The relationship between endothelial dysfunction and serum aminotransferase levels in nonalcoholic fatty liver disease

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Objectives: We assessed endothelial dysfunction, a precursor finding of atherosclerosis, and its severity in relation with aminotransferase levels in patients with nonalcoholic fatty liver disease (NAFLD).

Study design: Fifty-two patients without chronic alcohol ingestion were found to have NAFLD on routine abdominal ultrasonographic examination. Aminotransferase enzyme levels were normal in 26 patients (mean age 47±10 years), and elevated in 26 patients (mean age 48±12 years). The brachial artery was examined with Doppler ultrasonography to determine endothelium-dependent flow-mediated dilation in response to reactive hyperemia. Nonendothelium-dependent vasodilation was assessed following sublingual nitroglycerine administration. The results were compared with those of a control group of 27 age- and sex-matched patients (mean age 52±11 years) without NAFLD.

Results: Patients with NAFLD had significantly higher fasting plasma glucose, HDL-cholesterol and total cholesterol levels, but the two patient groups did not differ in this respect. Baseline brachial diameters were similar in all the groups. Flow-mediated dilation in response to reactive hyperemia significantly decreased in both patient groups compared to controls, but this decrease was more prominent in patients with an elevated aminotransferase level (p=0.03). No significant differences were found between the three groups following nitroglycerine administration (p=0.05). Multivariate analysis showed NAFLD as an independent determinant of reduced endothelium-dependent vascular relaxation (beta=-0.574, p=0.000).

Conclusion: Our data suggest that elevated aminotransferase enzyme levels in patients with NAFLD may predict endothelial dysfunction and the risk for cardiovascular events.
Nonalcoholic fatty liver disease represents a spectrum of hepatic disorders characterized by macrovesicular steatosis, with histology ranging from “simple” steatosis to nonalcoholic steatohepatitis. The latter represents a shift from fatty infiltration to an inflammatory/fibrosing disease that may progress to cirrhosis. For a long time, the presence of hepatic steatosis was considered a benign manifestation with unimportant clinical significance. However, recent studies indicate a broad spectrum of clinical and pathological manifestations that develop in individuals with nonalcoholic hepatic steatosis, a condition which is termed as nonalcoholic fatty liver disease (NAFLD).[1,2] This condition affects approximately 15-30% of the general population, with an increased prevalence up to 70-90% in people with obesity or type 2 diabetes.[3-5]

Both NAFLD and atherosclerosis have common risk factors such as obesity, hyperlipidemia, and insulin resistance which are associated with high morbidity and mortality.[6,7]Moreover, NAFLD itself may result in endothelial dysfunction, which is a precursor finding of atherosclerosis in patients without overt atherosclerosis.[8,9] In this study, we aimed to assess endothelial dysfunction in NAFLD patients and its severity in relation with normal and increased aminotransferase levels.

**PATIENTS AND METHODS**

This study was performed by cardiology and gastroenterology departments. Fifty-two patients without chronic alcohol ingestion were included. All the patients had mild degree of hepatosteatosis (NAFLD) on routine abdominal ultrasonographic (USG) examination. Of these, the level of aminotransferase enzyme was normal in 26 patients (mean age 47±10 years), and 1.5-2 times above the normal level in 26 patients (mean age 48±12 years). The control group consisted of 27 age- and sex-matched patients (mean age 52±11 years) without NAFLD. All the subjects participating in the study gave informed consent. Ingestion of alcohol was less than 40 gr/week in all the patients.

Exclusion criteria included the following: viral hepatic diseases; autoimmune hepatic diseases; ingestion of more than 40 gr ethanol/week; alpha-1 antitrypsin deficiency; hemochromatosis/transferrin saturation >60%; Wilson’s disease; toxic hepatic disease diagnosed within the past six months; use of hepatotoxic drugs within six months; use of medications for NAFLD within six months; gastrointestinal surgery, gastropexy, jejunoileal bypass, extensive small bowel resection, and biliopancreatic diversion surgery; biliary obstruction and primary biliary cirrhosis; pregnancy; loss of more than 20% of total body weight within three months; cancer; impaired cognitive functions; age older than 65 years; treatment with total parenteral nutrition within six months; known atherosclerotic vascular disease or a positive treadmill test with two or more risk factors for atherosclerosis.

After 12 hours of fasting, serum aspartate transaminase (AST), alanine transaminase (ALT), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, total cholesterol, triglyceride, and fasting glucose levels, and hepatitis markers were determined. Height, weight, waist circumference, and body mass index were measured and recorded.

