Non-alcoholic Fatty Liver Disease Related to Metabolic Syndrome: a Case-control Study

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ABSTRACT

***Background**: Non-alcoholic fatty liver disease (NAFLD) is a benign condition, but it can go for years and progress to liver cirrhosis or eventually to liver cancer. Metabolic syndrome (MS) is a condition associated with NAFLD. This study was aimed to know the risk factors of NAFLD related to metabolic syndrome.*

***Method**: A case-control study was performed in NAFLD patients with or without MS and healthy individuals. All subjects were recruited from population that underwent routine medical check-up at Sardjito Hospital, Jogjakarta, during March 2007–August 2008. Diagnosis of NAFLD is defined based on clinical and liver ultrasound findings. Diagnosis of MS is defined by International Diabetes Federation on criteria for the diagnosis of MS. Data were analyzed by using T-test, ANOVA and linear regression. Odds ratio (OR) (95% CI and $p < 0.05$) was calculated by cross-tab analysis.*

***Results**: There were 84 patients enrolled in the study (group I = 30 NAFLD + MS subjects; group II = 26 NAFLD patients; group III = 28 healthy). The data showed statistically significant results in waist circumference, systole blood pressure, fasting glucose, triglyceride, high density lipoprotein (HDL) cholesterol level, homeostasis models assessment index ratio (HOMA-IR), free fatty acid (FFA), and adiponectin. The ANOVA and linear regression test among NAFLD groups showed significant difference only on HDL-cholesterol and FFA level. The lowest OR was 1.674 for HDL-cholesterol and highest OR was 13.571 for triglyceride.*

***Conclusion**: The independent factors of NAFLD related to metabolic syndrome are FFA and HDL-cholesterol level, even though a decreasing of HDL-cholesterol level has a lowest risk of NAFLD.*

***Keywords:** NAFLD, metabolic syndrome, FFA, adiponectin, HDL-cholesterol*

ABSTRAK

***Latar belakang:** Penyakit perlemakan hati non-alkohol merupakan penyakit jinak. Namun dalam jangka waktu lama penyakit ini dapat berkembang secara progresif menjadi sirosis hati dan kanker hati. Sindroma metabolik (SM) merupakan kondisi yang berkaitan dengan kejadian penyakit perlemakan hati non alkohol. Penelitian ini bertujuan untuk mengetahui faktor-faktor risiko dari kejadian penyakit perlemakan hati non-alkohol yang terkait dengan sindroma metabolik.*

***Metode:** Penelitian kasus kontrol dilakukan pada populasi dengan penyakit perlemakan hati non-alkohol dengan dan tanpa SM dan subjek sehat. Subjek didapatkan dari pasien yang menjalani pemeriksaan kesehatan rutin di Rumah Sakit Sardjito, Yogyakarta, pada bulan Maret 2007-Agustus 2008. Diagnosis penyakit perlemakan hati non alkohol ditegakkan berdasarkan pemeriksaan klinis dan ultrasonografi. Sedangkan diagnosis SM berdasarkan kriteria International Diabetes Federation. Data dianalisis menggunakan uji T untuk perbedaan rerata, ANOVA dan uji regresi linier, untuk melihat odds ratio (OR) (IK 95%; $p < 0.05$) dilakukan analisis tabulasi silang.*

***Hasil**: Terdapat 84 subjek penelitian terdiri dari grup I = 30 pasien perlemakan hati non-alkohol dan SM; grup II = 26 pasien hanya perlemakan hati non-alkohol; grup III = 28 subjek sehat. Data secara bermakna ditunjukkan pada lingkar pinggang, tekanan darah, gula darah puasa, trigliserida, HDL-kolesterol, homeostasis models assessment index ratio, asam lemak bebas dan adiponektin. Berdasarkan uji ANOVA dan regresi linier diantara grup perlemakan hati non-alkohol, hanya high density lipoprotein (HDL), dan asam lemak bebas yang berbeda bermakna. Nilai OR paling rendah adalah HDL-kolesterol (1,674) sedangkan OR paling tinggi pada trigliserida (13,571).*

***Simpulan**: Asam lemak bebas dan HDL-kolesterol merupakan faktor risiko yang bermakna terhadap kejadian perlemakan hati non-alkohol pada sindroma metabolik, meskipun penurunan kadar HDL-kolesterol memiliki risiko paling rendah terhadap perlemakan hati non-alkohol.*

