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The Roles of Metabolic Syndrome and Several Biomarkers in Incidence and Severity of Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Backgrounds: The prevalence of NAFLD is increasing in the world. The mechanism of pathogenesis and severity has not been clearly understood. Metabolic syndrome and some biomarkers though to play a role in incidence and severity of NAFLD. **Objective:** To clarify the role of the metabolic syndrome and biomarkers: insulin resistance, adiponectin, plasma levels of TNF-α in the incidence and severity of NAFLD. We calculate diagnostic value of the metabolic syndrome with biomarkers and liver function test as non-invasive method of diagnosis severity of NAFLD. **Methods:** Conducted a case control study for risk factors of NAFLD and cross-sectional study for the diagnostic test severity of NAFLD. Cases were NAFLD patients and healthy subjects as controls. NAFLD diagnosis and severity classification based on liver biopsy with NAFLD activity score (NAS). Metabolic syndrome and insulin resistance evaluated based on IDF classification and HOMA IR index. Levels of insulin, adiponectine, and TNF-α were measured by ELISA. **Results:** Eighty cases and 75 healthy controls were included in the study. The independent risk factors for NAFLD significantly were hypo-adiponectinemia, metabolic syndrome, of insulin resistance and high plasma levels of TNF-α consecutively. Hypo-adiponectinemia was proven as independent risk factor and might be potential as diagnostic test severity of NAFLD while the metabolic syndrome, insulin resistance, high plasma levels TNF-α, were not proven as risk factors severity of NAFLD **Conclusions:** The presence of metabolic syndrome, insulin resistance, hypo-adiponectinemia high levels of plasma TNF-α was risk factors of NAFLD, while hypoadiponectinemia was proven to be risk factor and might be as diagnostic test severity of NAFLD.

Keywords: *metabolic syndrome, adiponectine, TNF- α, NAFLD*

Non-Alcoholic Fatty Liver Disease (NAFLD) was the spectrum of lesions in the liver that showed hepatic component of the metabolic syndrome (type 2 diabetes, insulin resistance, dyslipidemia, and hypertension). NAFLD is typically characterised by steatosis macrovesicular, from simple steatosis to the inflammation and persistant lesion will progress into fibrosis and cirrhosis.(1)(2)(3)

Several studies indicated that the prevalence rate of NAFLD and NASH have increased ranging from 17 to 33% for NAFLD and 5.7 to 17% for NASH.(4) Hasan Irsan (2002) reported that NAFLD was found in 30% of the population in Jakarta (5) In dr. Kariadi Hospital Semarang, the

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number of patients with fatty liver has increased by the year. The percentage of fatty liver was 4% in 2005, 4,5% in 2006, 5% in 2007, 6% in 2008 and 7% in 2009.(6) The prevalence is expected to increase in accordance with increasing population of obesity, metabolic syndrome, and development of diabetes.

NAFLD severity is defined as the most severe form of NAFLD with histopathology showing NASH characterized by fatty infiltration of lobular inflammation, hepatocyte ballooning with or without fibrosis (NAFLD activity score ≥ 5).(7) Currently NAFLD progression is also associated with various complications outside the liver. Cardiovascular complication is one of the many conditions associated with NAFLD.(8)

Pathogenesis of NAFLD have not yet clearly understood. The currently accepted explanation is the "Multiple hits hypothesis". Metabolic syndrome and several biomarkers such as: insulin resistance, adiponectin, plasma levels of TNF-α and polymorphisms of promoter gene of TNF-α may be involved.(9)(10)(11)(12)

Recent studies indicated that hypoadiponectinmia was responsible for the hepatic fat accumulation and insulin resistance. Complexity of NASH pathogenesis involves reciprocal role between adiponectin and proinflammatory cytokines produced by mononuclear cells of peripheral blood and infiltration of lymphocytes and macrophages, which are embedded in white fat tissue.(10)(13)

Insulin resistance is a major mechanism in the pathogenesis and progression of NAFLD. However, only a minority of patients with risk factors for NAFLD develop NASH, fibrosis and cirrhosis. It was still unclear why not all patients with insulin resistance develop NASH, allegedly specific changes in the liver responsible for the development of fatty accumulation and progression to inflammation. Studies in families suggest that genetic factors have a role in determining susceptibility to NASH.(14)(15)(16)

The role of TNF-α in NAFLD is crossroad of many pathogenic pathways. Specific differences between patients with hepatic steatosis and NASH is the serum level of TNF-α, which is usually higher in patients with NASH. However, this difference did not always reach statistical significance.(14)

The roles of metabolic syndrome and several biomarkers, such as insulin resistance, adiponectin, plasma levels of TNF-α in the pathogenesis of NASH require further investigation.

