The Risk Factors Of Non-Alcoholic Fatty Liver Disease Prevalence In Obesity With Or Without Diabetes Mellitus


Citation

Abstract
Background. The main cause of non-alcoholic fatty liver disease (NAFLD) is insulin resistance and the one of the most common risk factors for insulin resistance is obesity. The abnormality of lipid metabolism can be seen in NAFLD such as: High level of free fatty acid (FFA) in plasma, formation of reactive oxygen species and high level of lipid peroxidation (malondialdehyde (MDA) and trans-4-hydroxy-2-nonenal). Objective. The aim of the study is to find the risk factors of NAFLD prevalence in obesity with or without diabetes mellitus. Methods. Case control study. The population was taken from general check up patients in Internal Medicine Department, Dr. Sardjito General Hospital Yogyakarta - Indonesia, March 2007 until August 2008. The case group is obesity who suffered diabetes mellitus and the control group is obesity without diabetes mellitus. The Subjects who fulfilled inclusion and exclusion criteria are enrolled in this study. Diagnosis of NAFLD is defined by Liver Ultrasound (Bright Liver appearance). AST, ALT, GGT, cholesterol, triglyceride, fasting glucose, 2 hour post-prandial glucose, insulin, Apo-B, FFA, MDA and adiponectin were examined. Data analyzed by computer using t-test for different of mean, Anova and linear regression test for significant factors (CI 95% and p < 0.05), and odd ratio for risk factors. Results. The forty six obese patients (case 23 pts, control 23 pts) are matched to age and sex. Significant difference are shown in triglyceride, FFA and adiponectin level (p: 0.013; p: <0.001; and p: 0.045). There are no significant difference in insulin resistance, cholesterol, MDA and Apo-B level. By linear regression test, triglyceride, FFA and adiponectin level are predicted as significant factor (p<0.05) with odd ratio 1.64, 12.4, and 0.9. Conclusion. Increasing of triglyceride, FFA and adiponectin level in obesity with diabetic have risk of NAFLD prevalence with odd ratio 1.64 time (triglyceride), 12.4 (FFA) and 0.9 (adiponectin).

INTRODUCTION
Fatty liver disease (hepatic steatosis) based on etiopathogenesis was divided into two categories which is Alcoholic Fatty Liver Disease (AFLD) and Non Alcoholic Fatty Liver Disease (NAFLD). Non Alcoholic Fatty Liver Disease (NAFLD) is a common disease with natural disease and benign during 5-10 years and survival rate 67-59%. However, for long time NAFLD can be growth to liver fibrosis, liver cirrhosis, and liver cancer.1,2

Prevalence of Non Alcoholic Fatty Liver Disease (NAFLD) is 10-51%, increased in obesity, hyperlipidemia, and diabetes mellitus (Metabolic Syndrome / MS).1,2,3 Previous studies of NAFLD noted that prevalence in obesity 30-100%, diabetes type II 10-75%, hyperlipidemia 20-92%, respectively.4 There is no data base for National prevalence of NAFLD and obesity in Indonesia. According to Angulo (2002), the prevalence of NAFLD is 10-24% in general population. It can reach 57.5% in obesity. Based on the third National Health and Nutrition Examination Survey (NHANES III), the prevalence of NAFLD in United States reached 16-23% 1 and in Shanghai during 2002 reached 12.9%.5

The prevalence of Type 2 Diabetes Mellitus (T2DM) is increasing globally and the Asia–Pacific region is at the forefront of the current pandemic. The prevalence of NAFLD among diabetics is reported to be from 30% to 90% in Japan, China, Korea, India, and Indonesia. In a prospective study of Indian patients with diabetes, histological significant liver disease (fibrosis grade 3 or 4) was seen in 10% of patients with NAFLD. The prevalence of NAFLD in obese people has been reported to be from 15% to 80%, with no apparent differences between countries. The prevalence of NAFLD among patients with dislipidemia ranges between 25% and 60% in reports from the Asia–Pacific region.6

