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Research Article

THE ROLE OF GENETIC VARIATION SINGLE NUCLEOTIDE POLYMORPHISM T45G AND SINGLE NUCLEOTIDE POLYMORPHISM G276T OF THE ADIPONECTIN GENE IN FATTY LIVER PATHOMECHANISM ON OBESE MALE SUBJECT

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ABSTRACT

Objective: The aim of this study was to observe the role of genetic variation T45G and G276T of adiponectin gene in pathomechanism of fatty liver on obese subjects.

Methods: An observational study with case–control design was conducted on 94 obese male subjects (50 subjects are obese male with fatty liver and 44 subjects are obese male without fatty liver). The research is taken place in Prodia Clinical Laboratory Makassar to determine genetic variation single nucleotide polymorphism (SNP) T45G (Genotype TT, thyroglobulin [TG], and GG) and SNP G276T (Genotype GG, GT, and TT) of adiponectin gene, technique of polymerase chain reaction-restriction fragment length polymorphism was used. The level of adiponectin, soluble tumor necrosis factor-α receptor 2 (sTNF-αR2), and insulin serum were measured by enzyme-linked immunosorbent assay method, meanwhile, fatty liver was detected by ultrasonography.

Results: The result of the study showed genetic variation, T45G of adiponectin gene was genotype TT 62.8 %, genotype TG 30.9%, and genotype GG 6.3%; meanwhile, genetic variation G276T of adiponectin gene was genotype GG 43.6%, genotype GT 38.3 %, and genotype TT 18.1 %. There was no significant correlation of genetic variation T45G as well as G276T of adiponectin gene, to the level of adiponectin serum (>0.05). Insulin resistance was more frequent on genotype TT genetic variation T45G of adiponectin gene as compare to that on TG+GG (p=0.069). Genotype TT on genetic variation T45G of adiponectin gene was significant correlated with fatty liver (p=0.010). Genotype TG+TT on genetic variation G276T of adiponectin gene was more likely to have insulin resistance and fatty liver than that of genotype GG. Allele T carrier on genetic variation T45G and G276T of adiponectin gene had a higher chance to have insulin resistance and fatty liver as compare to that of allele G carrier. The odds ratio of having fatty liver insulin resistance is 5.3, genotype TT on genetic variation T45G of adiponectin gene is 3.8, low level of adiponectin is 3.4, and high level of sTNF-αR2 is 3.3.

Conclusion: Genotype TT on genetic variation T45G of adiponectin gene has a role in fatty liver on obese subjects. Genotype TG+TT on genetic variation G276T of adiponectin gene was more high frequent to have fatty liver compare to that on genotype GG. Allele T carrier on genetic variation T45G of adiponectin gene had higher frequency on the occurrence of insulin resistance than that on allele G carrier. Insulin resistance has the highest influence as compare to genotype TT on genetic variation T45G of adiponectin gene, low level of adiponectin serum, high level of sTNF-αR2 serum in the pathomechanism of fatty liver in obese subjects.

Keywords: Genetic variation, Adiponectin, Fatty liver.

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INTRODUCTION

Fatty liver is a condition characterized by liver steatosis, an excessive buildup of triglycerides and other fats in liver cells caused by an imbalance between the production and release of triglycerides by the liver [1,2].

Insulin resistance is a key component in obesity and is known as a risk factor for the occurrence of non-alcoholic fatty liver disease (NAFLD) [3,4].

Adiponectin and leptin each play a role in increasing the oxidation rate of fatty acids and reducing fat in muscle, which is the key for improving insulin's effect [5].

Molecularly, adiponectin may increase insulin sensitivity by enhancing the effects of insulin suppression on gluconeogenesis and increasing the oxidation of fatty acids in liver cells and muscle cells through the mechanism of adenosine monophosphate kinase and peroxisome proliferator-activated receptor [6,7].