Aim of this study was to clarify the roles of metabolic syndrome, insulin resistance, adiponectin and plasma levels of TNF-α as risk factors for the presence and severity of NAFLD, and to analyze the diagnostic value of the metabolic syndrome with several biomarkers as non-invasive methods of diagnosis and early detection of NASH.

MATERIALS AND METHODS

Study designs were case-control to clarify risk factors of NAFLD and cross sectional for diagnostic test of NASH. Study was held in outpatient clinic of dr. Kariadi General Hospital Semarang on January 2009 – December 2011.

Inclusion criteria for case group includes: aged > 14 years old having metabolic syndrome components and proven to have NAFLD based on abdominal ultrasound. Subjects will be excluded if known to have hepatitis A, B, C virus infection (AST and ALT > 5 times higher than normal with positive IgM anti HAV or positive HBsAg or positive anti HCV); autoimmune hepatitis (positive ANA test); alcoholic hepatitis; history of alcohol consumption (> 30 gr/ day for male and > 20 gr/day fpr female); history of taking drugs causing fatty liver (glucocorticoid, estrogen, tamoxifen, amiodarone, metothrexate, valproate, diltiazem).

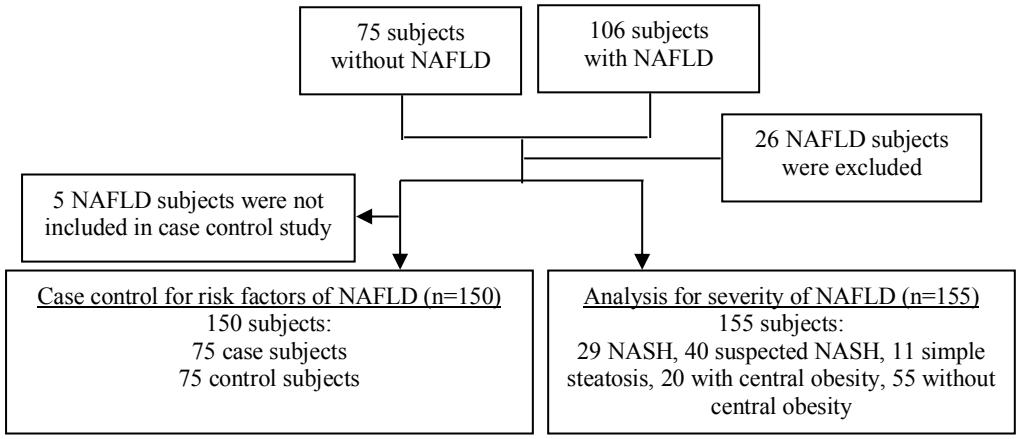
Inclusion criteria for control group includes no history, and clinical symptoms of liver disease (with normal AST and ALT, negative HbsAg, negative anti HCV) and no liver abnormality found on abdominal ultrasound. Subjects will excluded if known to have liver disease by anamnesis, physical examination and laboratory findings; hepatitis virus infection (A,B,C); autoimmune hepatitits, alcoholic hepatitis; history of alcohol consumption; and history of taking drugs causing fatty liver.

Histopathology of liver biopsy in classification according to NAS (NAFLD activity score) was performed in case group to assess the diagnosis and severity of NAFLD.(7) Diagnosis of metabolic syndrome was based on IDF classification,(17)(18) insulin resistance by index HOMA IR,(17)(18) measurement of the levels of insulin, adiponectin, and TNF-α using ELISA method.(19)(20)(21)

RESULTS

Clinical characteristics

During the study period 80 NAFLD cases and 75 controls were enrolled. To analyze risk factors for NAFLD, only 75 subjects in each group were involved in the study (figure 1). Analysis for severity of NAFLD involved all 155 subjects (figure 1).



