Clinical Picture, Insulin Resistance, and Adipocytokines Profiles of Nonalcoholic Steatohepatitis (NASH) Patients in Indonesia


* Department of Internal Medicine, Faculty of Medicine, University of Indonesia-dr. Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta Pusat. ** Department of Gastroenterology, Pertamina Central Hospital, Jakarta. *** Department of Anatomical Pathology, Faculty of Medicine, University of Indonesia-dr. Cipto Mangunkusumo Hospital, Jakarta.

Correspondence mail to: ijim@inaactamedica.org

ABSTRACT

Aim: to know the clinical picture of subjects with NASH in Jakarta, Indonesia and the prevalence of insulin resistance, TNF-α, and adiponectin levels among them.

Methods: this was a comparative cross-sectional study between patients with histopathologically confirmed NASH and normal subjects. The population of study was patients with fatty liver without history or significant consumption of ethanol. Patients were consecutively enrolled in the study if the ultrasonography showed fatty liver appearance with or without increased liver transaminases.

Results: thirty patients and thirty normal subjects were recruited between February 2005 and January 2006. Median age of the patients was 45 years while the median age of the control group was 32 years. More than 80% of the patients were overweight (BMI 23-25 kg/m²) and obese (BMI >25 kg/m²). Increased alanine aminotransaminase levels were found in almost two thirds of the patients. Other comorbidities included hypertension, hypertriglyceridemia, and type-2 diabetes mellitus. In patients with NASH, fasting insulin level, insulin resistance, and TNF-α level were significantly higher, whereas adiponectin level was significantly lower than the control group.

Conclusion: most of the metabolic syndrome determinants were found in patients with NASH. HOMA-IR and TNF-alpha levels in subjects with NASH are higher than those in controls. Adiponectin levels in subjects with NASH are lower than those in controls. Further epidemiological studies are still needed to elaborate the causal relationship of insulin resistance and cytokine profiles to the development of NASH in Indonesia.

Key word: nonalcoholic steatohepatitis, insulin resistance, adiponectin, TNF-α.

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a progressive form of liver disease which could lead to cirrhosis and liver failure.¹ The condition is believed to start from a simple, “benign” fatty liver (steatosis).³ Steatosis and NASH both represent a spectrum of disease called nonalcoholic fatty liver disease (NAFLD) which is now the most common chronic liver disease found in many countries.⁴⁻⁵ The pathogenesis of NAFLD involved insulin resistance⁶ although NASH is not universal among those with metabolic (insulin resistance) syndrome.⁷⁻⁸ However, NASH should be considered in people with metabolic syndrome.⁹ The presence of metabolic syndrome carried a high risk of NASH among NAFLD subjects, 88% of patients with NASH had a metabolic syndrome.¹⁰ The pathogenesis of NASH involved events that are known as “two-hit hypothesis” whereby the first hit, i.e. steatosis, sensitizes the liver to a variety of second “hits” which lead to necro inflammation and fibrosis.¹¹

Most studies on NASH were conducted in Western countries. Study on clinical pictures, insulin resistance and adipocytokine levels in subjects with NASH in Indonesia has not been reported yet. The objectives of this study were to know the clinical picture of subjects with NASH in Jakarta, Indonesia, and to know the prevalence of insulin resistance, TNF-α, and adiponectin levels in subjects with NASH.
METHODS

Study Design and Patient Recruitment
This was a cross-sectional study comparing patients with histopathologically confirmed NASH and normal subjects. The population of study was patients with fatty liver without history of significant consumption of ethanol. Patients were consecutively enrolled in the study if the ultrasonography showed fatty liver appearance or without increased liver transaminases. Other inclusion criteria were negative hepatitis B and C viral markers and anti-nuclear antibody (ANA), no history of alcohol abuse and drug use.

Body mass index (BMI) was calculated as body weight in kilograms divided by body height in meter square (kg/m$^2$). Obesity was defined by a body mass index of more than 25, according to World Health Organization. The diagnosis of type II diabetes mellitus was based on the Indonesian Consensus of Management and Prevention of Type 2 Diabetes Mellitus. Hyperlipidemia was diagnosed on the basis of treatment with lipid-lowering medication or raised total cholesterol and/or triglyceride levels on at least two occasions. Hypertension was diagnosed if patients were on antihypertensive therapy or the blood pressure was 140/90 mmHg or more. A complete history was obtained and physical examination was performed in all patients. Hepatomegaly was diagnosed by ultrasound.