Pathogenesis of NAFLD and steato-hepatitis until now,
The Risk Factors Of Non-Alcoholic Fatty Liver Disease Prevalence In Obesity With Or Without Diabetes Mellitus

combination between genetic and environment maybe predicted as a precursor. The multiple hit hypotheses is a theory that can explain the pathogenesis of NAFLD. First hit: insulin resistance will help triglyceride accumulated in hepatocytes cell, and can produce a large contain Free Fatty Acid (FFA) in serum which will be metabolized and enter into the liver. That condition can result decreasing of degradation fatty acid and triglyceride from liver, thus hyperinsulinemia, insulin resistance, and syndrome metabolic are occurred. Second hit: appearing of stress oxidative (lipid peroxides) and cytokine tumor necrosis factor and Interleukin (TNF-α; IL) which induced simple steatosis to be a steatohepatitis by activating stellate cell and necrosis of hepatocyte cell and may contribute injury cell and fibrosis too. Probability of third hit is increasing of leptin, primer protein from adipocyte that can induce resistance insulin. Occurring of leptin with stress oxidative and cytokine will induce fibrosis of the liver.1,3,4,7

The aim of the study is to find the risk factors of NAFLD prevalence in obesity subjects with or without diabetes mellitus.

MATERIALS AND METHODS

DESIGN OF THE STUDY

The case control study was performed in obesity subjects from general medical check-up population. The study take place at Internal Medicine Department, Dr. Sardjito General Hospital, Yogyakarta, Indonesia, started on March 2007 until August 2008. The subjects divided to two groups which were matched on age and sex. The case group is obesity who suffered diabetes mellitus and the control group is obesity without diabetes mellitus.

SUBJECT

The inclusion criteria: the subjects must be between 18 to 60 years old, with no history of alcohol consumption ≥ 20 gram of alcohol/day (2 glasses/ day), obese patient if Body mass index ≥ 23 kg/m² (WHO criteria for Asia) and T2 DM if fasting glucose ≥126 g/dl and 2 hour post-prandial glucose ≥ 200 g/dL (ADA criteria). Subjects are in clinical finding and already signed informed consent. The exclusion criteria: all patients that related to elevation of enzyme transaminase (AST, ALT, GGT) ≥2x above normal threshold level such as in hepatitis virus B, hepatitis virus C, hepatophaty ischemia, hepatophaty congestive. Other diseases that have similar on liver ultra sound appearance (“bright liver”) such as malnutrition, rapid weight loss, post surgery of intestines in obese, consumption of drugs that causes steatosis. The suspicious criteria of NAFLD: The liver ultra-sound shows “bright liver” (homogeneous hyper echoic in liver parenchyma compared to right kidney). Liver ultra-sound is useful in detection of steatosis (sensitivity 89%; specificity 93%), and liver fibrosis (sensitivity 77%, specificity 89%). Interpretation of liver US was conducted by two hepatologists (kappa value 0.95). Alanine Transf erase (ALT) and Aspartate Transf erase (AST) are normally or slightly elevated (>30 U/L), ratio AST/ALT < 1. Gamma Glutamyl Transf erase (GGT) is normally or slightly elevated (> 35 U/L). HBsAg and Anti HCV both are negative.

VARIABLES

The independent variables are the risk factors of NAFLD on Obesity population such as: diabetes mellitus, hyperlipidemia, insulin resistance, Apolipoprote in-B (Apo-B), Free Fatty Acids (FFA), Malondealdehyde (MDA) and adiponectin. The dependent variable is non alcoholic fatty liver disease. The variables that was examined in this study: age, height, weight, Body Mass Index (BMI), cholesterol, triglyceride, insulin, HOMA-IR, fasting blood glucose, 2-hours post-prandial blood glucose, Apo-B, MDA, FFA, adiponectin, GGT, ALT, AST. Before blood examination all subjects were fasting 10 hours and normal diet and daily activity during 3 days before blood examination.

RESULTS

Population of the study is recruited from general check-up patients. All subjects that enroll in this study are 46 obese patients: 23 subjects as case group (obesity with diabetes mellitus, and 23 subjects as control group (obesity). All both groups are matched to age and sex. The characteristic of subject is shown in table 1.

Figure 1

Table1. Base line data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obesity +DM (case group)</th>
<th>Obesity (control group)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs old)</td>
<td>47.76 ± 5.84</td>
<td>46.60 ± 5.55</td>
<td>0.514</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>34.82 ± 4.24</td>
<td>30.95 ± 4.05</td>
<td>0.566</td>
</tr>
<tr>
<td>Glucose</td>
<td>9.12 ± 6.4</td>
<td>5.50 ± 1.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting (mg/dL)</td>
<td>13.16 ± 3.31</td>
<td>6.17 ± 1.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 hour post-prandial (mg/dL)</td>
<td>9.72 ± 4.85</td>
<td>15.66 ± 2.68</td>
<td>0.282</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>1.56 ± 1.46</td>
<td>4.20 ± 7.68</td>
<td>0.696</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.59 ± 0.94</td>
<td>0.723</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>5.79 ± 4.03</td>
<td>5.65 ± 2.94</td>
<td>4.07 ± 2.19</td>
</tr>
<tr>
<td>triglycerides (mg/dL)</td>
<td>1.30 ± 0.32</td>
<td>1.40 ± 0.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Free Fatty Acids (mU/L)</td>
<td>1.14 ± 0.34</td>
<td>1.15 ± 0.25</td>
<td>0.519</td>
</tr>
</tbody>
</table>