Characteristic distribution of subjects in case and control groups were showed sex, age years and height did not differ significantly between both groups. (44,7 ±10,28 vs 44,3 ± 9,98 years old, 162,1±8,37 vs 161,1±6,31 Cm). Mean weight, body mass index and waist circumference of subjects in case group was found significantly higher than that of control group 77,0 (54,0 - 115,0) vs 56,0 (41,0 - 74,0) kg, 29,0 (22,0 - 47,4) vs 22,5 (17,3 - 27,5)kg/M2, 99,0 (77,0 - 134,0) vs 80,0 (64,0 - 98,0) cm (p< 0,001) consecutively.

Metabolic syndrome and several biomarkers of NAFLD

Distribution level of 5 components metabolic syndrome (waist circumference, blood pressure, fasting blood glucose , HDL cholesterol, fasting triglyserides) in case and control groups were significantly different (p<0,001). There was a significant relationship between each MS component and the frequency of NAFLD incidence (p <0.001). The majority of subjects in the case group (77.0%) had central obesity, 83.8% hypertension, 100% fasting blood glucose> 100 mg / dl, 78.6% low-level HDL cholesterol and 87.3% hypertriglyceridemia. Based on the number of MS components in the case group with MS, most (42.1%) had four, 44.4 % had three and 17.5% had 5 MS components. Presence of insulin resistance was greater significant in cases (86,2%) compared to the control (13,8%) group. (p<0,001) Median HOMA-IR index and insulin level in case group were higher than that of control group, 3,67 (0,40-29,00) vs 0,62 (0,02-23,58) , 12,40 (0,80-116,56) uIU/ml vs 2,74 (0,09-86,95) uIU/ml consecutively. Statistical test showed a significant correlation between insulin resistance status and presence of NAFLD (p <0,001).

Mean plasma levels of TNF-α in case group was significantly higher than in control group 7,98 (0,00 - 332,56) vs 2,24 (0,04 -77,65) pg/ml (p <0,001), while mean serum level of adiponectin in case group was significantly lower than that of control group, 2148,40 (493,80-9766,40) vs 8351,20 (1080,50 - 22364,00) (p <0,001). Cut off level for TNF-α and adiponectin as risk factors for incidence of NAFLD was > 3645 ng /cc, and < 4512 ng/cc respectively. There was significant correlation between serum levels of TNF-α and adiponectin with incidence of NAFLD (p <0,001).

Risk factors of incidence of NAFLD

Metabolic syndrome, insulin resistance, level of TNF- and adiponectin were analyzed for risk factors of incidence of NAFLD. With 95% confidence interval, all four variables were statistically significant as risk factors for the incidence of NAFLD. Highest to lowest odds ratio (OR) consecutively were level of adiponectin < 4511,94 ng/ml, OR 60,4 (21.4 - 170.4), presence of metabolic syndrome, OR 44,3 (15.5-126.8), presence of insulin resistance, OR 21,6 (9.1-51.6) and high level of TNF- >3,645 pg/ml,OR 10,8 (5.1-23.0).

Multivariate regression test for risk factors of NAFLD was performed as shown in table 1. Adiponectin < 4511,94 ng/ml and metabolic syndrome were two biggest risk factors for the incidence of NAFLD with adjusted OR 35,5 and 25 respectively

Table 1. Analysis of multivariate regression test for risk factor for NAFLD

Risk factors	*Crude*§ OR	*Adjusted* OR□ (95% confidence interval)	p*
Metabolic syndrome	44,3	25,0 (4,7 - 133,6)	< 0,001
Insulin resistance	21,6	12,4 (2,5 - 61,8)	0,002
TNF- α > 3,645	5,0	6,1 (1,3 - 19,9)	0,020
Adiponectin < 4511,94	60,4	35,5 (7,5 - 167,5)	<0,001

§ OR from bivariate test
□ OR from multivariate logistic regression test
* p value of multivariate logistic regression test

Severity of NAFLD

Severity of NAFLD was based on Histological Scoring System for Non Alcoholic Fatty Liver Disease Score and Fibrosis Staging (NASH activity score = NAS). Histopathology biopsy of the liver was performed in all subjects in case group (75 subject) and the results were NASH (38,7%), Possible NASH (50,7%) and Simple Steatosis (10,6 %). Simple steatosis and possible NASH in case group combined with the normal healthy control group were later categorized as non NASH.

