Review Article

Non-Alcoholic Fatty Liver and Cardiovascular Disease: An Emerging Relationship

Katerina Baou[1], Charalambos Vlachopoulos[1], Emanuel Manesis[2], Athanasios Archimandritis[2], Christodoulos Stefanadis[1]

[1]Peripheral Vessels Unit, First Cardiology Department, [2]Second Department of Internal Medicine, Athens Medical School, Hippokration General Hospital, Athens, Greece

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Address:
Charalambos Vlachopoulos

17 Kerassoundos St. 11528 Athens, Greece
e-mail:
cvlachop@otenet.gr

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological condition characterised by a wide spectrum of histological abnormalities and clinical outcomes. The underlying histological abnormality is hepatic steatosis, characterised by fat accumulation, which may progress to non-alcoholic steatohepatitis (NASH), characterised in turn by steatosis, periportal inflammation, and ballooning degeneration, with or without fibrosis.[1] NASH may progress to cirrhosis and hepatocellular carcinoma.[2-4]

Epidemiological and clinical presentation

NAFLD is emerging as a major cause of liver-related morbidity and is the most common abnormality observed in hepatological practice. The true prevalence of NAFLD remains to be established. In general population studies its prevalence ranges between 10 and 39% and it is the most common liver disease in adults in the United States. The most common cases are obese, middle-aged women with diabetes.[5,6]

Patients with hepatic steatosis alone or steatosis with non-specific inflammation appear to have a mild clinical course.[4] In contrast, 50% of patients with NASH may progress to fibrosis or cirrhosis.[4,7,8] One retrospective study of 30 NASH patients reported a 67% 5-year survival rate and a 59% 10-year survival rate.[9] NASH-related cirrhosis has been recognised as a distinct entity that can cause severe liver disease and death.[10]

The majority of patients with NAFLD are asymptomatic, though generalised fatigue, mild epigastric or right upper quadrant pain may occur. On physical examination, hepatomegaly is found in up to 25% of patients.[4]

Most commonly, the detection of the disease is incidental, and patients are found to have elevated levels of aminotransferases or hepatomegaly when examined for other conditions. The most common laboratory abnormality is the elevation of alanine aminotransferase (ALT), while aspartate aminotransferase (AST) may be the predominantly elevated aminotransferase in cases with advanced liver fibrosis. Several studies have demonstrated that an AST/ALT ratio >1 is correlated with increasing fibrosis stage in patients with steatohepatitis.[11] Several studies have examined factors that correlate with the presence of fibrosis, such as age, AST/ALT ratio, diabetes mellitus type 2, and hypertension. Less than 50% of patients have elevated alkaline phosphatase (ALP) and only 10-15% of patients have increased levels of serum bilirubin. Hypoalbuminaemia, thrombocytopenia and prolonged clotting time indicate advanced liver disease.[12] Hepatic iron content may be elevated. Increased ferritin and trans-

ferrin levels, when they occur, may indicate the presence of hepatocyte necrosis.[13]

When NAFLD is suspected in a patient with abnormal liver function tests, the most common, although not highly reliable, screening imaging technique used is ultrasound.[4,14] On the other hand, the diagnosis of NASH depends entirely on liver biopsy.[1] Liver biopsy is an invasive procedure requiring hospitalisation of the patient and is associated with a low but definite morbidity. It is therefore performed to establish the diagnosis of NASH (NAFLD type 3 and 4) as opposed to simple liver steatosis (NAFLD type 1 and 2) not requiring treatment.[4] Histologically confirmed NASH is characterised by the presence of macrovesicular steatosis, lobular, polymorphonuclear or mixed infiltrate, Mallory's hyaline inclusions, focal hepatocyte ballooning degeneration, and fibrosis. This condition has the potential for progression to cirrhosis and hepatocellular carcinoma.[4,8]

Liver biology and pathogenesis of NAFLD

Upon ingestion, free fatty acids (FFA) are transferred to the liver and are metabolised through mitochondrial oxidation to complex lipids. They are then stored in the liver or converted to lipoproteins and secreted into the plasma. Increased FFA influx to the liver leads to their conversion to triglycerides and storage in the cytoplasm, leading in turn to steatosis. Triglycerides are also secreted into the plasma as very low-density lipoproteins (VLDL), resulting in hypertriglyceridaemia. Insulin resistance and obesity cause increased release of FFA from the adipocytes and transfer to the liver.[15]

