulin values with age groups.

Table 3 Status Data of Weight, Height, IMT, Blood Sugar, Insulin, HOMA-IR, and ICAM-1 based on sex

Variables	Male			Female			P
	Mean	SD	Median	Mean	SD	Median	
Weight (kg)	87,25	14,91	86,80	81,19	12,03	80,50	*<0,05
Height (cm)	163,98	7,90	163,50	156,68	9,18	157	*<0,05
BMI	32,43	4,39	31,40	32,92	4,93	31,60	**0,62
Blood sugar	87,03	6,36	85	86,02	7,07	84	**0,15
Insulin	23,18	14,02	19,57	23,72	13,68	19,66	**0,71
HOMA-IR	4,99	3,11	4,19	5,04	2,89	4,32	**0,76
ICAM-1	592,81	458,50	560	696,72	471,29	656	**0,06

*T-test, **Mann-Whitney test

In table 3 there is a significant difference between body weight and height for gender. The weight and height of the male sex are greater than female in obese adolescents. BMI, blood sugar, insulin, HOMA-IR, and ICAM-1 were insignificant.

The difference in the mean BMI between the age groups in this study was influenced by sexual maturity

which generally differed in each group, besides that it could be due to changes in dietary patterns with age. In children and adolescents who are still developing, BMI is interpreted differently from adults. BMI changes with age and sex, as body weight and height increase. Besides, other factors such as ethnicity, social, and culture also affect obesity.

Table 4. Gene ADIPOQ +45T>G Polymorphism Genotype Distribution Analysis Based on Sex

Genotype Distribution	n(%)	Sex		*p=1,00
		M	F	
Wild type (TT)	111(61,70)	65	46	*p=1,00
Mutant heterozygote (TG)	62 (34,4)	36	26	
Mutant homozygote (GG)	7 (3,9)	4	3	

* Chi-square

The relationship of ADIPOQ + 45T> G gene polymorphisms and sex is shown in table 4. The results of the analysis had no relationship between genotype distribution and sex. Analysis of correlation data between alleles and sex is shown in table 5. There was no relationship between allele distribution and sex.

In this study, heterozygote and homozygote mutants were found in some obese children. There was no

significant difference between sex, body mass index, and age group on the genotype distribution of ADIPOQ +45T>G gene polymorphisms. This is also similar to the research of Kasap et al. there was no significant difference in each adiponectin genotype on obesity. There was also no relationship between the polymorphism of the +45 T>G SNP gene from the adiponectin gene and obesity in children and its complications.¹⁴ In this study, we did not group ethnically that may made bias in the result.

Table 5. Correlation Analysis of Polymorphism ADIPOQ +45T>G and HOMA-IR

Genotype Distribution	HOMA-IR		p
	Insulin Resistance	Non-resistance insulin	
Wild	77	34	*0,74
Mutant	46	23	

*Chi-square

Table 5 shows no correlation between the distribution of ADIPOQ +45 T>G gene polymorphisms and the HOMA-IR value in obese adolescents. In this study, there was no significant difference between the HOMA-IR values based on gender, age group, and the distribution of the genotype of the ADIPOQ + 45T> G gene polymorphism. Similar to this study, several previous studies did not find a relationship between ADIPOQ + 45T> G gene polymorphisms and HOMA-IR.¹⁵⁻¹⁷

Some studies conclude that the G allele in + 45T> G SNP of the adiponectin gene is associated with decreased fasting insulin levels and lower HOMA-IR scores.^{7,18} In another study, adiponectin concentrations were positively correlated with insulin sensitivity and significantly decreased with worsening glucose tolerance in Pima Indians and Caucasians.¹⁹

Table 6. Correlation Analysis of Gene Polymorphism ADIPOQ+45T>G and ICAM-1

Genotype Distribution	ICAM-1		p
	Normal	High	
Wild	30	81	*0,60
Mutant	16	53	

*Chi-square

Table 6 shows that there is no correlation between the genotype distribution of the ADIPOQ + 45T> G gene polymorphism and the ICAM-1 value in obese adolescents. Intercellular adhesion molecule-1 / ICAM-1 is a member of the immunoglobulin superfamily and is expressed on a wide variety of cells under inflammatory conditions. One of the functions of ICAM-1 is the leukocyte adhesion receptor in response to inflammatory stimuli, usually expressed on the surface of endothelial cells as a mediator for transferring leukocytes to tissues.²⁰

In previous study of sICAM-1 (Serum ICAM-1) levels were analyzed in the serum of obese mice due to a long-term (6 months) high-fat diet. The mean level of sICAM-1 was higher in male rats than in female rats (p <0.05). When associated with body weight, sICAM-1 levels increased by an average of 10 mg/ml for every 10g of body weight gain, with a correlation of r = 0.50

(P <0.001). This relationship did not differ statistically between men and women.²¹ It is consistent with human studies, that elevated levels of sICAM-1 have been associated with several pathological conditions, including obesity and its complications.

Several human studies have also shown that sICAM-1 levels are also increased in obesity, and are positively correlated with central obesity²² and insulin resistance.^{23,24} The difference in the results of this study was due to the endothelial inflammatory process in adolescents which did not occur at the time of examination but could arise over time. Also, adolescents are still classified as active in physical activity which is a factor in increasing insulin sensitivity to suppress the inflammatory process in the endothelium, but in this study, physical activity factors were not recorded and analyzed.

Table 7. Correlation between HOMA-IR and ICAM-1 groups

HOMA_IR	ICAM-1		p
	normal	high	
Non-insulin resistance	17	40	*0,46
Insulin Resistance	29	94	

*Chi-square

In Table 7, there is no correlation between the HOMA-IR and ICAM-1 groups in obese adolescents. Insulin resistance has been recognized as an independent risk factor for cardiovascular disease by causing dyslipidemia.²⁵ Besides, insulin resistance is often associated with metabolic syndrome, whose components are central obesity, high blood pressure, high triglycerides, low HDL, and impaired fasting plasma glucose.²⁶

Obese children who are resistant to insulin have a significantly greater risk of cardiovascular disease.

Insulin resistance in childhood predicts cardiovascular risk.²⁷ Insulin resistance in early vascular smooth muscle abnormalities can be seen with markers of endothelial dysfunction (ICAM and E-selectin).²⁸

There was an association between ICAM-1 and insulin resistance found (p <0.0001) in other studies that were consistent with clinical evidence related to insulin resistance and inflammation.²³ No studies have directly measured in vivo insulin sensitivity and its association with atherosclerotic abnormalities in children. Very limited observation shows an association between HOMA-IR, arterial stiffness, and fasting insulin levels

in children.²⁹

The difference in this study could be due to differences in the classification of insulin resistance using the HOMA-IR value with different cut-off values, while other studies have used FSIVGTT so that it affects the results obtained. Also, the physical activity factor of the sample was not analyzed in this study so that it could be the one that affects the results on insulin and ICAM-1 values.

Conclusion

There is no association between ADIPOQ +45T>G gene polymorphisms to insulin resistance and ICAM-1 value. No association of insulin resistance and ICAM-1 values. If further research is carried out, it is preferable to add non-obese subjects to the study sample for control. Physical activity and race of each research subject are recorded and analyzed on the research results.

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Ethical Clearance: This study had got permission from the ethics committee of The Faculty of Medicine, Airlangga University Before the subject recruitment, the explanation was done to the subjects and their parents.

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