Risk factors of severity of NAFLD

Bivariate analysis showed all clinical characteristics and biomarkers had significant correlation with severity of NAFLD (incidence of NASH) (table 2). Further multivariate regression test revealed that the most significant parameters correlated to the severity of NAFLD were adiponectin <2230,50 ng/ml, Exp(B)=11.5 (3.2-41), p=0.000, ALT >59,5 IU/L, Exp (B) = 8.6 (2.8-26), p =0.000 and waist circumference >95,5 cm, Exp(B) = 6.0 (1.8-20)p=0.003

Table 2. Correlation between clinical characteristic, biomarkers and severity of NAFLD

Clinical characteristic and biomarker	**Group**		**p***
	NASH n=29 n (%)	**Non NASH n=126 n (%)**	
Waist circumference (cm)			
- >94,5	23 (39,7%)	35 (60,3%)	
- ≤94,5	6 (6,2%)	91 (93,8%)	<0,001
Fasting blood glucose (mg/dL)			
- > 102,5	16 (28,1%)	41 (71,9%)	
- ≤102,5	13 (13,3%)	85 (86,7%)	<0,020
Triglycerides (mg/dL)			
- >142,5	23 (32,4%)	48 (67,6%)	
- ≤142,5	6 (7,1%)	78 (92,9%)	<0,001
HDL (mg/dL)			
- <43,5	23 (30,3%)	53 (69,7%)	
- ≥43,5	6 (7,6%)	73 (92,4%)	<0,001
ALT (IU/L)			
- >59,5	20 (54,1%)	17 (45,9%)	
- ≤59,5	9 (7,6%)	109 (92,4%)	<0,001
AST (IU/L)			
- >27,5	24 (37,5%)	40 (62,5%)	
- ≤27,5	5 (5,5%)	86 (94,5%)	<0,001
Homa IR			
- >2,21	23 (34,8%)	43 (65,2%)	
- ≤2,21	6 (6,7%)	83 (93,3%)	<0,001
TNF-α (ng/mL)			
- >9,41	18 (39,1%)	28 (60,9%)	
- ≤9,41	11 (10,1%)	98 (89,9%)	<0,001
Adiponectin (ng/mL)			
- <2230,50	25 (44,6%)	31 (55,4%)	
- ≥2230,50	4 (4,0%)	95 (96,0%)	<0,001

* x^2 test

Risk factor of severity of NAFLD was analyzed by comparing several risk factors: metabolic syndrome, insulin resistance, level of TNF-α and adiponectin. All parameters were statistically significant as risk factors of severity of NAFLD with prevalence ratio as listed in table 3.

Table 3. Analysis for risk factor of severity of NAFLD

Risk factors	Group		Prevalence ratio (95% confidence interval)	p
	NASH n=29 n (%)	Non NASH n=126 n (%)		
Metabolic syndrome (MS)				
- MS (+)	23 (35,9%)	41 (64,1%)	5,4 (2,3 - 12,6)	< 0,001*
- MS (-)	6 (6,6%)	85 (93,4%)		
Insulin resistance (IR)				
- IR (+)	23 (34,3%)	44 (65,7%	5,0 (2,2 - 11,7)	< 0,001*
- IR (-)	6 (6,8%)	82 (93,2%)		
TNF- α (pg/mL)				
- > 9,41	18 (39,1%)	28 (60,9%)	3,9 (2,0 - 7,5)	< 0,001*
- ≤ 9,41	11 (10,1%)	98 (89,9%)		
Adiponectin (ng/mL)				
- < 2230,50	25 (44,6%)	31 (55,4%)	11,0 (4,0 - 30,1)	< 0,001*
- ≥ 2230,50	4 (4,0%)	95 (96,0%)		

* x^2 test

Multivariate regression test for risk factor of severity of NAFLD is shown that Matabolic syndrome Adj OR (95% CI) 3.3 (0.9-11.5), p = 0.060, HomaIR >2.21 AdjOR 1.4 (0.4-4.8), p = 0.600, TNF-TNF- α > 9,41, AdjOR 1.6 (0.5-4.5) , p=0.400, and Adiponectinin < 2230,50 ng/ml, AdjOD 9,6 (2,6 - 34,4),p=0.001. Based on the test result, adiponectin < 2230,5 ng/ml was the most significant risk factor of severity of NAFLD. Results of analysed among clinical characteristic and biomarkers for diagnostic test severity of NAFLD (presence of NASH) proved that adiponectin < 2230 ng/ml has Sensitivity 86 %, Specificity 75%, Positive predictive value 45%, Negative predictive value 96 %, and Accuracy 77%.