Base on table 1, significant difference are shown in
triglyceride, FFA and adiponectin levels with p < 0.05. There are no significant difference in HOMA –IR, cholesterol, MDA and Apo-B levels (p > 0.05). The insulin resistance is defined if HOMA-IR > 2.77. The mean of HOMA-IR are more than 2.77 in both groups, but the values are not significant different. The mean of MDA value is not normal distribution because the range of mean is wide.

The three significant predicted factors (triglyceride, FFA and adiponectin) are tested by linear regression test (ANOVA test). The Anova test showed that all three predicted factors are still significant with p<0.05. (Table 2)

Figure 2
Table 2. Linear regression test for significant risk factors (Anova test)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>DM</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>1</td>
<td>6.760</td>
<td>0.013</td>
</tr>
<tr>
<td>Free Fatty Acids</td>
<td>1</td>
<td>28.139</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>1</td>
<td>4.262</td>
<td>0.045</td>
</tr>
</tbody>
</table>

The data have been continued by cross table analyzed due to find the odd ratio of significant predicted factors that was analyzed by ANOVA test before. There are not cut of point value of FFA and adiponectin, thus the data are continued analyzed by ROC curve for finding the normal concentration of FFA and adiponectin in this study. Base on ROC curve analyzed the normal value of FFA is 0.815mE, with 88% sensitivity and 72% specificity, and the normal value of adiponectin is 3.090µg/mL with sensitivity 88% and specificity 72%. The cut of point of triglyceride is 150mg/dL (1.69mmol/L).

Base on table 3, alteration of FFA have the highest odd ratio than triglyceride and adiponectin (odd ratio 1.64, 12.4, and 0.9), its mean that FFA is the strongest risk factors than adiponectin and triglyceride.

Figure 3
Table 3. The odd ratio of risk factors of NAFLD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>CI 95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td>2.391</td>
<td>1.848 to 3.077</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>1.636</td>
<td>1.034 to 2.640</td>
<td>0.034</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.933</td>
<td>0.599 to 1.454</td>
<td>0.763</td>
</tr>
</tbody>
</table>

DISCUSSION

The number of people who suffering from Type 2 Diabetes Mellitus (T2DM) 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. Asians who develop diabetes have a less degree of obesity at a younger age than Caucasians, but suffer from a higher rate of complications and premature death. The similar increases in obesity and metabolic syndrome prevalence in Asia with increasing rates of NAFLD indicate that the overall prevalence of NAFLD is likely also to increase progressively in the next decade.

Liver histology remains the gold standard for the diagnosis of NAFLD, particularly in defining steatohepatitis (NASH) versus simple steatosis, and for assessing the stage of hepatic fibrosis, both of which have prognostic implications. However, a requirement of liver biopsy to define NAFLD is often impractical for several reasons. First, poses logistic problems (cost, access, acceptability) in a region already overwhelmed with chronic viral hepatitis, both hepatitis B and hepatitis C. Second, risks of intraperitoneal bleeding pain and death (1 in 10,000). Third, arise with biopsy interpretation in NAFLD due to sampling variability and inter-observer variation in interpreting some aspects (necro-inflammatory activity). Unlike hospital-based studies, population surveys have defined NAFLD by biochemical criteria (increased serum aminotransferases and/or alkaline phosphates and gamma-glutamyl transeptidase) or by hepatic imaging (hepatic ultrasound, computerized tomography, magnetic resonance imaging), or both. In both Western series and in the large Asian population NAFLD is identified as the principal underlying cause of abnormal liver tests in persons without excessive alcohol use or viral hepatitis. The diagnosis of NAFLD in this study is based on biochemical criteria and imaging (hepatic ultrasound). The liver biopsy had not be done because of the study population is out patient without any health complaints and all subjects refused to perform biopsy.