The concentration of adiponectin in plasma was found to be lower in obese subjects compared to non-obese subjects. The mechanisms underlying the decrease in plasma adiponectin concentrations are unclear, one of which is the inhibition of synthesis and secretion by large-scale locally produced tumor necrosis factor-α (TNF-α) tumors, beginning with the entry of macrophages into the adipose tissue [7,8]. Activation of TNF-α can be measured by measuring the soluble TNF-α receptor 2 (sTNF-αR2). sTNF-αR2 is associated with an increase in insulin resistance [9].

Decreased levels of adiponectin may also be due to several genetic variations that occur in the promoter, exon, intron 2, and rare mutations in exon 3 in human genes where the phenotype is obesity, insulin resistance and diabetes risk and coronary heart disease [8].

The genetic variation of adiponectin gene that is reported to be associated with insulin resistance, obesity, and risk of Type 2 diabetes in intron 2 is G276T and on exon 2 is T45G [11,12].

Research conducted by Xita *et al*. found the presence of polymorphism in the T45G position associated with insulin resistance in polycystic ovary syndrome [11]. Research conducted by Zacharova *et al*. found the G allele at position 45 as a predictor of changes in impaired glucose tolerance to Type 2 diabetes [13]. Suspected genetic variation of adiponectin also plays a role in fatty liver of obese men's subjects.

Lawrence *et al*. proposed the theory of translating pendulum hypothesis (personal communication) that occurs in the condition of impaired

glucose tolerance that is the balance of oxidative stress components and antioxidants continuously occur in the journey of life [14]. The phenomenon of translating pendulum hypothesis is also thought to occur in various metabolic disorders [14].

Pathogenesis of fatty liver is a long journey that begins one of them is the interaction between adiponectin genetic factors and the environment that causes decreased levels of adiponectin (phase molecular phenotype) [14]. Decreasing adiponectin as an anti-inflammatory marker will cause the anti-inflammatory - inflammatory balance to be impaired. If the position of inflammation is more, then there will be interference in insulin resistance (cellular phenotype phase) that potentially the happening of the liver (clinical phenotype), but if the body can do the compensation so that the inflammation can be rebalanced, then the process of fatty liver can change back to normal. Hence, the treatment for fatty liver can be done by improving insulin sensitivity by treatment and dietary abdominal decrease.

METHODS

An observational study with case–control design was conducted on 94 obese male subjects (50 subjects are obese male with fatty liver and 44 subjects are obese male without fatty liver).

This study received the approval of the Ethics Committee of Biomedical research on humans of the Faculty of Medicine, Hasanuddin University.

Subject

The population of the study was male who underwent medical checkup at Prodia Clinical Laboratory in Makassar. Research subjects are research populations that have met the criteria that have been set.

Sampling was collected by consecutive sampling, i.e., every sample who had undergone medical checkup during the research period at the Prodia Clinic Laboratory Makassar that fulfilled the above inclusion and exclusion requirements, was taken as the subject of the study, until a large sample was reached.

Inclusion criteria consist of male subjects aged ≥29 years with waist circumference of >90 cm, experiencing fatty liver and without fatty liver, subject not alcoholic, and subject not consuming angiotensin receptor blocker.

Exclusion criteria consisted of individuals high-sensitivity C-reactive protein (Hs-CRP) >10 mg/L, subjects with hepatitis B and C disease, subjects with sugar content >126 mg/dl, and subjects who were on a weight loss diet program with or without medication.

Determination of blood sugar levels

The concentration of fasting blood sugar level was measured from serum in units of mg/dl, taken in fasting state 12–14 h, measured with COBAS 501, and using enzymatic method of hexokinase. Reagents are manufactured by Roche® Mannheim - Germany with catalog number 04404483190.

Determination of Hs-CRP levels

The Hs-CRP concentration was measured from serum in units of µg/ml, with an Immulite 200 device and using chemiluminescence immunoassay method. The reagents are manufactured by Diagnostic Product Corporation with LKCRP1 catalog number.

Determination of insulin levels

Insulin concentration was measured from serum in units of mU/mL, measured by chemiluminescence immunoassay method using DPC No. reagent L2KN2 catalog in Immulite 2000 (Siemens) tool.