***Kata kunci:** penyakit perlemakan hati non-alkohol, sindroma metabolik, asam lemak bebas, adiponektin, HDL*

INTRODUCTION

Spectrum of liver disorders in non-alcoholic fatty liver disease (NAFLD) are characterized by macro-vesicular hepatic fat accumulation only (steatosis liver), or it may be accompanied by signs of liver cell injury, infiltrate of mixed inflammatory cells, and variable hepatic fibrosis (non-alcoholic steatohepatitis/ NASH), through to cirrhosis.[1] NAFLD is a common disease with natural history of illness. It remains benign in 5-10 years period and the survival rate is 67-59%. NAFLD may progress to liver cirrhosis, and liver cancer.[2,3] The prevalence of NAFLD is 10-15%, which is increased with obesity (30-100%), hyperlipidemia (20-92%), and type 2 diabetes mellitus (T2DM) (10-75%).[2-4] Based on the third National Health and Nutrition Examination Survey (NHANES III), the prevalence of NAFLD in United States reached 16-23% and in Shanghai, it reached 12.9% in 2002.[2,5]

The prevalence of obesity in Asia is relatively low compared to Western countries; however, it is growing up into a significant public health problem.[6] The multiple hit hypotheses are important reasons for explaining the pathogenesis. The central role of insulin resistance in NAFLD had been studied. One or more characteristic(s) of the metabolic syndrome (MS) were observed in at least a third of participants. The pathogenesis of NAFLD and NASH is a combination between genetic and environment factors, which may be predicted as precursor.[2-4,7-9] Serum free fatty acid (FFA) derived from lipolysis of visceral adipose tissue are the main source of hepatic triglycerides in NAFLD, although hepatic *de novo* lipogenesis and dietary fat supply contribute to the pathogenesis of NAFLD. Approximately 10-25% NAFLD patients may develop NASH, the evolutive from of hepatic steatosis. Presumably in a genetically predisposed environment, this increased lipid overload overwhelms the oxidative capacity and reactive species are generated, leading to lipid peroxidation, cytokine induction, chemo attraction of inflammatory cells, hepatic stellate cell activation and finally fibrogenesis with extracellular matrix deposition.[10,11]

The aim of the study was to know the risk factor of NAFLD related to metabolic syndrome patients. The hypothesis of the study was that the variables occurred in metabolic syndrome may contribute to increased risk of NAFLD prevalence.

METHOD

A case-control study was performed in patients with fatty liver with or without metabolic syndrome, who were recruited from population that underwent routine medical check-up at Sardjito Hospital, Jogjakarta, Indonesia during March 2007–August 2008. Healthy patients in the control group were recruited from general population. The patients were matched for age and sex.

The inclusion criteria were patients aged 18 to 63 years old, non-alcoholic (alcohol consumption not more than ≥ 20 g/day or 2 glasses/day), fulfilled the criteria for the diagnosis of metabolic syndrome by the International Diabetes Federation (Table 1). According to definition of MS as proposed by the International Diabetes Federation criteria, metabolic syndrome is defined by central obesity plus any of two of the risk factors.[1]

Subjects with positive clinical findings signed their informed consent prior to their participation in the study. The suspicious criteria of NAFLD are "bright liver" ultrasound appearance (homogenous hyper echoic in liver parenchyma compared to right kidney), normal or slightly elevated alanine aminotransferase (ALT) and aspartate transaminase (AST) (> 30 U/L), AST/ALT ratio < 1, normal or slightly elevated gamma

glutamyl transferase (GGT) (> 35 U/L), HBsAg and anti-HCV were negative. The interpretation of liver ultrasound was conducted by two hepatologist with kappa value 0.95.

The exclusion criteria were all patients with elevated liver transaminase level (AST, ALT, GGT ≥ 2 times above the normal range), i.e. patients with hepatitis B, hepatitis C, ischemic hepatopathy, congestive hepatopathy. The exclusion criteria also included other diseases that have similar ultrasonographic findings ("bright liver"), such as malnutrition, rapid weight loss, after intestinal surgery in patients with obesity, and use of drugs that may cause steatosis.

