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

Alkole bağlı olmayan karaciğer yağlanması olan olgularda endotel disfonksiyonu ile serum aminotransferaz enzim düzeyleri arasındaki ilişki

Aydın Yıldırım, M.D.,[1] Özer Soylu, M.D.,[1] Alper Aydın, M.D.,[1] Hakan Güveli, M.D.,[2] Mehmet Ergelen, M.D.,[1] Seden Çelik,[1] M.D., Barış Güngör, M.D.,[1] Ender Özgün Çakmak, M.D.,[1] Fatih Uzun, M.D.,[1] Tuna Tezel, M.D.[1]

[1]Department of Cardiology, Siyami Ersek Cardiovascular Surgery Center, İstanbul;

[2]Department of Gastroenterology, Haydarpaşa Numune Training and Research Hospital, İstanbul

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 dilatation 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.

Key words: Brachial artery/ultrasonography; coronary arteriosclerosis/physiopathology; endothelium, vascular/ultrasonography; fatty liver/complications; risk factors.

Amaç: Alkole bağlı olmayan karaciğer yağlanması olan hastalarda, ateroskleroz gelişiminin bir öngördürücüsü olan endotel disfonksiyonu değerlendirildi ve bu durumun aminotransferaz düzeyleriyle ilişkisi araştırıldı.

Çalışma planı: Kronik alkol alımı öyküsü olmayan 52 hastanın rutin abdominal ultrasonografik incelemesinde karaciğer yağlanması saptandı. Bu hastaların 26'sında (ort. yaş 47±10) aminotransferaz enzim düzeyi normal bulunurken, 26'sında (ort. yaş 48±12) yüksek bulundu. Tüm hastalarda reaktif hiperemiye yanıt olarak, brakiyal arterden endotele bağımlı damar genişlemesi Doppler ultrasonografi ile ölçüldü. Endotele bağımlı olmayan vazodilatasyon ise dilaltı nitrogliserin uygulaması sonrası değerlendirildi. Sonuçlar, yaş ve cinsiyet uyumlu ve karaciğer yağlanması olmayan 27 hastanın (ort. yaş 52±11) sonuçlarıyla karşılaştırıldı.

Bulgular: Karaciğer yağlanması olan hastalarda açlık plazma glukoz, HDL-kolesterol ve toplam kolesterol düzeyleri kontrol grubundan anlamlı derecede yüksek bulundu; ancak, enzim düzeyi normal ve yüksek olan hastalar arasında bu açıdan fark yoktu. Reaktif hiperemi sonrasında akıma bağımlı dilatasyon, kontrol grubuyla karşılaştırıldığında her iki hasta grubunda da anlamlı derecede düşük bulundu; bu düşüklük, aminotransferaz düzeyi yüksek olan grupta daha belirgindi (p=0.03). Nitrogliserin uygulaması sonrasında gruplar arasında anlamlı farklılık gözlenmedi (p>0.05). Çokdeğişkenli analizde, alkole bağlı olmayan karaciğer yağlanmasının endotele bağımlı vasküler relaksasyondaki azalma için bağımsız belirleyici olduğu görüldü (beta= -0.574, p=0.000).

Sonuç: Bulgularımız, alkole bağlı olmayan karaciğer yağlanmasında görülen aminotransferaz yüksekliğinin endotel disfonksiyonu ve kardiyovasküler olaylar için öngördürücü olabileceğini göstermektedir.

Anahtar sözcükler: Brakiyal arter/ultrasonografi; koroner arterioskleroz/fizyopatoloji; endotel, vasküler/ultrasonografi; karaciğer yağlanması/komplikasyon; risk faktörü.

Received: January 8, 2007 *Accepted:* June 13, 2007

Correspondence: Dr. Aydın Yıldırım. Acıbadem Mah., Zeamet Sok., B 19, D: 21, 34722 Acıbadem, İstanbul.
Tel: 0216 - 349 91 20 Fax: 0216 - 326 51 81 e-mail: draydinyildirim@yahoo.com

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.[1-4]

Both NAFLD and atherosclerosis have common risk factors such as obesity, hyperlipidemia, and insulin resistance which are associated with high morbidity and mortality.[4-6] Moreover, NAFLD itself may result in endothelial dysfunction, which is a precursor finding of atherosclerosis in patients without overt atherosclerosis.[5-9] 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 nonendothelium-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