Insulin sensitivity was assessed by the formula homeostasis model assessment of insulin resistance (HOMA-IR)=(fasting sugar X fasting insulin level)/22.5.

Determination of adiponectin levels

The concentration of adiponectin was measured from serum in units of µg/ml, by means of reader, by enzyme-linked immunosorbent assay (ELISA) method. Reagents are manufactured by Daiichi Sekisui with lot number 004RKC ED 2006-06.

Determination of sTNF-αR2 levels

The sTNF-αR2 concentration was measured from the serum in units of pg/ml, by means of the reader, by ELISA method. Reagents are produced by Quanticine from R and D systems with DRT200 catalog numbers.

HbsAg check

HBsAg was examined from serum in units of µg/ml, with Axymm device, using microparticle enzyme immunoassay (MEIA) method with Axsym HBsAg Ver. 3 packet catalog 3B44-20.

Antihepatitis C virus (HCV) inspection

Total anti-HCV of serum in units of µg/ml was inspected, with Axymm device, by MEIA method, Axsym HBsAg Ver. 2 pack reagent, no. catalog 7A40-22.

Ultrasound examination

The ultrasound examination was performed by radiologist using the well-calibrated Medison SA-606 (Product Medison Co. Ltd - Seoul Korea) brand.

Examination of genetic variation

The genotyping of genetic variations of single nucleotide polymorphism (SNP) T45G and SNP G276T adiponectin gene was performed by allele polymerase chain reaction (PCR) amplification method from deoxyribonucleic acid (DNA) genome. DNA is extracted from blood containing ethylenediaminetriacetic acid. The PCR reaction consisted of amplification of 100 ng template DNA up to a 25 µL volume through denaturation at 950C for 5 min, followed by 35 cycles (950C for 45 s, 550C for 45 s, and 720C for 1 min and extension at 720C for 2 min) [5].

The genetic variation of SNP T45G adiponectin gene using the forward primer 5'GAAGTAGACTCTGCTGAGATGG 3' and reverse primer 5'TATCAGTGTAGGAGGTCTGTGATG 3' with the cutting enzyme of SmaI (NEB) and the cuts were then electrophoretically separated on 2% agarose gel.

The genetic variation of the SNP G276T adiponectin gene using the forward 5'CAGGAAACCACGACTCAAGG3' and reverse primer 5'GTCTAGGCCCTAGTTAATAATGAAGG3' with the cutting enzyme Stu I (Takara) and the cuts were then separated electrophoresis on 2% agarose gel.

Statistics

The data obtained were analyzed with SPSS program for Windows version 16.0. The analysis results were narrated and clarified in tables and graphs. For the statistical test, the significance level used is 5%. Statistical test of univariate method, Spearman one-tailed correlation analysis, Mann–Whitney U-test, Chi-square cross tabulation test, and multiple logistic regression test.

RESULTS

Characteristics of age and biochemical marker of fatty liver and non-fatty liver subjects

Characteristic of age and biochemical marker of fatty liver and non-fatty liver subjects were in Table 1. There were no significant differences of age between fatty liver and non-fatty liver subjects. There were statistically significant differences of the level of insulin, sTNF-αR2, HOMA-IR, level of adiponectin between fatty liver and non-fatty liver subject ($p<0.05$).

Genetic variation of SNP T45G and G276T adiponectin gene

Genotype and allele frequency of genetic variation SNP T45G and SNP G276T of adiponectin gene can be mentioned in Table 2.

Analysis of HOMA-IR, genotype TT of genetic variation SNP T45G of adiponectin gene, level of sTNF-αR2, and level of adiponectin

The correlation of genotype TT of genetic variation SNP T45G adiponectin gene, genotype GT+TT of genetic variation SNP G276T of adiponectin gene, level of adiponectin, level of sTNF-α R2, and HOMA-IR with occurrence of fatty liver can be analyzed with binary logistic analysis. The result of analysis can be shown in Table 3.