Table 1. International Diabetes Federation Criteria for the diagnosis of metabolic syndrome[1]

Criteria	Cut-off point
Central obesity[#]	Waist circumference ≥ 94 cm (Europid male), ≥ 80 cm (Europid female); ≥ 90 cm (Asian male) and ≥ 80 cm (Asian female)
Triglycerides	≥ 150 mg/dL (1.7 mmol/L) or receiving specific treatment for this lipid abnormality
Reduced HDL-cholesterol	< 40 mg/dL (1.03 mmol/L) in male and < 50 mg/dL (1.29 mmol/L) in female or receiving specific treatment for this lipid abnormality
Raised blood pressure (BP)	Systolic BP ≥ 130 or diastolic ≥ 85 mmHg or receiving specific treatment for hypertension
Fasting plasma glucose	≥ 100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes

[#]MS is defined by central obesity plus any of two of the risk factors

The independent variables were NAFLD risk factors in population with metabolic syndrome such as hypertension (on systole and diastole blood pressure), fasting glucose and fasting insulin level, homeostasis model assessment (HOMA) index ratio, waist circumference, the serum level of triglyceride, high density lipoprotein (HDL) cholesterol, FFA, malondealdehyde (MDA), apoB, leptin, adiponectin, interleukin 6 (IL6), and tumor necrosis factor-α (TNF-α).

Prior to blood examination all subjects were instructed to have 10-hour fast, maintain normal dietary and normal daily activity during 3 days before examination. Data were analyzed by SPSS program using T-test for mean differences. ANOVA test and linear regression test for calculating significant variables with 95% confident interval (CI) and $p < 0.05$ was considered significant. The odds-ratio (OR) was calculated by cross-tab analysis for significant variables as risk factors of NAFLD.

RESULTS

There were 56 eligible patients with ultrasonographic findings of "bright liver" appearance and 28 healthy subjects. According to the criteria of metabolic syndrome (MS) proposed by the International Diabetes Federation, the 56 subjects of fatty liver ("bright liver") were divided into a treatment group patients with MS (n = 30) and a control group patients without MS (n = 26). Subjects characteristics are shown in Table 2.

Table 2.The characteristics of baseline data

Variable	Fatty liver + MS group I (mean ± SD)	Fatty liver group II (mean ± SD)	Healthy control group III (mean ± SD)	p
Age (years)	46.40 ± 5.68	46.15 ± 7.15	46.11 ± 4.56	0.979
Sex (n)				
Female	17	16	16	0.714
Male	13	10	12	
Waist circumstance (cm)	92.62 ± 8.33	90.29 ± 10.29	75.18 ± 7.78***	< 0.001
Blood pressure (mmHg)				
Systole	136.33 ± 21.81*	127.29 ± 16.15	115.53 ± 8.75***	< 0.001
Diastole	85.17 ± 10.79	81.45 ± 8.79	75.00 ± 7.58***	< 0.001
Fasting glucose (mmol/L)	7.57 ± 2.72*	5.02 ± 0.57	5.39 ± 2.37	0.002
Triglyceride (mmol/L)	2.57 ± 1.26*	1.61 ± 0.92	1.31 ± 0.49***	0.002
HDL cholesterol (mmol/L)	1.27 ± 0.19*	1.46 ± 0.33	1.49 ± 0.28	< 0.001
HOMA index ratio	4.28 ± 6.54	2.11 ± 1.67	1.42 ± 2.12**	0.030
FFA (mE)	0.91 ± 0.32*	0.60 ± 0.14	0.670 ± 0.174	< 0.001
MDA (µmol/L)	0.77 ± 0.278	0.773 ± 0.29	0.745 ± 0.331	0.935
ApoB (g/L)	115.82 ± 22.20	103.41 ± 25.34	101.73 ± 27.50	0.070
Leptin (µg/mL)	17.46 ± 14.23	22.99 ± 18.03	15.18 ± 20.48	0.346
Adiponectin(µg/mL)	3.55±1.34*	5.06 ± 2.33	7.38 ± 4.05***	< 0.001
IL6 (pg/mL)	20.071 ± 7.50	19.49 ± 9.47	18.64 ± 8.50	0.814
TNF-alpha (pg/mL)	24.07 ± 10.52	23.39 ± 12.16	22.35 ± 11.28	0.852

MS: metabolic syndrome; HDL: high density lipid; HOMA: homeostasis model assessment of insulin resistance (cut off > 2.77); FFA: free fatty acid; MDA: malondialdehyde; ApoB: apolipoprotein-B; IL: interleukin; TNF: tumor necrosis factor; ANOVA test with post hoc analysis; *significant different between group I and group II; ¨significant different between group I and group III; ¨¨significant different between group II and group III

The data showed statistically significant results in waist circumference, blood pressure (systole and diastole), fasting glucose, triglyceride, HDL cholesterol level, HOMA index ratio and adiponectin ($p < 0.05$).