The most prevalent general theory is the "two-hit" hypothesis, which was proposed by Day and James in 1998.[16] The theory has two stages or "hits". At the first "hit", fat accumulation is generated. The increase of insulin levels causes lipolysis (in the adipocytes) and stimulation of the synthesis of fatty acids (in the hepatocytes).[17] Concurrently, synthesis of apolipoprotein B is decreased in patients with NAFLD, impairing the ability of the hepatocyte to export VLDL and causing accumulation of triglycerides and cholesterol esters in the liver.[2] Increased levels of leptin are considered to promote insulin resistance and alter insulin signalling in hepatocytes, resulting in increasing hepatocellular fatty acid production.[12] Studies have shown a correlation between insulin resistance and γ-glutamyl transferase (γ-GT),[18] and between fasting insulin, uric acid and NAFLD.[19] The increased flow of fatty acid from the adipose tissue to the liver causes abnormal lipid metabolism, and this results in increased accumulation of triglycerides in the liver, impaired glucose metabolism and decreased insulin sensitivity, initially in the muscle and later in the liver, thus accentuating liver steatosis.[20]

At the second "hit", steatosis is converted to steatohepatitis. Oxidative stress is thought to be one of the major factors that affect the progression of steatosis to NASH; it is caused by elevated enzymes CYP2E1 and CYP3A4, and oxidation of free fatty acids. Chronic oxidative stress leads to depletion of the natural antioxidant pool, and results in excess reactive oxidative species within the hepatocyte. In addition, excess iron may increase oxidative stress.

Serum oxidised low-density lipoproteins (ox-LDL) and thiobarbituric acid reacting substances (TBARS) are higher in NASH patients, and the homeostatic model assessment (HOMA) index is an independent factor associated with ox-LDL and TBARS.[21] The association of ox-LDL with the initiation and progression of atherosclerosis could explain the early atherosclerotic process in NASH patients. The release of tumour necrosis factor-α (TNF-α), I kappaB kinase-beta and nuclear factor-kappa B could be induced by the generation of excess reactive oxygen species. Another factor is the depletion of important nutrients, causing the increase of lipid peroxidation and the decrease of VLDL production. Obesity leads to impaired Kupffer cell function.[22] Kupffer cell dysfunction leads to decreased phagocytic activity, altered cytokine profiles and increased fibrinogenesis. The result of continued oxidative stress and accumulated fat in hepatocytes is the reduction of ATP homeostasis, the accession of UCP-2 (mitochondrial uncoupling protein) and a subsequent additional increase in oxidative stress. Stellate cells are primarily responsible for fibrinogenesis, and are a principal cytokine target in liver fibrosis. The activation of stellate cells is promoted by products of lipid peroxidation.[2]

NAFLD and cardiovascular disease

Recent studies have pointed out the association between NAFLD and high cardiovascular risk. NAFLD is associated with an increased risk of future cardiovascular events among type 2 diabetic patients. This association is independent of classical risk factors, liver enzyme levels, or the presence of the metabolic syndrome, and is strongly correlated with NAFLD.[23] In a recent study, it was noted that individuals with elevated

ALT had not only NAFLD but also an increased calculated risk of coronary heart disease, as estimated using the Framingham risk score.[24] In another study, the survival of NAFLD patients was shown to be lower than expected compared to the general population. In that particular study, a higher mortality was observed among NASH individuals, and this mortality was primarily cardiovascular, rather than liver-related.[25]

NAFLD has been shown to be strongly associated with the major components of the metabolic syndrome. Among 30 NAFLD patients included in one study, 67% were overweight, 47% had central obesity, 57% had impaired glucose metabolism, 47% had elevated triglycerides, and 17% had hypertension.[26] The association of hypertension with NAFLD has also been investigated. In a recent study the prevalence of fatty liver was higher in a group of non-obese, non-diabetic, hypertensive patients with normal liver enzymes than in matched normotensive controls, suggesting that hypertension may be associated with fatty liver.[27] The results from the third National Health and Nutrition Survey (NHANES III) have shown that individuals with the metabolic syndrome have a significantly higher prevalence of unexplained elevations in ALT levels, supporting the hypothesis that NAFLD is part of the spectrum of the metabolic syndrome.[28] A multivariate analysis identified insulin resistance and body mass index (BMI) as factors independently associated with fatty liver.[29] Patients with diabetes and elevated BMI and low fibrosis stage were at risk for higher rates of fibrosis progression.[30] These findings fit with the hypothesis that NASH, as part of the metabolic syndrome, contributes to a higher risk of cardiovascular disease.[31,32]