Subject with insulin resistance (HOMA ≥2) has ×5.3 higher risk in fatty liver occurrence than non-insulin resistance subject (HOMA <2). Subject with genetic variation SNP T45G of adiponectin gene has ×3.8 higher risk in fatty liver occurrence than genotype thyroglobulin (TG)+GG subject. Subject with the level of adiponectin <3.30 µg/dl has ×3.4 higher risk in fatty liver occurrence than subject with the level of adiponectin ≥3.30 µg/dl. Subject with the level of sTNF-αR2 ≥21.78 pg/mL has ×3.3 higher risk in fatty liver occurrence than subject with the level of sTNF-αR2 ≥21.78 pg/mL.

The pathomechanism of fatty liver can be proposed with resulting of correlation analysis of genotype TT of genetic variation SNP T45G adiponectin gene, genotype GT+TT of genetic variation SNP G276T of adiponectin gene, level of adiponectin, level of sTNF-αR2, and HOMA-IR was described in Fig. 1.

Table 1: Characteristic of age and biochemical marker of fatty liver and non-fatty liver

Variable	Unit	Mean±SD		p
		Fatty liver	Non-fatty liver	
		n=50	n=44	
Age	Year	42±9	44±11	0.415
Adiponectin	µg/ml	3.11±1.12	3.54±1.06	0.050
sTNF-αR2	µg/ml	23.63±5.91	20.57±5.34	0.004
HOMA-IR		2.54±1.97	1.36±0.93	0.000

p*: Mann–Whitney U-test, sTNF-αR2: Soluble tumor necrosis factor-α receptor 2, HOMA-IR: Homeostasis model assessment of insulin resistance. SD: Standard deviation

Table 2: The genotype and allele frequency of genetic variation SNP T45G and SNP G276T of adiponectin gene

Genetic variation T45G	
Genotype	n (%)
TT	59 (62.8)
TG	29 (30.9)
GG	6 (6.3)
Allele frequency	
T	137 (78.2)
G	41 (21.8)
Genetic variation G276T	
Genotype	n (%)
GG	41 (43.6)
TT	36 (38.3)
TT	17 (18.1)
Allele frequency	
G	118 (74.6)
T	70 (25.4)

SNP: Single nucleotide polymorphism, TG: Thyroglobulin

DISCUSSION

Decreased levels of adiponectin may be due to genetic or inflammatory factors that develop in the body. Genetic variations of SNP T45G and SNP G276T adiponectin gene have been studied in association with obesity, insulin resistance, and risk of Type 2 diabetes.

The results of our study showed genetic variation of T45G adiponectin genes is TT 60%, TG 29% genotype, and GG genotype 5%. The results of González-Sánchez research in Spain 2006 obtained genotype TT 61.1%, TG 31.9%, and GG 8% in men, and TT 62.6%, TG 33.5%, and GG 3.9% in women [9]. Mackevics *et al*. obtained TT 77.88% genotype, TG 21.20% genotype, and genotype GG 0.92% [13]. Xita *et a*l. (2005) in his 2002 study found TT genotype 48%, TG 40% genotype, and GG genotype 11% [12].

Our results, genetic variation of G276T adiponectin genes is GG 41%, genotype GT/TG 36%, and TT genotype 17%. The results of González-Sánchez research in Spain 2006 obtained genotype GG 51.8%, GT 42.5% genotype, and genotype TT 5.7% in men, and genotype GG 49.0%, genotype GT/TG 44.0%, and TT 7.0% genotype in women [9]. Mackevics *et al*. in his research in Austria in 2006 obtained genotype GG 50.60%, genotype TG 39.83%, and genotype TT 9.57% [13]. Xita *et a*l. (2005) found genotype GG 53%, GT genotype 39%, and TT genotype 8% [12].

The association of T45G adiponectin genetic variation with adiponectin levels was not statistically significant. In the genetic variation of T45G adiponectin gene on adiponectin levels, the TT genotype has a lower frequency of adiponectin levels than the TG+GG genotype. The association of genetic variation of G276T adiponectin gene with adiponectin levels was not statistically significant. In the genetic variation of G276T adiponectin gene showed GT+GG genotype has a lower frequency of adiponectin levels than with GG genotype. This study also showed that T allele carriers have a lower frequency of adiponectin levels than the G allele carriers. The results of our study were similar to those of González-Sánchez 2005, Mackevics *et al*. 2006 found no significant difference in adiponectin levels in the genetic variation of adiponectin G276T [11,15].

