

The Role of Polymorphisms in Adiponectin Genes and Aerobic Exercises towards Adiponectin Levels on the Overweight and Obesity Individuals

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ABSTRACT

In Indonesia, National research of Basic Health Research reported that there is an increase in national prevalence of obesity (combination of overweight and obese category) in 2010-2013 according to the male sex, there were 15% in 2010 and increased to 20% in 2013, and according to the female sex, there were 26% in 2010 and increased to 35% in 2013 (Ministry of Health in 2010 and 2013). This study aims at determining the role of genetic variations in adiponectin genes and aerobic exercise towards adiponectin levels. The research applied quasi experimental design with one group pre-post test design. The research subjects were 36 women with overweight problem. Overweight ranges are determined by using inclusion criteria. The research variables are genetic variation of adiponectin genes and adiponectin levels. In order to know the genetic variation, PCR check was examined in the Nechri laboratory with standardized protocol. The research results showed that variations of adiponectin genes were 75.0% TT genotype, 22.2% GT genotype, 2.8% GG genotype. The mean score of Adiponectin levels in pretest was 24.09 ± 12.47 and in posttest was 39.93 ± 23.79 . Based on the results of paired t-test statistical value of 0.001 ($P < 0.05$), it is indicated that there is a difference between adiponectin levels before and after combining the aerobic exercise combined with OCD diet. While the relationship between gene variation and the adiponectin levels after aerobic exercise showed that respondents with TT genetic variation and adiponectin levels increased into 21 respondents or 77.8%, and GT genetic variation increased into 7 respondents or 87.5%, and GG genetic had 1 respondent or 100%. The statistical test result of Kruskal-Wallis test showed that p-value of 0.74 or $P > 0.05$, it can be concluded that there is no relationship between genetic factors with adiponectin levels after aerobic exercise is combined with OCD diet. There is a difference between adiponectin levels before and after combining aerobic exercise and OCD diet with p-value of 0.001. However, there is no relationship between genetic factors and adiponectin levels after combining aerobic exercise and OCD diet with p-value of 0.74.

KEYWORDS: genetic variation of adiponectin gene, adiponectin levels, aerobic exercise, overweight, obesity.

INTRODUCTION

In Indonesia, National research of Basic Health Research (Riskesda) reported that there is an increasing in the national prevalence of obesity (combination of overweight and obese category) in 2010-2013 based on sex. According to the male sex, there were 15% in 2010 and increased to 20% in 2013, and according to the female sex, there were 26% in 2010 and increased to 35% in 2013 [1].

Obesity is caused by an imbalance of calories intake and expenditure. Calories are obtained from food while its expenditure through body activity and sport. The amount of basic caloric needs is determined by genetic or hereditary. However, physical activity and exercise can increase the amount of calorie expenditure. Therefore, this calorie imbalance can be determined by heredity but triggered by lifestyle and environment. Relaxed lifestyle habits, lazy move, always assisted by others and overeating will increase calorie intake and lower calorie expenditure. Several studies have shown or identified that lifestyle, socioeconomic status, excessive energy consumptions, low levels of physical activity are risk factors for the development of CHD in adults. The importance of exercise (sport) regularly to prevent CHD in adolescents and adults is not yet clear.

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Genetic variations of Adiponectin gene were most reported to be associated with insulin resistance, obesity and the risk of type 2 diabetes in intron 2 with G276T and in exon 2 with T45G (Xita et.al, 2012). In a study conducted in Japan found that subjects with GG genotype had lower plasma adiponectin levels than in TT genotype. Odd ratio in these conditions was more than 2 [2].

The large number of overweight and obesity events that are caused by an imbalance between food intake and the body's energy needs where the excessive intake was stored into fats. The balance of energy requirement was affected by various factors such as regulation of metabolism and physiological regulation from inside body or lifestyle (environment) impact from outside body that will influence eating habits and physical activity. Metabolism and physiological regulation is influenced by genetics and environment. Environmental factors have 70% while genetic factors have 30% role in obesity. The environmental factors include food and physical activity, and also drugs, toxins and viruses factors [3].

Adiponectin may cause large and diversity effects, it is necessary to do further research to see whether the role of genetic variation in adiponectin gene with aerobic exercise interventions to increase levels of adiponectin that can prevent disease type 2 diabetes, coronary heart disease, cancer (endometrial, breast, colon), hypertension, dyslipidemia, liver, gall bladder disease, insomnia, apnea, respiratory problems, osteoarthritis, and gynecological problems.

Based on the increased incidence of obesity and the incidence of diseases caused by obesity and overweight, it can be formulated the problem "How the role of adiponectin gene polymorphism and aerobic exercise towards adiponectin levels in overweight and obese individuals."