Patients then underwent blood chemistry tests, measurement of insulin resistance and cytokine assays. Subjects with normal body mass index, blood glucose level, transaminase level and ultrasound were recruited to serve as controls.

Liver Biopsy
All patients underwent ultrasound-guided liver biopsy using a 16-gauge Menghini type needle (Hepafix, B. Braun Melsungen AG, Germany) under local anesthesia. Specimens were graded and staging was done according to Brunt criteria. The degree of steatosis was graded 1 to 3, according to the percentage of cells with fatty droplets (grade 1: 0-33%, grade 2: 34-66%, grade 3: 67-100%). The stage of fibrosis was measured on a 5-point scale (F0 = normal connective tissue; F1 = foci of perivenular and/or perisinusoidal fibrosis in zone 3; F2 = perivenular or pericellular fibrosis affecting zones 3 and 2; F3 = septal or bridging fibrosis; and F4 = cirrhosis).

Measurement of Insulin Resistance
Insulin resistance was measured with homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (iU/mL) x plasma glucose (mg/dL)/405. This model was developed by Matthews and modified later.

Cytokine Assays
TNF-α and adiponectin levels were measured with enzyme-linked immunosorbent assay technique (Quantikine ELISA; R&D Systems, Minneapolis, MN) which were done in a private laboratory (PRODIA Laboratory, Jakarta).

Data Analysis
Characteristics of the patients and controls were presented descriptively. Comparative analysis was performed between subjects with NASH and controls on fasting insulin level, insulin resistance, adiponectin and TNF-α levels. Statistical analysis was done using computer software SPSS for Windows PC version 12 (SPSS Inc, Chicago, IL). A significant level of 5% was used.

RESULTS
A total of 30 patients were recruited between February 2005 and January 2006. Almost two-thirds of the patients were men. The median age of the patients was 45 years, with a range of 18 to 61 years. Among the control group, both sexes were equally represented; the median age was 32 years ranging from 19 to 52 years. The patients’ characteristics were significantly different from the controls because there are no evidence on age yet in previous NASH study. (Table 1)

Almost half of the patients presented with dyspepsia and hepatomegaly. More than 80% of patients were overweight or obese. Increased liver transaminase levels were found in almost two-thirds of the patients. The AST/ALT ratio was less than 1 in all NASH patients. Other comorbidities included hypertension, hypertriglyceridemia, and type 2 diabetes mellitus. (Table 2)
Fasting insulin level, insulin resistance, and TNF-α level significantly higher in patients with NASH than controls. Adiponectin level was significantly lower in patients with NASH than the control group for both male and female group. Adiponectin level still showed significantly lower in male NASH group compared to normal male group and normal female group as we know that adiponectin level is lower in men compared to women. (Table 3 and 4)

Liver biopsy showed hydropic degeneration and lobular inflammation typical for NASH in all patients. More than half of the patients were classified as having F2 and 70% of them had grade 2-3 steatosis. (Table 5)

DISCUSSION

This study was previously designed to distinguish clinical characteristics and cytokine profiles of nonalcoholic fatty liver patients with NASH and without NASH. Surprisingly, all subjects who met the inclusion criteria have already had NASH, the most severe form of NAFLD. This might be due to the fact that our hospitals are referral hospitals. A recent study in Malaysia showed that NASH was found in 84.3% of the patients, using the same inclusion criteria as ours. The presence of NASH in all patients could also be due to selection bias; subjects in this study were highly selected patients who had chronic liver disease with fluctuating serum ALT for years and were referred to a hepatologist for further evaluation. These patients had neither positive serologic test for hepatitis B or C markers and ANA nor a history of excessive alcohol consumption or drug abuse. Thus, ‘normal subjects’ were recruited to serve as controls for insulin resistance and cytokine profiles. Apparently, the normal subjects who had normal BMI, ALT, blood glucose, triglyceride levels and ultrasonogram were significantly younger than the patients with NASH.