Adiponectin levels that did not differ significantly on this examination were likely due to the relatively small number of samples examined, or the likelihood of measuring adiponectin instead of the active substance in the form of high-molecular-weight (HMW). In obese individuals, oligomers adiponectin has different distributions compared with lean controls. Low-molecular-weight and HMW are closely related to obese with metabolic complications. Increasing the ratio of HMW to total adiponectin is closely related to improved insulin sensitivity during the treatment of insulin with thiazolidinedione in both humans and in mice [16]. Both *in vitro* and animal studies support the role of HMW oligomers as the main active ingredient mediating the various roles of adiponectin in liver tissue [13]. Hsieh *et al* (2015) found that serum levels of adiponectin were negatively associated with NAFLD and that percentage of body fat, BMI, and serum levels of TG and ALT were positively associated with NAFLD [17].

Table 3: The result of binary logistic analysis genotype TT of genetic variation SNP T45G adiponectin gene, genotype GT+TT of genetic variation SNP G276T of adiponectin gene, level of adiponectin, level of sTNF-αR2, and HOMA-IR with occurrence of fatty liver

Factors	B	SE	Wald	p	Exp (B)	95.0% CI for Exp (B)	
Genotype TT of SNP T45G	1.337	0.532	6.324	0.012	3.808	1.343	10.797
Genotype GT+GG of SNP G276T	0.118	0.508	0.054	0.817	1.125	0.415	3.046
Adiponectin	1.240	0.525	5.585	0.018	3.456	1.236	9.664
sTNF-αR2	1.202	0.502	5.725	0.017	3.327	1.243	8.907
HOMA	1.674	0.584	8.219	0.004	5.336	1.698	16.763
Constanta	(7.305)	1.979	13.628	0.000	0.001		

HOMA: Homeostasis model assessment of insulin resistance, sTNF-αR2: Soluble tumor necrosis factor-alpha receptor. SNP: Single nucleotide polymorphism, SE: Standard error, CI: Confidence interval