DISCUSSION

This study proved the presence of metabolic syndrome, insulin resistance, low adiponectin levels, high plasma levels of TNF-α as independent risk factors for the incidence of NAFLD. Various studies have shown that obesity, type 2 diabetes, dyslipidemia, hypertension and insulin resistance (metabolic syndrome) were associated with NAFLD.[2,3,23] Multiple hit hypothesis that explained the pathogenesis of simple fatty liver to the occurrence of progression to inflammation (NASH, fibrosis and cirrhosis) involves thorough metabolic syndrome components. The first hit is the presence of fatty liver as results from imbalance formation and breakdown of triglycerides.(22)(23)(24)(25)(26) Mitochondrial dysfunction is likely; disorder of beta-oxidase in mitochondria is the main cause of fatty liver. Insulin resistance is a key abnormality underlying the metabolic syndrome, and progression to NASH. Insulin resistance will enable secretion of several adipocytokines (TNF-α, IL-6), if excessive will be dangerous for the liver tissue, changes the speed and transports triglyceride synthesis in hepatocytes and finally

increases lipolysis in adipose tissue that causes exposure to the liver tissue with more free fatty acids.(15)(22)(27)

Other factors such as inflammatory and oxidative stress will aggravate the progression of fatty liver to NASH, fibrosis, and necrosis. Metabolic syndrome is closely related to inflammation and oxidative stress conditions, individuals with metabolic syndrome, there were an increase in lipid peroxidation, IL-6, some adipocytokine eg TNF- α, reactive oxygen species (free radicals) decrease of adiponectin, and will activate stelate cells and liver fibrogenesis.(2)(3)(27)

Multivariate analysis of risk factors for the severity variables of NAFLD showed that only adiponectin which was proven to be an independent risk factor of severity of NAFLD. In accordance with these results, Hui et al (2004) also reported that adiponectin levels of NASH patients were significantly lower compared to patients with simple fatty liver,(28) although there were differences in the criteria used NAFLD degrees where the Hui, et al using Brunt criteria and this study used NAFLD activity score (NAS). Another study by Lemoine et al (2009) also reported a significant difference in adiponectin levels between NASH group with simple fatty liver.(29)

Adiponectin concentrations decreased in patients with obesity, insulin resistance, and type 2 DM and NAFLD, correlated negatively with the degree of fatty liver(30)(31) Hyperinsulinemic condition will lower adiponectin receptor expression and biological activity. Adiponectin has anti-lipogenic effects that would protect non-adipose tissues such as liver and muscle. Adiponectin was hepatoprotective by increasing the sensitivity of liver cells to insulin, which leads to increased adenosine monophosphat protein kinase (AMPK) activity of fatty acid oxidation and the inhibition of the inflammatory process and lowering sterol regulatory element,(32)(33) hepatic cell proliferation and increased apoptosis thus lowering severity NAFLD and antagonists against TNF-α .(34)(35)(36) Decrease in adiponectin causes an increase of inflammatory response characterized with the increased of TNF-α level and fibrosis of the liver tissue.(34)(35)(36)(37)(10)

Adipose tissue is a major presence of macrophage accumulation as the main source of local expression of TNF-α.(37) TNF- α is one of adipocytokine known to have antagonistic effects with adiponectin and contribute to insulin resistance, and recent studies have also alleged role in the metabolic syndrome and the progression / severity of NAFLD.(10)(38) TNF- α in animal model proved to play an important role in obesity and insulin resistance, there was also a link between levels of TNF- α and mitochondrial dysfunction that underlying liver abnormalities in NAFLD and NASH.(14)(39)

This study suggest that adiponectin may potential it is a non-invasive diagnostic parameter for determining the existence of NASH. Although liver biopsy is the gold standard for diagnosis NASH, not easily be done in clinical practice for a variety of reasons accompanying. Expected future examination of adiponectin may be one option for diagnosis NASH.

CONCLUSION

This study showed that the risk factors proven to influence the incidence of NAFLD were metabolic syndrome, insulin resistance, plasma adiponectin levels <4511 ng / ml, plasma of TNF-α levels > 3,645 pg / ml consecutively.