MATERIALS AND METHODS

This research is an experimental study or experimental design (quasi experimental) which is researched to know causal relationships where randomization is not carried out and there is no a control group. The study design used one-group pre-posttest design. In this study, it is selected a group of subjects or experimental unit. Before being given treatment, measurement (pre-test) is done first. In this case, blood samples for adiponectin levels test were taken by Elisa test and genes were tested by PCR and then were given treatment in the form of aerobic exercise with weight training program in maximum capacity of 60% for 30 minutes, 1 set with moderate / low rhythm exercises, the frequency of exercise were 18 times and at the end of aerobics or after treatment, re-measurement (post-test) with a blood sample to recheck the levels of adiponectin. The results of measurement pre and post test will be compared to see the difference between adiponectin levels in pre and post workout aerobic then connected with genetic.

RESULTS AND DISCUSSION

Characteristics of Respondents

Table 1. Characteristics of study subjects

Age	N	%
17	5	13.9
18	17	47.2
19	9	25
20	5	13.9
Total	36	100

Source : Primary data 2015

Table 1 presents that the average result of 18 years old respondents was 47.2% The number of respondents in this research consisted of 36 people from several institutions of nursing and midwifery in Makassar. Characteristics of research subjects include gender, age, and education. The sex of the respondents were 100% female, 17-20 years old respondents with an average respondent was 18 years old and the percentage was 47.2%, education with 100% female students. from the 36 samples, after the genetic variation test of adiponectin gene, each genetic variation was represented by each of them, but the amount of any gene variation were not balance. The imbalance gene variations were 27 TT, 8 GT, and only 1 GG.

Table 2. Genetic variation of adiponectin gene on research

Gene Variations	N	%
TT	27	75
GT	8	22.2
GG	1	2.8
Total	36	100

Source : Primary data 2015

Table 2 presents the average respondent has genetic variation of TT with average was 75%. The results of this study showed that from 36 nursing students as samples, there were 27 students have genetic variation of TT with average was 75.0%, while the genetic variation of GT with 8 students were at average of 22.2% and genetic variation of GG with only one student was at average of 2.8%.

In this study, dominant gene variation of TT is possible caused by race where the research was conducted in the United States compares whites and blacks. The result showed that there was a dominant gene variation of TT in whites than gene variation of GG in blacks and also levels of adiponectin in whites were higher than adiponectin levels in blacks, it is in line with our results because our sample is based on skin color of brown skin or race.

In this research, it is conducted aerobic exercise for 6 weeks and 3 times in a week or 18 times exercise. The aerobic exercise was combined with OCD diet that leads the students to do fasting during aerobic exercise schedule. There were only 8-hour for meal time from 7 am until 3 pm and in the next hour, they should not to eat but they may drink. From the results of student interviews, it is showed that the average student groups come from overweight families.

The results of this research found that there was a trend of adiponectin gene variation on nursing student groups from various districts in the province of South Sulawesi with gene variation of TT was at average of 75.0%. In individuals with visceral obesity and type 2 diabetes, adiponectin was decreased significantly. Wicek (2012), comparing the levels of adiponectin and also lipid profile between obese and normal weight adolescents. From these studies, it is known that obese adolescents have adiponectin up to 50% lower than adolescents with normal weight.

Polymorphisms in adiponectin gene have been identified in humans and have been studied with adiponectin concentrations in plasma and insulin resistance index. The studies lead to two types of polymorphism, namely the substitution of T to G in exon 2 (45T> G) and substitution of G to T in intron 2 (276G> T). Both types of polymorphisms are associated with obesity, insulin resistance and type 2 diabetes (Freitag, 2010).

Table 3. Description of differences in adiponectin levels pre and post workout aerobics

Variables	Aerobic exercise			
	Pre		Post	
	N	mean ± sb	mean ± sb	P
Levels of adiponectin	36	24.09 ± 12.47	39.93 ± 23.79	0.001

Table 3 presents adiponectin levels in pretest with mean score was 24.09 + 12.47 and adiponectin levels in posttest with mean score was 39.93 + 23.79. Based on the test results of paired t-test statistical, it is obtained significant value of 0.001 (P <0.05) which indicates that there is a difference between adiponectin levels before and after aerobic exercise that is combined with OCD diet.

Adiponectin gene genetic variation relationship with adiponectin levels after aerobic exercise

Based on the results of Kruskal-Wallis test, it is showed that genetic variation of TT decreased weight after aerobic exercise was combined with OCD diet as many as 19 people or 70.4% and GT decreased weight of 7 people or 87.5%, while GG 0%. From the statistical test of Kruskal-Wallis test, it is obtained value P 0:20 P> 0.05, so it can be concluded that "there was no relationship between genetic factors with weight loss after aerobic exercise was combined with OCD diet.