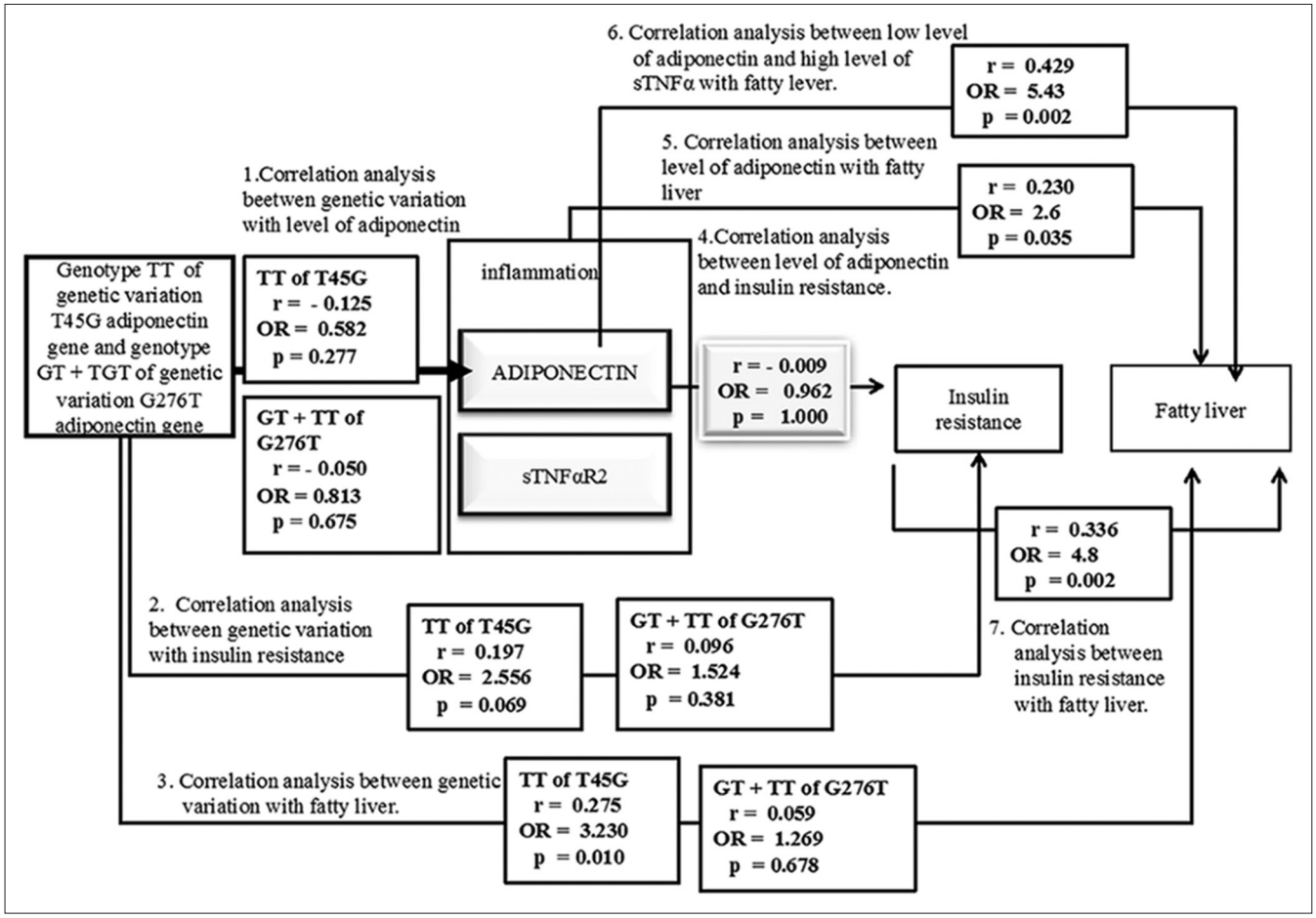

Fig. 1: Correlation of the correlation of genotype TT of genetic variation single nucleotide polymorphism (SNP) T45G adiponectin gene, genotype GT+TT of genetic variation SNP G276T of adiponectin gene, low level of adiponectin, high level of soluble tumor necrosis factor-α receptor 2 and high level of homeostatis model assesment of insulin resistance in fatty liver occuurance

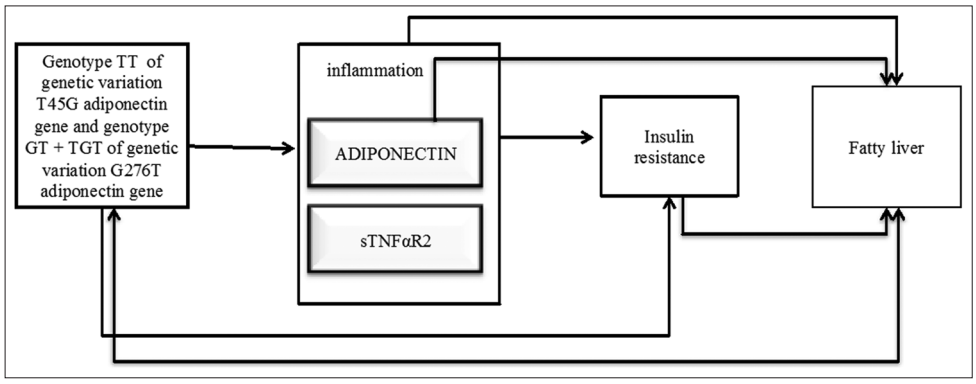

Fig. 2: The predictive pathomechanism of fatty liver occurrence on obese subject

In the genetic variation of T45G adiponectin gene, TT genotype has a higher frequency of insulin resistance than TG+GG genotype. While on the genetic variation of G276T adiponectin gene, GT+TT genotype has more frequency of insulin resistance than GG genotype. The results showed that genetic variation of T45G adiponectin gene of T allele carrier has frequency of insulin resistance incidence 34.1% while carrier of G allele 20.0%.