Several studies examined the relation of NAFLD with functional and structural alterations of the arteries. A study carried out in Japanese university students has shown that the brachial-ankle pulse wave velocity (baPWV) in male subjects was significantly higher in the obese than in the overweight group, and higher in those with than in those without NAFLD. Fatty liver was diagnosed in 64% of the obese group.[33] Another study has reported that baPWV values were positively associated and serum adiponectin levels negatively associated with NAFLD and liver enzyme titre, especially in females.[34] Villanova et al studied the relation between flow-mediated dilatation of the brachial artery and NAFLD and found evidence of endothelial dysfunction and an increased risk of cardiovascular events in these patients.[10] Furthermore, patients with fatty liver have more components of the metabolic syndrome and carotid atherosclerosis, as expressed by higher carotid intima-media thickness (IMT) than controls.[31] Finally, a relation was found between carotid artery wall thickness and the severity of liver histological lesions in subjects with NAFLD.[35] Specifically, carotid IMT was strongly associated with the degree of hepatic steatosis, necroinflammation, and fibrosis. In preliminary studies from our laboratory, NAFLD subjects had significantly increased carotid-femoral PWV and mean IMT, and a higher frequency of plaques in the carotid arteries compared with control subjects.[36] Plasma C-reactive protein concentrations were also higher in these patients (Figure 1). Arterial stiffness, endothelial function of the brachial artery and IMT of carotid arteries have all been identified as markers of cardiovascular disease and predictors of the corresponding risk.[37-48]

NASH is associated with inflammatory markers and fat-produced hormones, which have been linked with increased cardiovascular risk. Fat tissue produces hormones, such as leptin, resistin, and adiponectin, and

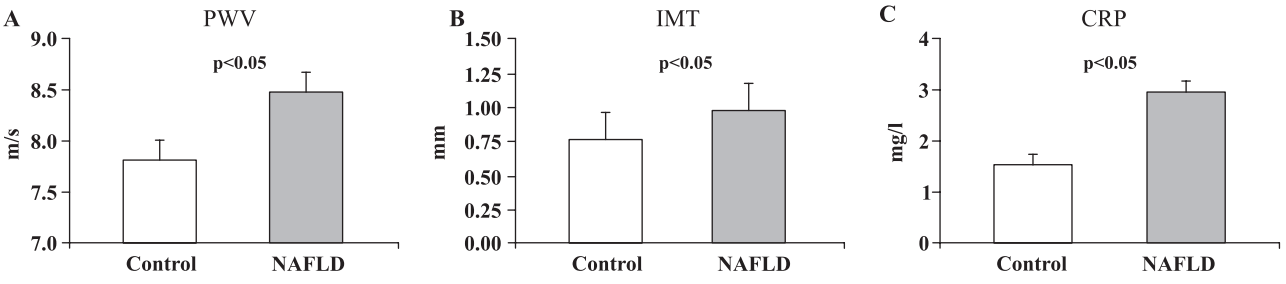

Figure 1. Patients with non-alcoholic fatty liver disease (NAFLD) show increased pulse wave velocity (PWV) (A), increased carotid intima-media thickness (IMT) (B), and increased levels of plasma C-reactive protein (CRP) (C), compared to controls.

it is a source of neurotransmitters (noradrenaline and angiotensin II) and immunomodulatory cytokines, particularly TNF-α and interleukin-6.[20] TNF-α interferes with insulin signalling, favouring steatosis and playing a proinflammatory role in the pathogenesis of NASH. Adiponectin, which has anti-inflammatory and anti-atherogenic properties, has independently been related to cardiovascular disease. Adiponectin is also known to act directly on hepatic tissue, to inhibit glucose production and to protect against lipid accumulation.[32] Adiponectin levels are lower than normal in obese patients, in other insulin-resistance states, and in NAFLD patients. In a recent study, low adiponectin levels have also been associated with increased aminotransferase and γ-GT levels, suggesting a role in liver cell integrity.[49] NAFLD patients have resistance to leptin action. The increased leptin levels worsen insulin resistance and lipid metabolism.[50] Resistance to insulin and high levels of leptin could favour fatty acid oxidation in the mitochondria, and increase the production of free oxygen radicals, promoting hepatic fibrogenesis.

Conclusion

While several studies have identified the association between NAFLD and enhanced cardiovascular risk, the mechanisms have not been fully explored at this stage. The atherogenic impact of the metabolic syndrome may be involved. NAFLD is associated with surrogate markers of cardiovascular function, such as inflammatory markers, arterial stiffness, endothelial function, and structural changes in the great arteries. It is not known whether an improvement in the histological status of NAFLD blunts the progression of cardiovascular disease, but it is worth noting that preventive measures for cardiovascular disease, such as weight reduction and treatment for diabetes and triglyceridaemia have the potential to favourably affect this liver disease.

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