Tabel 4. Relationship of genetic variation with adiponectin levels

Genetic variation	Changes in adiponectin levels				P
	Decreased		Increased		
	N	%	N	%	
TT	6	22.2	21	77,8	0,74
GT	1	2,5	7	87,5	
GG	0	0.0	1	100	
Total	7	19,4	29	80,6	

From the table 4 above shows that respondents with genetic variation of TT had adiponectin levels increased by 21 respondents with a percentage of 77.8% and compared with genetic variation of GT which increased 7 respondents with a percentage of 87.5%, and genetic variation of GG with 1 respondent or 100%. From the statistical test of Kruskal-Wallis test showed that p-value was 0.74 or $P > 0.05$ and it can be concluded that there is no relationship between genetic factors with adiponectin levels after aerobic exercise is combined with OCD diet. In this research, aerobic exercise was conducted for 6 weeks in 3 times a week or 18 times the exercise that is combined with OCD diet where the respondents do not fasting during aerobic exercise schedule for 8-hour of meal time from 7 am until 3 pm and then they should not to eat but they may drink. The result showed that genetic variation of TT had higher adiponectin levels with an increased sample 21 people with a percentage of 77.8%, while the GT increased 7 people with a percentage of 87.5%, and GG 1 or 100%. With p-value of 0.74 or $P > 0.005$, it can be concluded that there is no relationship between genetic factors and the levels of adiponectin.

It can be influenced by the combination of exercise with diet where food intake of samples for the research program was less and the level of activities was low. It indicates that there is a change in increasing adiponectin levels after aerobic exercise was combined with diet. This diet is included in light diets on OCD programs and is not continued on the next OCD diet program because it is combined with exercised. And then it is continued with more genetic variation of TT. The result showed that the change of adiponectin levels tend to be increased rapidly where it is in line with the theory who said that variations of TT tend to lead to normal adiponectin levels than in GT and GG variations. However, the results of our study showed that gene variation of GT and GG also had a change of adiponectin levels increased but the frequency of increasing is slightly different from variation of TT, it can be affected by an imbalance between the amount of sample variation TT, GT and GG.

In this research, it was found that genetic variations in adiponectin gene with adiponectin levels were not significant where the genetic variation of TT increased higher than the GT and GG genetic variation. It indicates that the carrier of G allele has lower frequency adiponectin levels than the percentage of carriers of T allele. However, the percentage of changes in levels of adiponectin were higher in GT and GG variation, it can be caused by the imbalance amount of genetic variation, so the percentage of three variations do not appear.

Energy metabolism system to produce ATP may work aerobically (with oxygen) and anaerobic (without oxygen). Both of these processes may work simultaneously in the body during exercise. In low-moderate intensity exercise, the aerobic component is high, the body's energy metabolism running aerobically in the presence of oxygen through combustion deposits of carbohydrates, fats and proteins. Among all forms of stored energy contained in the body, carbohydrates and fat deposits are the main source of nutrients that will be used to provide energy for muscle contraction. Both will be the main source of energy for the body during exercise where its contribution to energy production will be determined by the intensity of the exercise and the duration of exercise [5]. In a study of the relationship between sport and adiponectin Esposito et al, it was implemented changes in diet and aerobic exercise for obese middle-aged women and observed weight loss and a significant increase in adiponectin levels.

Obesity is defined as an excessive accumulation of adipose tissue which leads to health problems and in terms of pathological has functioned as a major risk factor for metabolic syndrome. Metabolic syndrome refers to a group of disorders consisting of obesity, insulin resistance, hypertension, and hyperlipidemia which can increase the risk of developing chronic diseases.

Adiponectin is known to be associated with obesity and insulin resistance even from an early age. Adiponectin is a plasma protein with larger size of 30-kd which is secreted specifically by adipose tissue. Levels of adiponectin in obese individuals tend to decline than the levels of adiponectin with normal weight. Adiponectin is adipocytokines that acts as an important mediator in insulin and glucose metabolism. Adiponectin mechanism is currently a major

focus of research in obesity and metabolic syndrome. The name comes from the term adipocytes adiponectin and protein, thus signaling protein secreted by adipose tissue. Plasma concentrations are in the range of 5-30 mg / dl which is 1000 times higher than the hormone leptin and cortisol and approximately 1 million times higher than the cytokine tumor necrosis factor (TNF) - α and interleukin (IL) -6. In particular with high molecular weight (HMW) adiponectin are biologically active form of adiponectin and more functionally important than low molecular-weight (LMW) and medium molecular weight (MMW) adiponectin.