The role of insulin resistance as a “first hit” of ethiopathogenesis of NAFLD is important because insulin resistance and compensatory hyperinsulinemia have central etiologic roles in the development of MS. The hepatic very low density lipoprotein (VLDL) overproduction as a critical underlying factor in the development of hypertriglyceridaemia, have one of the main features of MS. The reduction of HDL-cholesterol is a consequence of changes in HDL composition and metabolism. Obesity has been also described as the central causative component in the development of the MS. In both muscle cells and adipocytes of obese individuals, insulin binding to its receptor, receptor phosphorylation, tyrosine kinase activity, and phosphorylation of IRSs are reduced. Increased adipose
energy storage in obesity results in increased FFA flux to other tissues and increased triglyceride storage in these tissues, which promote insulin resistance and other adverse effects. Chronic inflammation is frequently associated with the MS and the main inflammatory mediators are adipocytokines and FFAs. Pro-inflammatory cytokines that have been associated with MS include CRP, TNF-a, IL-6 and others and they result in more insulin resistance and lipolysis of adipose tissue triglyceride stores, in enhanced hepatic glucose and VLDL production. Cytokines and FFA also increase the production of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) by the liver that complements the overproduction of PAI-1 by adipose tissue, so inducing pro-thrombotic state.  

There were some studies that look for the risk factor of NAFLD or NASH, however the risk factors are varies depended on population and methods of the study. Alterations in glucose metabolism and insulin resistance in subjects with normal ALT should also be considered in the selection of NAFLD cases for histological assessment of disease severity and progression, as we know that normal ALT usually one of exclusion criterions for biopsy in NAFLD.  

Interestingly, while AST was associated with NASH and advanced fibrosis, the majority of the patients with either NASH or advanced fibrosis had normal AST. There is no definite noninvasive test that helps to predict liver fibrosis however AST, ALT levels and AST: ALT ratio may help to determine the fibrosis in patients of NASH with diabetes in majority of cases. Our study did not analyze association between AST with NAFLD, even though most of subject with normal AST, only few subjects increased liver enzyme.

The fatty acid delivery mechanisms to NASH development in severely obese individuals is important, because hypertriglyceridemia increased the likelihood of NASH 3.4-fold, whereas high-density lipoprotein (HDL) levels predicted no NAFLD (p < 0.01), and Concentrations of TNF-alpha, leptin, and RBP4 did not differ among histological groups and did not identify NASH; however, there was a trend for adiponectin to be lower in NASH vs. no NAFLD (p=0.061). The complex interaction between adipocytokines and the pathogenesis of NAFLD seen in some obesity studies. Increasing of serum TNF-α, IL-8, visfatin and decreasing of adiponectin significantly were higher in NAFLD patients when compared with both obese and non-obese controls. The four factors were independently associated with NASH: age, ALT, IL-8 and adiponectin (P < 0.05). Multivariate analysis indicated that TNF-α was the only independent predictor of fibrosis in NASH (P < 0.0004). A circulating of adiponectin in NAFLD is related to hepatic insulin sensitivity (insulin resistance) and to the amount of hepatic fat content. Hypoadiponectinemia in NAFLD is part of a metabolic disturbance characterized by ectopic fat accumulation in the central compartment.

Our case control study is performed in obese subject with or without DM, the data showed that all subjects suffered insulin resistance (HOMA-IR >2.77). There are no significant difference of cholesterol, MDA and APO-B, however significant difference is shown in triglyceride, adiponectin and FFAs. Base on Anova test, ROC curve (FFAs and adiponectin) and two-table test, all significant variables (triglyceride, adiponectin and FFAs) are still significant by Anova test, even though only FFAs and triglyceride have significant OR (12.4 ; 1.6). Its mean that increasing of FFA and triglyceride serum in obesity with DM had risk of NAFLD 12.4 times for FFA and 1.64 times for triglyceride.

CONCLUSION
Non alcoholic fatty liver is the object of significant scientific and clinical interest which is going to increase in the following years. Epidemiological studies demonstrate that increasing prevalence of obesity and T2DM may impact to increasing of NAFLD prevalence, automatically may influence to public health. Triglyceride, FFA and adiponectin level in obesity with diabetic have risk of NAFLD prevalence with odd ratio 1.6 (triglyceride), 12.4 (FFA) and 0.9 (adiponectin). The targets of future investigations are to clarify the pathogenesis and to establish effective treatment in both NAFLD and MS.

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References
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