An assessment of oxidized LDL in the lipid profiles of patients with obstructive sleep apnea and its association with both hypertension and dyslipidemia, and the impact of treatment with CPAP

Marcia C. Feres [a, *], Francisco A.H. Fonseca [b], Fatima D. Cintra [a, b], Luciane Mello-Fujita [a, c], Altay Lino de Souza [a], Maria C. De Martino [d], Sergio Tufik [a, c, d], Dalva Poyares [a, c]

[a] Psychobiology Department, Universidade Federal de Sao Paulo — UNIFESP, Sao Paulo, Brazil
[b] Medicine Department, Universidade Federal de Sao Paulo — UNIFESP, Sao Paulo, Brazil
[c] Instituto do Sono de Sao Paulo, São Paulo, Brazil
[d] Associaçao Fundo de Incentivo à Pesquisa — AFIP, Sao Paulo, Brazil

ABSTRACT

Objective: Obstructive sleep apnea (OSA) has been linked to increased oxidative stress, lipid peroxidation and worsening atherosclerosis. This study investigated oxidized low-density lipoprotein (oxLDL) as a marker of lipid peroxidation, and total LDL cholesterol (direct LDL-C), as a marker of the lipid profile among individuals with OSA, and its association with hypertension (HYP) and dyslipidemia (DYS). The impact of one year of continuous positive airway pressure (CPAP) was also assessed.
Methods: Blood was collected after 12 h of fasting from 99 consecutive patients who were diagnosed with OSA via polysomnography, and were also diagnosed with both HYP and DYS via clinical and laboratory studies. The patients were classified into the following three groups: GI [OSA with comorbidities (HYP or DYS)], GII [OSA without comorbidities], and GIII [control]. Thirty-five patients with an apnea/hypopnea index >20 per hour of sleep were randomized to groups that received either Sham-CPAP or CPAP treatments over 12 months.
Results: In a binary regression controlled for sex, age, body mass index, and glycemia, model 1 which analyzed direct LDL-C, demonstrated significant levels of risk in the setting of DYS but not in the settings of HYP and OSA. In model 2, which analyzed oxLDL, DYS (p = 0.01), HYP (p = 0.032), and OSA (p = 0.039) were statistically significant. Significant alterations were observed in only the sleep parameters following one year of CPAP.
Conclusions: Based on the statistical regression model, only the presence of DYS (p = 0.001) was associated with the levels of direct LDL-C. The remaining comorbidities (OSA and HYP) were not significantly related to the levels of direct LDL-C. Regarding oxLDL, OSA, HYP and DYS each added significant score values to the levels of oxLDL.
These findings are suggestive of the importance of assessing oxLDL among patients presenting with OSA, both with and without comorbidities.

1. Introduction

Atherosclerosis has been found in patients with obstructive sleep apnea (OSA) who are without additional cardiovascular risk factors, and is associated with nocturnal hypoxemia [1—3]. Another consequence of OSA is arterial hypertension (HYP), which is common among patients with OSA and may result in arterial inflammatory processes that favor the development of oxidative stress, aggravating atherosclerosis [4]. Dyslipidemia is the most important factor in the development of atherosclerosis. When dyslipidemia is associated with the above-mentioned conditions, the effects of lipid peroxidation may become potentiated, resulting in higher

* Corresponding author. Psychobiology Department, Universidade Federal de Sao Paulo — UNIFESP, Brazil. ST; Napoleão de Barros n°925- Vl. Clementino, 04020-060, Sao Paulo, Brazil. Associação Fundo de Incentivo a Pesquisa — AFIP, Sao Paulo, Brazil.
E-mail addresses: marcia.feres@unifesp.br, marcia.feres@afip.com.br (M.C. Feres).

levels of oxidized low-density lipoprotein (oxLDL) in the bloodstream [5,6].

Oxidative modifications to LDL (oxLDL) represent an important physiological mechanism underlying atherogenesis [7−9]. OxLDL and anti-oxidized LDL antibodies (anti-oxLDL) have been detected in both human serum and atherosclerotic lesions [10,11]. OxLDL accumulates in atherosclerotic lesions over time as result of its uptake by scavenger receptors, resulting in the accumulation of cholesterol within foam cells [6]. This process subsequently promotes the formation of atherosclerotic lesions. This mechanism also explains the formation of atheroma plaques via the accumulation of non-modified total LDL-cholesterol (direct LDL-C), which occurs at high cholesterol concentrations [7]. Non-modified LDL is recognized by cell membrane receptors, internalized, and degraded, which results in the release of the cholesterol needed for cellular metabolism. However, when LDL is modified by external agents and becomes oxidized, it is no longer recognized by cellular receptors and causes both inflammation and plaque formation on the internal surfaces of blood vessels [8].

These plaques give rise to atherosclerosis, which may lead to either acute myocardial infarction or stroke. Therefore, atherogenesis may be potentiated in the setting of oxLDL formation. Therefore, modified LDL (i.e., oxLDL) appears to be more harmful than LDL-C [9].