The role of adiponectin is described in promoting fat oxidation of liver and muscles to improve insulin sensitivity. Anti-inflammatory effect by pressing the cytokine secretion of endothelial cells, and anti-atherosclerosis effect by pressing the proliferation and migration of vascular smooth muscle cells is promoted by and platelet-derived growth factor (PDGF).

The results of other studies support that there is no relation with the amount of adiponectin, plasma lipid levels and body composite variable. However, haplotype analysis 45T / + 276G haplotype have lower adiponectin levels than in noncarriers. In addition, there are significant differences between women who have a high body fat than normal.

On the results of research conducted in the United States which compares between obesity in whites and blacks, where the result showed that adiponectin levels in whites were higher than in blacks. The research results of Agus sulaeman (2011) explained that the genetic variation of adiponectin gene has no significant relationship with adiponectin levels [6]. It is in line with the theory that genetic variation of TT tends to increase adiponectin levels of GG.

Based on population studies, it is known that the concentration of adiponectin in several races in the world is not the same. Children who come from African-American race had lower adiponectin concentrations compared to the Caucasian race.

Giannopoulou et al in his observations found that there is no change in adiponectin after 6 months of aerobic exercise, though found a decrease in blood insulin concentrations and improved insulin sensitivity [7]. In a study conducted for 6 months which aims to see their weight loss through diet and medication to reduce insulin sensitivity, it is obtained total adiponectin levels unchanged despite increased HMW adiponectin.

Various studies showed that how the effect of exercise on adiponectin levels, some of them showed conflicting results. Further studies on the effects of long-term exercise intervention on adiponectin in middle-aged women, who generally experience changes in body weight, is currently not strong enough to produce a robust conclusion. Researchers believe that the judge HMW adiponectin along with the amount of adiponectin can more effectively determine the effect of exercise on specific populations.

Adiponectin concentrations will increase if the individual is experiencing weight loss. In individuals with visceral obesity and type 2 diabetes, it is known that the expression of adiponectin decreased significantly. Based on several studies that have been conducted, found that adiponectin plays an important role in the development of insulin resistance. This cytokine levels in circulation is positively related to glucose tolerance and insulin sensitivity.

Gene expression (apM1) adiponectin in fat tissue as opposed to decline, despite there were addition in fat tissue mass of obesity. It is explained by the antagonism role between the TNF- α on adiponectin and vice versa. TNF- α experienced excessive expression in fat tissue on subjects with obesity, blocked insulin action in inhibiting the insulin receptor substrate-1 and inhibited the action of tyrosine kinase. Adiponectin has a mapping of genes on chromosome 3q27. Some analysis of SNPs (single nucleotide polymorphism) and a missense mutation found that adiponectin genes associated with metabolic syndrome. At this gene missense mutations are common; especially in the domain isoleucine at position 164 is replaced by threonine [Ile164 \rightarrow Thr (I164T)] which is common in type 2 diabetes mellitus and coronary heart disease [8].

Adiponectin is influenced by several factors such as environmental factors (excessive nutrition and lack of adequate physical activities) and genetic factors (SNPs [I164T]) [9]. Adiponectin may be a protective factor in the incidence of non-alcoholic fatty liver disease in obese children and that inspection diponektin levels should be the standard tests on obese children to evaluate the presence of non-alcoholic fatty liver disease [10].

Hipoadiponektinemia occurs in a variety of subjects with a variety of symptoms of metabolic syndrome [11]. Adiponectin will affect insulin sensitivity which will cause triglycerides in adipose cells to break down into excessive FFA and may cause a lot of free radicals and oxidation of fatty acids decreases so it will reduce levels of adiponectin and creates more triglycerides. Excessive triglyceride formation will lead to accumulation of liver cells that can be detected by means of ultrasound as fatty liver.

Insulin resistance occurs because of an imbalance between inflammatory factors (sTNF alpha R2) and anti-inflammatory factors (adiponectin). Insulin resistance and increased FFA considered as major factor in the pathogenesis mechanism of NSAH. Insulin resistance and hyperlipidemia cause accumulation of lipids, especially

triglycerides in hepatocytes and increase hepatocytes sensitivity to oxidative stress, expression of cytokines, inflammation and decreased ATP which causes liver cells shock [12].

In insulin resistance condition, there is acceleration of lipolysis of adipose tissue which causes an increase in hepatic FFA and increases fat oxidation. This process helps process fat accumulation in hepatocytes cells. Yoon, et, al obtain a good correlation between the results of the fat content of the liver and liver insulin resistance in normal individuals and those with diabetes mellitus [13].

Conclusion

There is a difference between adiponectin levels before and after aerobic exercise is combined with OCD diet with p-value of 0.001, but there is no relationship between genetic factors with adiponectin levels after aerobic exercise is combined with OCD diet with p-value of 0.74.

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