**Brachial artery assessment.** Echocardiographic examination was performed using the Vivid 7 system (General Electric, Horten, Norway) with a 2.5 to 3.5 MHz transducer. The brachial artery was examined with Doppler USG (Vivid 7) by experienced cardiologists using a 10 MHz linear array probe. Each participant was studied in the morning following abstinence from caffeine, tobacco, and food for eight hours before the study. Endothelium-dependent flow-mediated dilation of the brachial artery was assessed noninvasively in response to reactive hyperemia. Briefly, a nontortuous segment of the brachial artery above the antecubital fossa was identified and scanned in a longitudinal fashion. Once depth and gain settings were adjusted to optimize images of the lumen to arterial wall interface, baseline scanning was performed with images magnified in a 20x20-mm viewing window. A pneumatic blood pressure cuff positioned above the elbow was then inflated for five minutes at a pressure of 250 mmHg, after which the cuff was released and the artery imaged continuously for five minutes. After 10 minutes of allowing participants to return to baseline conditions, images of baseline and three minutes after 400 µg sublingual nitroglycerine administration were obtained to assess nondendothelium-dependent vasodilation. End-diastolic frames (coincident with the electrocardiographic R wave) from three consecutive cardiac cycles of the baseline, reactive hyperemia, and post-nitroglycerine images were digitized and analyzed by one observer blinded to the protocol. To minimize reader bias, a second observer who was also blinded to the protocol analyzed digitized
Endothelium-dependent flow-mediated dilation and nonendothelium-dependent vasodilation were calculated as the percent changes in brachial artery diameter compared to the baseline, one minute after reactive hyperemia and three minutes after sublingual nitroglycerine administration, respectively. The interobserver and intraobserver variabilities for the measurements of brachial artery diameter were less than 4%.

Statistical analysis. Data were expressed as mean± standard deviation (SD). All statistical analyses were processed using SPSS for Windows (version 11.5). One-way ANOVA test followed by Tukey’s post-hoc analysis was used for comparison of the study groups. Univariate correlations were sought using the Spearman’s correlation analysis. Multivariate analyses included stepwise regression and the general linear model to seek independent determinants of reduced endothelium-dependent vasodilation among increased serum lipids, glucose, HbA1c parameters, and NAFLD. All probability values were two-tailed, and a p value of less than 0.05 was considered statistically significant.

RESULTS

Demographic, clinical, and biochemical characteristics of the study groups are summarized in Table 1. Among the three groups, there were no significant differences with regard to age, smoking habit, LDL-cholesterol level, and family history of atherosclerosis. Patients with NAFLD exhibited significantly higher fasting plasma glucose, HDL-cholesterol and total cholesterol levels (Table 1), but the two patient groups did not differ in this respect.

Baseline brachial diameters did not differ significantly between the three groups (Table 2). Flow-mediated dilation in response to reactive hyperemia significantly decreased in both patient groups compared to controls, indicating impaired vascular response (Table 2). This decrease was more prominent in patients with an elevated aminotransferase level compared to those with a normal enzyme level (p=0.03). No significant differences were found between the three groups with respect to nitroglycerine-induced vasodilation rate (p>0.05).

The severity of NAFLD as assessed by enzyme elevation was inversely correlated with flow-medi-
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There was no relationship between NAFLD and nonendothelium-dependent vasodilation (p=0.6). After correction of factors negatively affecting endothelial functions, intergroup analysis showed that patients with elevated enzyme levels had more prominent endothelial dysfunction than those having normal levels.

In multivariate analysis including NAFLD, increased serum lipids, glucose, and HbA1c parameters adjusted as covariates, NAFLD was found as the only independent determinant of reduced endothelium-dependent vasodilation (beta= -0.574, p=0.000).

DISCUSSION

Endothelial dysfunction is an important process accepted as a predictor of atherosclerosis. Several clinical factors that play a major role in etiologies of both endothelial dysfunction and NAFLD are common, such as obesity, diabetes mellitus, dyslipidemia, metabolic syndrome, and carbohydrate-rich nutrition. This led us to seek a correlation between NAFLD and endothelial dysfunction and to assess the usefulness of NAFLD as a cardiac risk factor. The basic mechanism of endothelial dysfunction is impairment of endothelium-dependent vasodilation resulting from imbalance of mediators affecting endothelium for vasoconstriction. Release of nitric oxide from endothelial cells is mainly regulated by potassium channels and this mechanism is responsible for flow-induced vasodilation. Oxidative stress occurs when antioxidant defence mechanisms become inadequate and leads to endothelial dysfunction initially by impairing endothelium-dependent vasodilation. Persisting oxidative stress and inadequate antioxidant mechanisms could give rise to coronary artery disease evident by atherosclerotic plaques. Targher et al. showed that NAFLD was associated with an increased risk for future cardiovascular events among type 2 diabetic patients. Importantly, this association was independent of classical risk factors, liver enzymes, and metabolic syndrome, a highly atherogenic condition that is strongly correlated with NAFLD. As a strong indicator of the correlation between oxidative stress and atherosclerosis, reduced activity of superoxide dismutase (SOD) enzyme, one of the intracellular antioxidants, was shown in atherosclerotic cells of human coronary artery. Noninvasive methods such as flow-mediated dilation of the brachial artery have been developed to assess peripheral arterial endothelial functions as an alternative to invasive methods to evaluate coronary risk factors.