Table 1. Baseline characteristics of the two patient groups with nonalcoholic fatty liver disease with normal and increased aminotransferase levels compared to the control group

	Control (n=27)			Enzyme-normal (n=26)			Enzyme-elevated (n=26)				
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	p_1	p_2
Mean age (years)			52±11			47±10			48±12	NS	NS
Male	5	18.5		5	19.2		6	23.1		NS	NS
Smoking habit	1	3.7		2	7.7		1	3.9		NS	NS
Family history	4	14.8		5	19.2		6	23.1		NS	NS
Diabetes	–			6	23.1		5	19.2		0.033	NS
HbA1C (%)			5.01			5.97			6.01	0.001	NS
Body surface area (m^2)			1.88±1.1			1.91±0.8			1.87±1.0	NS	NS
Heart rate (beat/min)			70.7±12			67.4±14			73.6±11	NS	NS
Blood pressure (mmHg)											
Systolic			143±23			139±37			144±33	NS	NS
Diastolic			85±11			88±13			79±14	NS	NS
Ejection fraction (%)			69.4±10.3			64.1±8.8			62.9±9.1	NS	NS
Plasma glucose (mg/dl)			96.3±17.0			122.9±50.4			120.7±46.9	0.031	NS
Total cholesterol (mg/dl)			194.6±34.5			217.6±41.6			224.8±51.2	0.018	NS
HDL-cholesterol (mg/dl)			38.9±7.7			50.5±9.1			52.2±15.8	0.000	NS
LDL-cholesterol (mg/dl)			129.9±35.0			131.7±34.2			139.8±40.3	NS	NS
Triglyceride (mg/dl)			176.6±109.2			185.1±105.3			181.9±98.5	NS	NS
BUN (mg/dl)			23.9±9.8			26.8±10.3			25.7±7.0	NS	NS
Creatinin (mg/dl)			0.8±0.2			0.6±0.1			0.7±0.1	NS	NS
Uric acid (mg/dl)			5.0±1.7			5.2±1.4			5.4±1.6	NS	NS
AST (U/l)			20.8±5.3			22.9±6.0			41.2±11.3	0.01	0.000
ALT (U/l)			24.7±6.8			27.4±8.4			61.3±16.5	0.01	0.000
ALP (U/l)			201±88			205±57			220.7±91.7	NS	NS

BUN: Blood urea nitrogen; AST: Aspartate transaminase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; NS: Not significant; p_1: Comparison between the patients and the control group; p_2: Comparison between the two patient groups.

images from 35 randomly selected patients. 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-mediat-

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

		Aminotransferase level				
	Control (n=27)	Normal (n=26)	Elevated (n=26)	p_1	p_2	p_3
Baseline vessel size (mm)	3.7±0.4	3.6±0.3	3.6±0.4	NS	NS	NS
Flow-mediated dilatation (%)	9.7±3.5	5.0±4.0	3.9±0.4	0.013	0.013	0.030
Increase in blood flow during hyperemia (%)	298±157	158±121	142±115	0.001	0.001	0.04
Increase in diameter after nitroglycerine (%)	11.4±3.8	12.1±4.8	11.9±4.1	NS	NS	NS

p_1: Comparison between the control group and patients with normal enzyme level; p_2: Comparison between the control group and patients with elevated enzyme level; p_3: Comparison between the patients with normal and elevated enzyme levels; NS: Not significant

ed dilation (Spearman's analysis; (r= -0.621, p=0.00) (Fig. 1). 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.[1,2,10,11] 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.[8,9,12] The basic mechanism of endothelial dysfunction is impairment of endothelium-dependent vasodilation resulting from imbalance of mediators affecting endothelium for vasoconstriction.[13,14] Release of nitric oxide from endothelial cells is mainly regulated by potassium channels and this mechanism is responsible for flow-induced vasodilation.[1] Oxidative stress occurs when antioxidant defence mechanisms become inadequate and leads to endothelial dysfunction initially by impairing endothelium-dependent vasodilation.[11,13,15] Persisting oxidative stress and inadequate antioxidant mechanisms could give rise to coronary artery disease evident by atherosclerotic plaques.[16,17] Targher et al.[18] 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.[13] 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.[19-22]