Almost half of our patients presented with dyspepsia and hepatomegaly. These symptoms are not specific to NASH but vague discomfort over the liver with hepatomegaly is common among patients with NASH. Clinically, NASH is usually suspected if liver function tests show abnormalities in an apparently healthy, asymptomatic person. However, our study showed that one-third of patients had normal serum ALT level. However, a study has shown that advanced fibrosis (bridging fibrosis and cirrhosis) could be found in NAFLD patients with normal ALT level. Liver biopsy is necessary to find fibrosis or inflammation in the presence of fat which could not be seen by imaging techniques. Thus, it will confirm the diagnosis of NASH. Liver biopsies in our study were offered to the patients in order to make further diagnostic assessment of their symptoms, such as abnormal ALT or dyspeptic symptoms after no other cause of liver disease was found. None of them refused to undergo this procedure.

<table>
<thead>
<tr>
<th>Table 3. Insulin resistance and cytokine profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin level (µU/mL)</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>12.76 ± 26.26</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
</tr>
<tr>
<td>Adiponectin level (ng/mL)</td>
</tr>
<tr>
<td>TNF-α level (pg/mL)</td>
</tr>
</tbody>
</table>

*(Mann-Whitney U test)

<table>
<thead>
<tr>
<th>Table 4. Adiponectin between men and women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>5006.8 ± 2109.17</td>
</tr>
</tbody>
</table>
The majority of our patients were overweight or obese with several ‘metabolic’ determinants. NASH has been associated with many clinical characteristics including type 2 diabetes mellitus, hyperlipidemia, hypertension, and obesity which collectively have been termed the metabolic syndrome or the insulin resistance syndrome. As seen in our patients, NASH occurs in the fourth or fifth decade of life.

Our current study showed that the degree of insulin resistance in NASH patients was more than twice of the normal subjects. Previously, it has shown that HOMA-IR was nearly doubled in NALFD cases compared to matched controls as a result of increased insulin concentration and normal glucose level. Impaired insulin signaling leading to hyperinsulinemia and reduced insulin sensitivity has been thought to be the cause of NASH regardless of the presence of obesity or overt diabetes.

Accumulation of lipid in the liver stimulates cytokine production such as tumor necrosis factor (TNF)-α and interleukin-6 which then influence fatty acid metabolism in the liver to form fatty liver. In the development of NASH, interaction among excessive proinflammatory cytokines in the early stage of NAFLD (steatosis) produce hepatic necro-inflammation, which could progress to cirrhosis.

TNF-α is a proinflammatory cytokine that has been implicated in the pathogenesis of insulin resistance through increased release of free fatty acids by adipocytes, reduction of adiponectin synthesis, and impairment of insulin signaling. Further studies showed that TNF-α activates stress-related kinases such as Jun N-terminal kinase (JNK) and inhibitor kappa beta kinase beta that make cells resistant to the actions of insulin. The production and activity of TNF-α is antagonized by adiponectin. Our current study showed a significantly higher level of TNF-α in patients with NASH compared to controls which accompanied by lower level of adiponectin.

Adiponectin (Acrp 30 or AdipoQ) has been found in low circulating levels in individuals with obesity and type 2 diabetes. The serum adiponectin level might depend on the visceral fat tissue content. Increased visceral fat may cause insulin resistance and low adiponectin and may cause fat accumulation in the liver. However, recent evidence has indicated that low serum hypo adiponectin might play a role in the development of NASH independently of insulin resistance. Adiponectin in the liver increases the sensitivity of insulin to inhibit gluconeogenesis and regulates hepatic free fatty acid metabolisms via suppression of lipogenesis and activation of free fatty acid oxidation. Hypoadiponectinemia plays a crucial role in the development of hepatic steatosis independently of the amount of visceral adipose tissue content. Recent finding showed that hypoadiponectinemia are early features of NASH and could be a link between impaired glucose hemostasis and liver disease.

CONCLUSION

Our study has shown that most of the metabolic syndrome features were found in patients with NASH. HOMA-IR and TNF-alpha levels in subjects with NASH are higher than controls, while adiponectin levels in subjects with NASH are lower than controls. Further epidemiological studies are still needed to elaborate the causal relationship of insulin resistance and cytokine profiles to the development of NASH in Indonesia.

REFERENCES