In the genetic variation of G276T adiponectin gene frequency of occurrence of insulin resistance on T allele carrier 35.8% while G allele carriers 27.3%. When combined these two genetic variations of T45G and G276T adiponectin genes, it can be concluded that T allele carriers play a role in the incidence of insulin resistance while G allele carriers play a role in the absence of insulin resistance.

González-Sánchez in his research using glucose tolerance impaired subjects, the value of TT genotype HOMA-IR was lower than that of TG and GG with lower TT genotype adiponectin values than TG and GG. Stumvoll *et a*l. (2002) with German populations showed that individuals with G alleles were significantly associated with increased body mass index (BMI) and decreased insulin sensitivity [11].

González-Sánchez *et a*l. (2005) in his study found that the G allele on SNP 45 had a higher prevalence of glucose tolerance disorder than

the Huang *et a*l. (2010) allele with Taiwanese population indicating that individuals with T alleles had a risk of elevated blood sugar levels [11].

In the genetic variation of T45G adiponectin gene, TT genotype has frequency of fatty liver 62.7%, genotype TG+GG 36.7%. While on genetic variation G276T, GG genotype has frequency of fatty liver 48.8%, GT+TT genotype 54.7%. The result showed that genetic variation of T45G adiponectin gene of T allele carrier had 53.4% of fatty liver frequency compared to G 34.4% allele carrier, whereas on genetic variation of G276T adiponectin gene frequency of fatty liver incidence in T 54.7% allele carrier while carrier allele G 50.6%. When combined these two genetic variations of T45G and G276T adiponectin genes, it can be concluded that T allele carriers play a role in fatty liver events, whereas G allele carriers play a role in the absence of fatty liver.

Musso *et a*l. (2008) in the study using non-alcoholic steatohepatitis (NASH) subjects found a higher frequency of steatosis, higher necroinflammation and fibrosis degree in TT genotype on T45G genetic variation of adiponectin gene compared with TG+GG genotype [14]. While on genetic variation of G276T adiponectin gene found GT/TT genotype has frequency of occurrence of steatosis, existence of nekroinflamasi and degree of fibrosis higher than GG genotype [18].

Musso *et a*l. (2008) found a link between adiponectin genetic variation and fatty liver incidence was an increase in postprandial adiponectin response to fat intake [18]. Musso *et al*. also found a decrease in mRNA expression in adipocytes carrying T allele versus G allele carriers causing decreased transcription and stability of mRNAs that may indicate the genetic role of NASH events. In this study can be seen the level of adiponectin in obese people with fatty liver is lower than those without the fatty liver [18]. Yoon, hypoadiponectinemia occurs in NAFLD individuals [19].

Kowalska *et al*. obtained higher levels of sTNF-αR2 in patients with Type 2 diabetes mellitus than controls [20]. Dzienis-Straczkowska *et al*. obtained sTNF-αR2 levels in obese subjects with impaired glucose tolerance higher than normal obese people with glucose tolerance ($p<0.05$) in both men and women [21]. González-Sánchez *et a*l. (2005) suggested a significant positive association between sTNF-αR2 and obesity as measured by BMI [11]. Studies conducted by Hui *et a*l. showed decreased adiponectin levels, elevated levels of TNF-α, and elevated soluble levels of TNF-α receptor 2 occurred in NASH subjects compared to controls [10]. Our results show that the value of HOMA-IR in liver fatty subjects is higher than that of subjects without fatty liver. Our study demonstrates the close association between adiponectin with HOMA-IR values and is associated weakly with sTNF-αR2. Yoon *et a*l. (2005) found insulin resistance to be the greatest cause of NAFLD, using the HOMA-IR method, as a calculation of elevated levels of insulin in the circulation [19]. Our results indicate the role of adiponectin against insulin resistance which then causes fatty liver, as well as the role of adiponectin balance with sTNF-αR2 to the occurrence of fatty liver provides a picture of the course of obesity conditions causing fatty liver as in Fig. 2.

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