Risk factors proven to affect the severity of NAFLD / NASH events in patients with simple fatty liver was plasma adiponectin levels <2330,5 ng / ml, whereas metabolic syndrome, insulin resistance, plasma levels of TNF- α > 9,41 pg / ml were not proven statistically. Plasma adiponectin levels <2230,5 ng / ml have potential can be used as a non-invasive diagnostic tests of NASH.

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REFERENCES

1. William F, Balistreri M. Nonalcoholic Fatty Liver Disease: New Insight Into A Major Cause of Obesity-Related Morbidity and Mortality. Medscape Gastroenterol. 2005;
2. Jeffrey R, Lewis S. Nonalcoholic Fatty Liver Disease: A Review and Update. Dig Dis Sci. 2010;55:560–78.
3. Metin Basaranoglu, Gökcen Basaranoglu HS. From fatty liver to fibrosis: A tale of "second hit." World J Gastroenterol. 2013;13(8):1158–65.
4. Cullough A. The Epidemiology and Risk Factors of NASH. In: Farrell G, George J, M PDL, editors. Fatty Liver Disease: NASH And Related Disorders. 1st ed. USA: Blackwell

Publishing; 2005. p. 23–37.
5. Hasan I, Gani R, Machmud R. Prevalence and risk factors for nonalcoholic fatty liver in Indonesia. Vol. 17, J Gastroenterol Hepatol. 2002. A30 p.
6. Sasdesi L, Purnomo H. Data Kunjungan Pemeriksaan Ultrasonografi Abdomen Instalasi Radiologi RSUP Dr Kariadi Semarang. 2010.
7. Kleiner DE, Brunt EM, Natta M Van, Behling C, Contos MJ, Cummings OW, et al. Design and Validation of A Histological Scoring System For Nonalcoholic Fatty Liver Disease. Hepatology. 2005;41(6):1313–21.
8. Ahmed MH, Barakat S, Almobarak AO. Nonalcoholic Fatty Liver Disease and Cardiovascular Disease: Has The Time Come For Cardiologists To Be Hepatologists? J Obes. 2012;
9. Kang H, Greenson JK, Omo JT, Chao C, Peterman D, Anderson L, et al. Metabolic Syndrome Is Associated with Greater Histologic Severity, Higher Carbohydrate, and Lower Fat Diet in Patients with NAFLD. Am J Gastroenterol [Internet]. 2006 Oct 1;101:2247. Available from: http://dx.doi.org/10.1111/j.1572-0241.2006.00719.x
10. Jarrar M, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2008;27:412–21.
11. Das SK, Balakrishnan V. Role of Cytokines in The Pathogenesis of Non-Alcoholic Fatty Liver Disease. Indian J Chem Biochem. 2011;26(2):202–9.
12. Wang J, Feng Z, Li Y, Li Q, Tao X. Association of Tumor Necrosis Factor-Alpha Gene Promoter Polymorphism at Sites -308 And -238 with Non-Alcoholic Fatty Liver Disease: A Meta-Analysis. J Gastroenterol Hepatol. 2012;27:670–6.
13. Emmanuel A, Tsochatzis G, Athanasios J. Archimandritis Adipokines in Nonalcoholic Steatohepatitis: From Pathogenesis to Implications in Diagnosis and Therapy. Mediators Inflamm. 2009;
14. Méndez-Sánchez N, Arrese M, Zamora-Valdés D, Uribe M. Current Concepts In The Pathogenesis Of NAFLD. Liver Int. 2007;27(4):423–33.
15. Lonardo A, Bellentani S, Ratziu V, Loria P. Insulin Resistance In Nonalcoholic Steatohepatitis. Necessary but Not Sufficient -- Death of A Dogma from Analysis of Therapeutic Studies? Expert Rev Gastroenterol Hepatol. 2011;5(2):279–89.
16 Day C, Daly A. Nash is A Genetically Determined Disease. Fatty Liver Disease: NASH and Related Disorders. Massachusetts: Blackwell Publishing Ltd; 2007. 66-75 p.
17. Alberti S, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23(5):469–80.
18. Tina Landsvig Berentzen, Ängquist L, Kotronen A, Borra R, Yki-Järvinen H, Iozzo P, et al. Waist Circumference Adjusted For Body Mass Index and Intra-Abdominal Fat Mass. PLoS One. 2012;
19. Lee J, MJ O, Davis M, Herman W, Gurney J. Prevalence and Determinants of Insulin Resistance Among U.S Adolescents A Population-Based Study. Diabetes Care. 2006;29:2427–32.
20. Matsubara M, Maruoka S, Katayose S. Inverse Relationship Between Plasma Adiponectin and Leptin Concentrations in Normal Weight and Obese Women. Eur J Endocrinol. 2002;147:173–80.
21. Haitham T, Idriss J. TNF α and The TNF Receptor Superfamily: Structure-Function Relationship(S). Microsc Res Tech. 2000;50:184–95.
22. Sanyal A, Campbell-Sargent C, Mirshahi F, Rizzo W, Contos M, Sterling R, et al. Nonalcoholic Steatohepatitis: Association of Insulin Resistance and Mitochondrial Abnormalitie. Gastroenterology. 2001;120(5):1183–92.
23. Vernon G, Baranova A, Younossi Z. Systematic Review: The Epidemiology and Natural History of Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis in Adults. Aliment Pharmacol Ther. 2011;34(3):274–85.
24. Farrell G, Larter C. Nonalcoholic Fatty Liver Disease: From Steatosis to Cirrhosis. Hepatology. 2006;43:S99–112.
25. Russo M, Jacobson I. Nonalcaholic Fatty Liver Disease. Hosp Physician. 2002;67:36–41.
26. Angulo P. Nonalcoholic Fatty Liver Disease. N Engl J Med. 2002;346:1221–31.
27. Farrell GC, Rooyen D van, Gan L, Chitturi S. NASH is an Inflammatory Disorder: Pathogenic, Prognostic and Therapeutic Implications. Gut Liver. 2012;6(2):149–71.
28. Hui J, Hodge A, Farrell G, Kench J, Kriketos A, George J. Beyond Insulin Resistance in NASH: TNF-Alpha or Adiponectin? Hepatology. 2004;40:46–54.
29. Lemoine M, Ratziu V, Kim M, Maachi M, Wendum D, Paye F, et al. No Serum Adipokine Levels Predictive of Liver Injury in Non-Alcoholic Fatty Liver Disease. Liver Int.

