CHEST

Original Research

SLEEP DISORDERS

Nonalcoholic Fatty Liver Disease, Nocturnal Hypoxia, and Endothelial Function in Patients With Sleep Apnea

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Background: **Nocturnal hypoxia, the hallmark of OSA, is a potential contributing factor for nonalcoholic fatty liver disease (NAFLD). NAFLD severity and its implication in OSA-related endothelial dysfunction have not been investigated in a large, unselected OSA population, including nonobese subjects.**

Methods: **Noninvasive blood tests (SteatoTest, NashTest, and FibroTest) were used to evaluate steatosis, nonalcoholic steatohepatitis (NASH), and fibrosis in a large cohort of patients with OSA. In the same group, endothelial function and its links with NAFLD severity were assessed.**

Results: **Of the 226 subjects included who were referred for suspicion of OSA (men, 55%; median age, 56 years; median BMI, 34.2 kg/m^2 [33% with BMI < 30 kg/m^2]), 61.5% exhibited moderate or severe steatosis. By multivariate analysis, independent factors for liver steatosis were, as expected, triglyceride levels ($P < .0001$) and insulin resistance ($P = .0004$) as well as nocturnal cumulative time spent < 90% of oxygen saturation (CT90) ($P = .01$). Thirty-eight percent had borderline or possible NASH (N1 or N2 with NashTest). CT90 was significantly associated with borderline or possible NASH ($P = .035$) in univariate but not in multivariate analysis. The dose-response relationship between the severity of nocturnal hypoxia and liver injury was established only in morbid obesity and not in lean. Multivariate models showed that steatosis was independently associated with endothelial dysfunction after adjustment for confounders.**

Conclusions: **In a large, unselected OSA population, the severity of nocturnal hypoxia was independently associated with steatosis. Preexisting obesity exacerbated the effects of nocturnal hypoxemia. NAFLD is a potential mechanism of endothelial dysfunction in OSA.**

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Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CIH = chronic intermittent hypoxia; CT90 = nocturnal cumulative time spent < 90% of oxygen saturation; FT = FibroTest; HOMA-IR = homeostasis of model of assessment-insulin resistance index; lnPAT = natural logarithm of peripheral arterial tone; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NT = NashTest; PAT = peripheral arterial tone; RH-PAT = pulse amplitude augmentation in response to hyperemia; ST = SteatoTest

Nocturnal hypoxia, the hallmark of OSA, is strongly suggested as a contributing factor for nonalcoholic fatty liver disease (NAFLD).[1] Although both conditions are associated with higher cardiovascular risk,[2,3] it has never been investigated whether NAFLD is among the underlying mechanisms of endothelial dysfunction in patients with OSA.

OSA is a common medical condition characterized by repetitive partial or complete obstruction of the upper airway, causing repetitive nocturnal oxygen desaturation (ie, chronic intermittent hypoxia [CIH]). CIH induces oxidative stress and, consequently, promotes systemic and vascular inflammation, insulin resistance, endothelial dysfunction, and cardiovascular morbidity and mortality.[4] Studies in mice and humans have suggested that OSA also leads to liver injury.[5-8] In morbidly obese subjects referred for bariatric surgery, CIH contributes to the severity of liver fibrosis and fibroinflammation independently of obesity.[1] However, the consequences of nocturnal hypoxemia on

the development of NAFLD remain largely underinvestigated in large populations of less obese or overweight patients with OSA.

OSA has been associated with a higher cardiometabolic risk, and NAFLD might be one of the mechanistic pathways upregulated by CIH and finally leading to poor cardiometabolic outcomes.[9] Clinical cardiovascular complications are preceded by both endothelial function impairments and the development of morphologic atherosclerotic changes.[10] Digital pulse amplitude augmentation in response to hyperemia (RH-PAT) is one of the validated methods to measure endothelial function, and RH-PAT measurements allow for quantifying cardiovascular risk[10] and predicting late adverse cardiovascular events.[11,12] The role of NAFLD in OSA-related endothelial dysfunction is largely unexplored.

The diagnosis of NAFLD relies on histopathologic findings and includes a wide spectrum of lesions, including simple steatosis, steatohepatitis, and fibrosis, potentially leading to end-stage cirrhosis. Therefore, liver biopsy represents the gold standard to confirm NAFLD diagnosis and provide prognostic information, yet it is an invasive and costly procedure prone to either minor secondary effects, such as pain, or more severe complications, including a risk of death of 0.01%.[13] Notably, there is high sampling variability and high intrapathologist and interpathologist inconsistency.[14] Most importantly, facing the actual epidemic of diabetes and obesity[15] and the subsequent number of patients at risk for liver alterations, biopsy cannot be considered a practical, efficient, and large-scale tool to identify those at risk for nonalcoholic steatohepatitis (NASH) and advanced fibrosis. To address this issue, less invasive tests have been developed and validated to largely screen NAFLD in at-risk populations (see review by Machado and Cortez-Pinto[16]). These methods are either biologic tests or physical techniques, such as transient elastography (FibroScan; Echosens). However, FibroScan has some limitations (failure or unreliability) in obese patients, as suggested by Castéra et al.[17] Biologic tests prospectively validated by a predetermined scoring system equivalent to that of the METAVIR scoring system appear, then, to be the most appropriate tool in this population.[18] The Fibromax patented algorithm[19] developed by BioPredictive is a validated, noninvasive tool for NAFLD screening that uses the association of sex, age, weight, height, and numerous serum biomarkers. Fibromax includes SteatoTest (ST), NashTest (NT), and FibroTest (FT) for the noninvasive evaluation of steatosis, NASH, and liver fibrosis, respectively. The aims of this study were (1) to use noninvasive blood tests (ST, NT, and FT) to evaluate steatosis, NASH, and fibrosis in a large cohort of patients with OSA and a wide range of BMI values, including nonobese subjects, and (2) to assess endothelial function by peripheral arterial tone (PAT) as a marker of cardiovascular risk and evaluate its relationship with NAFLD in OSA.

Materials and Methods

Patients

Figure 1 displays the study flowchart diagram and exclusion criteria. We enrolled 291 adult subjects referred for suspicion of OSA. Women with an alcohol consumption $\geq$ 20 g/d and men with $\geq$ 30 g/d were excluded as well as patients with chronic liver disease, including viral hepatitis B or C (confirmed with renewed serologies), and patients on potentially hepatotoxic drug therapy. Patients with obesity-hypoventilation syndrome were excluded. Finally, patients with serious comorbidities, such as heart failure, severe renal impairment, and severe hypertension, were also excluded. Two hundred and twenty-six subjects were included in the study, all of whom had Fibromax analysis as a surrogate of NAFLD. All subjects provided written informed consent approved by the ethical committee at the Grenoble University Hospital (institutional review board numbers 2007-A00472-51 and 38/2006/2).

Laboratory Analysis

Fasting serum samples were obtained from all subjects to be frozen and stored at −80°C until Fibromax analysis (α2-macroglobulin, apolipoprotein A1, haptoglobin, γ-glutamyltransferase, total bilirubin, alanine aminotransferase [ALT], aspartate aminotransferase [AST], fasting blood glucose, triglycerides, and total cholesterol levels). Results were sent anonymously to BioPredictive, blinded to the severity of sleep apnea, and used in the algorithm to obtain Fibromax results.

Fibromax

Fibromax was validated initially in viral hepatitis C,[20] chronic hepatitis B,[21] and alcoholic liver disease[22] and demonstrated good diagnostic ability. In NAFLD, prospective studies have also demonstrated good reliability for prediction of liver abnormalities,

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