Drager et al. investigated the effect of 4 months of CPAP treatment on atherosclerosis parameters. They observed that CPAP significantly improved the early signs of atherosclerosis (reduced carotid intima-media thickness), supporting the idea that OSA is an independent risk factor for atherosclerosis [10].

CPAP reportedly reduces the levels reactive oxygen species, including inflammatory neutrophils and monocytes, among patients with OSA [11]. This finding has not been confirmed in other studies, and oxLDL has not been evaluated [12].

Given the potential contribution of ox-LDL to the pathogenesis of atherosclerosis, as well as the potential relationship among OSA, cardiovascular disease and oxidative stress [13], we hypothesized that ox-LDL is increased in patients with OSA, and that CPAP is able to decrease its levels. In this study, we evaluated the contribution of OSA, as well as its relationship with both hypertension and dyslipidemia, to the increases in both oxLDL and direct LDL-C.

2. Methods

Patients were consecutively selected at the cardiovascular risk stratification outpatient clinic of the Sleep Institute, Sao Paulo, Brazil. The individuals in the control group were selected from the community of Sao Paulo. All participants were subjected to clinical, laboratory, and polysomnographic assessments. This study was divided into two stages, a baseline evaluation (case control evaluation), and a randomized, double blind controlled CPAP and Sham-CPAP trial. This study was part of a larger study regarding sleep apnea, and was approved by the Ethics Committee of the Federal University of Sao Paulo and registered at http://clinicaltrials.gov/ under the following number: NCT00768625.

2.1. Baseline evaluation

In total, of first 127 consecutive patients with suspected OSA were selected, 10 patients were excluded because they exhibited either mild OSA or other sleep disorders based on their polysomnography results, and because they exhibited comorbidities other than those investigated in this study. An additional 18 patients exhibiting only HYP without OSA were also excluded. Ultimately, 99 patients were included (55 females and 44 males). Following polysomnography, the patients were grouped according to the severity of their diseases, based on their apneahypopnea indices (AHI). Patients with moderate (AHI = 15 to 30 events/hour) and severe (AHI > 30 events/hour) OSA were included; the controls exhibited an AHI < 5 events/hour. They were divided into the following three groups: GI (OSA with comorbidities (DYS or HYP) n = 48); GII (OSA without comorbidities, n = 24), and GIII (controls without OSA, HYP or DYS, n = 27).

All polysomnographic tests were performed and analyzed by technicians according to the guidelines for studies of sleep [14], and were reviewed by a specialist.

Patients presenting with recently diagnosed OSA who were free of other diseases except for HYP and DYS, diagnosed according the consensus cited [15,16], who had never been treated for OSA, and who were not using medication except for either HYP or DYS treatment were included in the study [17].

The following patients were excluded from the study: smokers, individuals with mild OSA, individuals with sleep or respiratory disorders other than OSA, individuals presenting with comorbidities other than HYP and DYS that were not identified during the initial screening, individuals with a body mass index (BMI) > 40, individuals with definite chronic lung disease with a forced expiratory volume in 1 s/forced vital capacity < 0.7 [18], individuals exhibiting severe systemic diseases, and pregnant women.

The selection of overweight individuals for the control group helped to ensure the comparability of the groups. All patients recently diagnosed with OSA presented following 12 h of fasting for a blood draw and a physical examination.

2.2. CPAP trial

Following the observation of the increased effect of ox-LDL on patients with OSA, we elected to determine the effect of CPAP treatment on ox-LDL levels in patients with moderate to severe OSA. In order to achieve this goal, 72 consecutive patients recently diagnosed with OSA by polysomnography were studied. They underwent a second polysomnography for CPAP titration and were then randomized into groups that would undergo 6 months of treatment with either CPAP or sham-CPAP. Following the 6-month period, the patients in the CPAP group remained on CPAP for another 6 months in an open label study and completed 12 months of CPAP treatment.

Participants were included if they were between 30 and 65 years of age (both genders) and reported no recent hospitalizations. The exclusion criteria were a body mass index (BMI) > 40 kg/m^2, pulmonary disease or New York Heart Association class III or IV heart failure, unstable angina, valvular heart disease, lifethreatening arrhythmia, atrial fibrillation, left bundle branch block, uncontrolled hypertension, renal disease, pregnancy and individuals receiving treatment for OSA. Doppler echocardiography (IE33®, Philips Medical Systems), a maximum, symptom-limited CPET on a treadmill and pulmonary function tests (Ergo PC 13, Micromed®, Brazil) in a quiet air-conditioned room with an average temperature of 21 °C and full resuscitation facilities were performed at baseline to rule out respiratory and cardiac disease [19]. Each of these exclusion criteria was utilized in a larger study involving an OSA patient cohort [3].

2.3. The CPAP trial was divided into 2 steps

Step 1: the 6-month randomized, controlled, double blind study

Once enrolled in the study, the patients were randomly allocated into the following two groups: G1 = CPAP (n = X) and G2 = Sham-CPAP (n = Y). They underwent polysomnography for CPAP titration (G1) and polysomnography with sham-CPAP (G2), in