Post hoc test analysis showed significant difference of blood pressure (systole and diastole), waist circumference, triglyceride level and adiponectin in group I and group II compared to healthy subjects (group III). However, fasting glucose, HDL cholesterol and FFA levels were significantly different in group I compared to group II. Data were further analyzed by one-way ANOVA test and linear regression. This study results demonstrated that variables that had been statistically significant by T-test, were still significant in one-way ANOVA test (Table 2) and should be tested further by linear regression (Table 3). The result of linear regression showed that there were two significant variables, i.e. waist circumference and FFA level, with $p < 0.05$ was considered as significant (Table 3).

Table 3. The significant variables of NAFDL risk factors in subjects with and without metabolic syndrome compared to healthy subjects

	Beta	T	95% CI	p*
Waist circumference*	-0.474	-4.527	-0.05;-0.019	< 0.001
Systole BP	-0.160	-1.141	-0.21; 0.006	0.258
Diastole BP	-0.067	-0.481	-0.03; 0.018	0.632
Fasting glucose	-0.098	-0.974	-0.005; 0.002	0.334
Triglyceride	-0.051	-0.497	0.002; 0.001	0.621
HDL cholesterol	0.135	1.662	-0.002; 0.025	0.101
HOMA index ratio	0.067	0.657	-0.061; 0.121	0.513
FFA*	-0.256	-2.673	-1.45; -0.21	0.010
Adiponectin	0.088	0.977	-0.024; 0.071	0.332

BP: blood pressure; HDL: high density lipid; HOMA: homeostasis model assessment of insulin resistance (cut off > 2.77); FFA: free fatty acid; *significance of linear regression test with $p < 0.05$

The role of metabolic syndrome as the etiopathology of fatty liver disease could be analyzed in this study. The significant variables of fatty liver with or without metabolic syndrome in this study were analyzed by ANOVA test and followed by linear regression tests. The linear regression test showed only two significant variables (HDL cholesterol and FFA) with $p < 0.05$ (Table 4).

Table 4. The significant independent risk factors for NAFDL patients with and without metabolic syndrome

	Beta	T	95% CI	p*
Systole BP	-0.328	-1.732	-0.017 ; 0.001	0.091
Fasting glucose	-0.078	-0.567	-0.004 ; 0.002	0.574
Triglyceride	0.138	0.966	-0.001 ; 0.078	0.340
HDL cholesterol	0.453	3.256	0.009 ; 0.038	0.009
FFA	-0.431	-2.845	-1.176 ; -0.199	0.007
Adiponectin	0.025	0.172	-0.066 ; 0.078	0.865

BP: blood pressure; HDL: high density lipid; FFA: free fatty acid; *linear regression test with $p < 0.05$

Based on linear regression analysis in group I compared to group II (Table 4), data were analyzed further to calculate the odd ratio of NAFLD prevalence in MS. Due to the absence of data for normal FFA range, ROC table was used to define the cut-off point for FFA level, which was determined as 0.615 mE (79% sensitivity and 50% specificity). The lowest OR was 1.674 for HDL-cholesterol and the highest OR was 13.571 for trigycerides level (Figure 1).

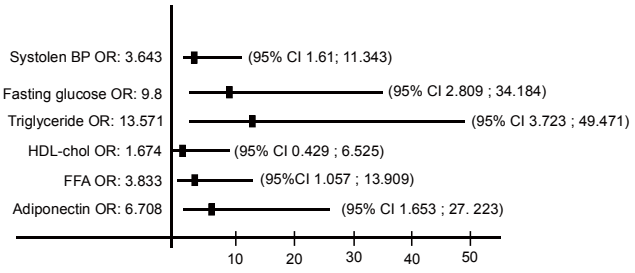

Figure 1. The odds ratio of NAFLD risk factors NAFLD in patient with metabolic syndrome

DISCUSSION

Diagnosis NAFLD in this study is defined by biochemical criteria and liver ultrasonographic findings, i.e. "bright liver" appearance with homogenous hyperechoic in liver parenchyma. It is brighter compared with right kidney and blurring of hepatic vessels. Although liver histology remains as the gold standard for diagnosis of NAFLD, particularly to distinguish steatohepatitis from simple steatosis and to assess the staging of hepatic fibrosis, but liver biopsy could not be performed in the study since there was no complaint on out-patient setting and all patients had refused the procedure. Based on population survey and The Asia-Pacific Working Party on NAFLD, the diagnosis of NAFLD is made by biochemical criteria or by hepatic imaging or both.[1]