2009;29(9):1431–8.
30. Djokomoeljanto R. Adiponectin And Insulin Resistance : Cardiovascular Perspective. PADVI Meet Jakarta. 2006;
31. Fernández-Real J, López-Bermejo A, Casamitjana R, W R. Novel Interaction of Adiponectin with The Endocrine System and Inflammatory Parameter. J Clin Endocrinol Metab. 2003;88(6):2714–8.
32. Perticone F, Maio R, Sciacqua A, Andreozzi F, Iemma G, Perticone M, et al. Endothelial Dysfunction and C-Reactive Protein Are Risk Factors for Diabetes in Essential Hypertension. 2008;57(January).
33. Whitehead J, Richards A, Hickman I, Macdonald G, Prins J. Adiponectin--A Key Adipokine in The Metabolic Syndrome. Diabetes, Obes Metab. 2006;8:264–80.
34. Rector R, Thyfault J, Wei Y, Ibdah J. Non-Alcoholic Fatty Liver Disease and The Metabolic Syndrome: An Update. World J Gastroenterol. 2008;14:185–92.
35. Targher G, Bertolini L, Scala L, Poli F, Zenari L, Falezza G. Decreased Plasma Adiponectin Concentrations are Closely Associated with Nonalcoholic Hepatic Steatosis in Obese Individuals. Clin Endocrinol (Oxf). 2004;61:700–3.
36. Ikejima K, Okumura K, Kon K, Takei Y, Sato N. Role of Adipocytokines in Hepatic Fibrogenesis. J Gastroenterol Hepatol. 2007;22(1):S87–92.
37. Rabelo F, Oliveira C, Faintuch J, Mazo D, Lima V, Stefano J, et al. Pro- and Anti-Inflammatory Cytokines in Steatosis and Steatohepatitis. Obes Surg. 2010;20(7):906–12.
38. Abiru S, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, et al. Serum Cytokine and Soluble Cytokine Receptor Levels in Patients with Non-Alcoholic Steatohepatitis. Liver Int. 2006;26(1):39–45.
39. Tomita K, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, et al. Tumour Necrosis Factor Alpha Signalling Through Activation of Kupffer Cells Plays an Essential Role in Liver Fibrosis of Non-Alcoholic Steatohepatitis in Mice. Gut. 2006;55(3):415–24.