It has been shown that non-diabetic patients with NAFLD have significantly decreased brachial artery endothelial flow-mediated vasodilation compared to matched healthy controls, and that this decrease is correlated with histological features of NAFLD independent of age, sex, body mass index, HOMA-insulin resistance, and other metabolic syndrome components. In our study, endothelium-dependent vasodilation was impaired in patients with elevated serum aminotransferase levels compared to those with normal enzyme levels. The relationship between endothelium-dependent vasodilation and the severity of nonalcoholic fatty liver disease in patients with normal and increased aminotransferase levels compared to the control group was analyzed using Spearman’s correlation coefficient. The results showed a significant negative correlation between endothelium-dependent vasodilation and the severity of NAFLD (r=-0.621, p=0.00). Figure 1 illustrates the analysis of correlation between endothelium-dependent vasodilation and the severity of nonalcoholic fatty liver disease in patients with normal and increased aminotransferase levels compared to the control group.

Table 2. Vascular parameters of the two patient groups with nonalcoholic fatty liver disease with normal and increased aminotransferase levels compared to the control group

<table>
<thead>
<tr>
<th>Vascular parameter</th>
<th>Control (n=27)</th>
<th>Normal (n=26)</th>
<th>Elevated (n=26)</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vessel size (mm)</td>
<td>3.7±0.4</td>
<td>3.6±0.3</td>
<td>3.6±0.4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Flow-mediated dilatation (%)</td>
<td>9.7±3.5</td>
<td>5.0±4.0</td>
<td>3.9±0.4</td>
<td>0.013</td>
<td>0.013</td>
<td>0.030</td>
</tr>
<tr>
<td>Increase in blood flow during hyperemia (%)</td>
<td>298±157</td>
<td>158±121</td>
<td>142±115</td>
<td>0.001</td>
<td>0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Increase in diameter after nitroglycerine (%)</td>
<td>11.4±3.8</td>
<td>12.1±4.8</td>
<td>11.9±4.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

p1: Comparison between the control group and patients with normal enzyme level; p2: Comparison between the control group and patients with elevated enzyme level; p3: Comparison between the patients with normal and elevated enzyme levels; NS: Not significant.

![Figure 1](image_url)
dependent vasodilation was found to be markedly reduced in patients with NAFLD compared to controls. Another important finding was that this decrease was even more prominent in NAFLD patients with elevated enzyme levels. This finding suggests that NAFLD patients with higher enzyme levels may have developed more severe and active metabolic processes causing worsened endothelial functions compared to those with normal enzyme levels. Thus, this patient group may have a higher probability for future cardiac events, requiring a close follow-up. Although high levels of fasting plasma glucose and cholesterol could be interpreted as the cause of endothelial dysfunction in the NAFLD group, the correlation between endothelium-dependent vasodilation and NAFLD was still prominent even after multivariate analysis. Furthermore, endothelial dysfunction was more pronounced in patients with higher enzyme levels, even though fasting plasma glucose and cholesterol levels were similar in the two patient groups.

Our study showed that endothelium-dependent vasodilation, an indicator of endothelial function, was significantly reduced in patients having both NAFLD and an elevated aminotransferase level.

In the presence of NAFLD, a higher risk for cardiovascular disease might be well explained by the close association of NAFLD with metabolic risk factors making up metabolic syndrome; nevertheless, cross-sectional and prospective studies support the hypothesis that NAFLD itself represents a high risk for cardiovascular disease independent of other prognostic risk factors, suggesting more complex and intertwined interrelationships between NAFLD, metabolic syndrome, and atherosclerosis. However, irrespective of what the initiating pathophysiological event is, the current body of evidence strongly emphasizes the importance of evaluating the global cardiovascular disease risk among patients diagnosed as having NAFLD. Our data suggest that the presence of elevated aminotransferase enzyme levels widely encountered in NAFLD patients may predict endothelial dysfunction and consequently the risk for cardiovascular events. Follow-up studies are necessary to determine to what extent this association affects long-term morbidity and mortality.

Limitations. Currently, Doppler USG of the brachial artery in reactive hyperemia is the most widely used method to analyze endothelial functions. In our study, peripheral Doppler USG was used to assess endothelial functions, which had been a frequently used method in previous studies. Our study results should be supported by invasive methods because peripheral Doppler USG is still a nonstandardized method and the gold standard for the diagnosis of NAFLD is percutaneous liver biopsy.

REFERENCES
16. West N, Guzik T, Black E, Channon K. Enhanced