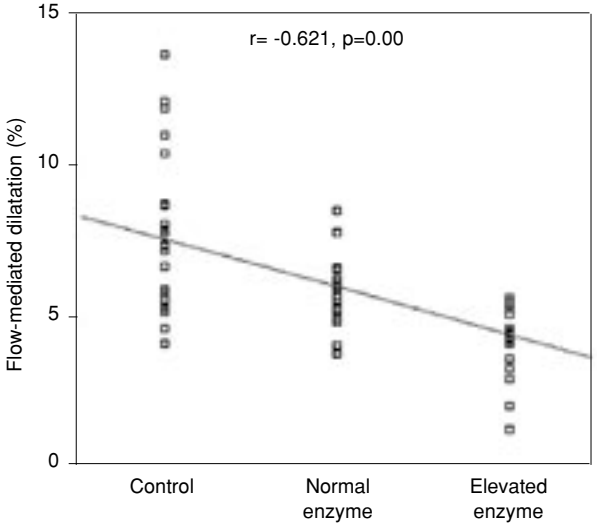

Figure 1. Analysis of correlation between endothelium-dependent vasodilation and the severity of nonalcoholic fatty liver disease in patients with normal and increased aminotransferase levels compared to the control group.

It has been shown that nondiabetic 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.[21] In our study, endothelium-

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,[23] 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

1. Adams LA, Angulo P. Recent concepts in non-alcoholic fatty liver disease. Diabet Med 2005;22:1129-33.
2. McCullough AJ. Pathophysiology of non-alcoholic steatohepatitis. J Clin Gastroenterol 2006;40(Suppl 1): S17-29.
3. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. Curr Opin Lipidol 2005;16:421-7.
4. Neuschwander-Tetri BA. Nonalcoholic steatohepatitis and the metabolic syndrome. Am J Med Sci 2005;330: 326-35.
5. Vanhoutte PM. Endothelial dysfunction and atherosclerosis. Eur Heart J 1997;18 Suppl E:E19-29.
6. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 2000;101:1899-906.
7. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Rand WM, Udelson JE, et al. Peripheral vascular endothelial function testing as a noninvasive indicator of coronary artery disease. J Am Coll Cardiol 2001;38: 1843-9.
8. Çengel A, Şahinarslan A. Nitric oxide and cardiovascular system. Anadolu Kardiyol Derg 2006;6:364-8.
9. Berberoğlu Z, Günever-Demirağ HN. Metabolik sendrom: Endotel disfonksiyonu, subklinik inflamasyon ve hiperkoagülabilite. Türkiye Klinikleri J Int Med Sci 2006;2:60-70.
10. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-8.
11. Herrmann J, Lerman A. The endothelium: dysfunction and beyond. J Nucl Cardiol 2001;8:197-206.
12. Schachinger V, Britten MB, Elsner M, Walter DH, Scharrer I, Zeiher AM. A positive family history of premature coronary artery disease is associated with impaired endothelium-dependent coronary blood flow regulation. Circulation 1999;100:1502-8.
13. Luft FC. Somatic DNA oxidative damage and coronary disease. J Mol Med 2005;83:241-3.
14. Wever RM, Luscher TF, Cosentino F, Rabelink TJ. Atherosclerosis and the two faces of endothelial nitric oxide synthase. Circulation 1998;97:108-12.
15. Sanyal AJ; American Gastroenterological Association. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002;123:1705-25.
16. West N, Guzik T, Black E, Channon K. Enhanced

superoxide production in experimental venous bypass graft intimal hyperplasia: role of NAD(P)H oxidase. Arterioscler Thromb Vasc Biol 2001;21:189-94.
17. Heinecke JW. Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. Am J Cardiol 2003;9(3A)1:12A-6A.
18. Targher G, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, et al. Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. Diabetes 2005;54:3541-6.
19. Vogel RA, Corretti MC, Plotnick GD. A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary risk factors. Clin Cardiol 2000;23: 571-5.
20. Vogel RA. Measurement of endothelial function by brachial artery flow-mediated vasodilation. Am J Cardiol 2001;88(2A):31E-4E.
21. Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001;121:710-23.
22. Kuvin JT, Karas RH. Clinical utility of endothelial function testing: ready for prime time? Circulation 2003;107:3243-7.
23. Targher G. Non-alcoholic fatty liver disease as a determinant of cardiovascular disease. Atherosclerosis 2007;190:18-19.