Most of the AST and ALT levels in this study were within normal range; therefore, we did not analyze the association between AST with NAFLD. It means NASH may not occur in most patients of our study. There were some studies that have looked for the risk factors of NAFLD and NASH; however, the risk factors vary depending on population and methods of studies. The studies revealed that there are alterations of glucose metabolism and insulin resistance in subjects with normal ALT level.[12] Male gender, AST and T2DM are independently associated with NASH, and waist-hip ratio, AST. Moreover, focal hepatocytes necrosis are independently associated

with advanced fibrosis.[13] It is interesting that although AST is associated with NASH and advanced fibrosis, but the majority of our patients with either NASH or advanced fibrosis had normal AST level. There is no definite non-invasive test that may help us to predict liver fibrosis; however, AST, ALT and AST/ALT ratio may provide some guidance to determine fibrosis in NASH patients with diabetes.[14]

There are some pro-inflammatory mediators which contribute to progressing chronic inflammation associated with MS, including adipocytokines (leptin, adiponectin), TNF-alpha, IL-6, C-reactive protein, and others.[15] However, Kasyap et al found that adiponectin level was to be lower in NASH than NAFLD ($p = 0.061$).[16] The contribution of adipocytokines in NAFLD etiopathogenesis has been shown in obese studies. Increasing of TNF-alpha, IL-8, visfatin and decreasing of adiponectin were significantly higher in NAFLD patients when compared with both obese and non-obese controls ($p < 0.05$). The four independently associated factors with NASH were age, ALT, IL-8 and adiponectin ($p < 0.05$), and multivariate analysis indicated that TNF-alpha was the only independent predictor of fibrosis in NASH ($p < 0.001$).[17] A study conducted by Bugianesi et al demonstrated that adiponectin circulation in NAFLD was related to hepatic insulin sensitivity (insulin resistance) and the amount of hepatic fat content.[18] Moreover, hypo-adiponectinemia in NAFLD is a part of metabolic disturbance characterized by ectopic fat accumulation in the central compartment.[18] Level of apoB was reported higher in T2DM patients with MS compared to without MS.[19] Another study revealed that obese patients with fatty liver have lower adiponectin level and high soluble TNF-α reseptor 2 (sTNFαR2).[20]

This study also analyzed leptin, adiponectin, FFA, apoB, and pro-inflammatory cytokines (TNF-alpha and IL-6), in addition to analyzing the component of MS criteria. Previous studies reported that fatty acid delivery mechanisms is important to NASH development in severely obese individuals as hypertriglyceridemia level would increase the likehood of NASH 3-4 fold; whereas HDL-cholesterol level predicted no NAFLD ($p < 0.01$).[17] This study found hypertriglyceridemia, T2DM and hypoadiponectinemia with high OR compared to increased FFA level and systole blood pressure. Moreover, it also found the lowest OR (1.674) for HDL-cholesterol level.

The number of people who suffered from type 2 diabetes appears to be rising exponentially in the Asia Pacific region, with prevalence rates increasing from 2 to 5 fold over a period of 20 years, also to increase progressively in the next decade.[21,22] A large follow-up study performed on a general healthy population revealed that a proportion of people might develop NAFLD in 7 years.[23] According to the study, T2DM had risk of NAFLD near 10 fold compare with fatty liver without T2DM. There were shown "bright liver" and significant risk variables of NAFLD in subjects without MS, such as waist circumference, blood pressure, triglyceride and adiponectin level, compared to healthy subjects.

There were limitations in our study since the well-established specific risk factors of MS (obesity, T2DM, dyslipidemia, and hypertension) were not separately analyzed for detecting etiology, prevalence, and risk factors of steatosis or steatohepatitis.

CONCLUSION

Systolic blood pressure, fasting glucose, triglyceride, HDL-cholesterol, FFA and adiponectin are the significant factors of NAFLD prevalence in MS; however, independent factors that statistically significant were only FFA and HDL-cholesterol. The lowest risk of NAFLD was below 2 fold for HDL-cholesterol and highest was 13 fold for triglyceride. Further studies are required to evaluate the impact of NAFLD in patients with well-established obesity and T2DM or combined with other criteria of MS.

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