Journal of Cardiology 67 (2016) 295–302

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

The relationship between neutrophil to lymphocyte ratio, endothelial function, and severity in patients with obstructive sleep apnea

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ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form 28 May 2015
Accepted 17 June 2015
Available online 4 September 2015

Keywords:
Sleep apnea syndrome
Endothelial progenitor cell
Flow-mediated dilatation
Neutrophil to lymphocyte ratio

ABSTRACT

Background: Obstructive sleep apnea (OSA) is characterized by repetitive intermittent hypoxia and reoxygenation during sleep with elevated oxidative stress and promotes the development of atherosclerosis, as demonstrated by vascular dysfunction and chronic inflammation. An increased neutrophil to lymphocyte ratio (NLR) has been recognized to be a novel inflammatory biomarker for systemic inflammation.

Objectives: We evaluated whether the NLR reflects the severity of OSA and if continuous positive airway pressure (CPAP) treatment ameliorates the endothelial function and NLR in patients with OSA.

Methods: We enrolled 95 patients with suspected OSA and 29 patients who received CPAP therapy for 3 months. We evaluated the number of endothelial progenitor cells (EPCs) and NLR, the levels of nitric oxide (NO_x) and asymmetric dimethylarginine (ADMA), and the endothelial function according to the flow-mediated dilatation (FMD) before and after CPAP treatment.

Results: The levels of apnea–hypopnea index demonstrated an inverse relationship with the FMD and a positive relationship with the NLR. Moreover, NLR is an independent factor suggested for the presence of severe OSA. CPAP therapy increased the levels of EPC and NO_x and decreased the level of ADMA. CPAP treatment also improved the FMD and decreased the NLR.

Conclusions: NLR and endothelial dysfunction significantly correlates with the severity of OSA and FMD and other biochemical parameters improved and NLR decreased significantly after CPAP treatment.

Introduction

Obstructive sleep apnea (OSA) is a chronic disorder associated with considerable cardiovascular risks that is characterized by repetitive episodes of transient oxygen desaturation that subsequently induces apnea and hypopnea during sleep, resulting in excessive sleepiness during the daytime in addition to an impaired quality of life and threatened health. OSA is considered to be an independent risk factor for a number of cardiovascular diseases, including hypertension (HT), ischemic heart disease, congestive heart failure (HF), and cerebral vascular events [1–4].

Treatment with nasal continuous positive airway pressure (CPAP) using a mask favorably ameliorates oxygen desaturation and mitigates the above adverse health consequences [5,6]. Although the pathophysiological basis of cardiovascular complications of OSA is multifactorial, involving sympathetic excitation, endothelial dysfunction, inflammation, and insulin resistance [7], it is likely that the intermittent episodes of hypoxia and reoxygenation are important mediators of these complications in evoking chronic inflammatory responses.

Endothelial dysfunction is on the causal pathway for both atherogenesis and destabilization of established plaques. Flow-mediated dilatation (FMD) as a noninvasive method to assess endothelial function has been shown to be affected by cardiovascular risk factors, to be related to structural arterial disease and to cardiovascular outcome, validating its use for studying the pathophysiology of arterial disease [8,9].

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http://dx.doi.org/10.1016/j.jjcc.2015.06.005
0914-5087/

Recently, the neutrophil–lymphocyte ratio (NLR) has been recognized to be a novel biomarker for the prediction of various diseases, including cardiovascular disease. Although chronic inflammatory biomarkers are elevated in patients with OSA, there is no evidence with respect to the pathogenesis of OSA.

Furthermore, CPAP treatment is the most important therapy for OSA; however, the effects of CPAP in promoting endothelial repair and improving chronic systemic inflammation, including that reflected by the NLR, are not fully understood. In the present study, we evaluated the effects of CPAP therapy in ameliorating endothelial dysfunction and chronic inflammation in patients with OSA.

Methods

Study design and protocol

The Institutional Review Board of Human Research at Saga University approved this study, and written informed consent was obtained from all participants. The study used a single-arm prospective observational study design that examined the impact of CPAP treatment on the endothelial function and inflammation in patients with OSA.

Patients were recruited for 2 years from July 1st 2012 to June 30th 2014. The inclusion criteria were as follows: ≥20 years of age and suspected OSA with subjective daily sleepiness. The exclusion criteria included severe HF with predominant central sleep apnea that required adaptive-servo ventilation, serious renal or hepatic dysfunction, inflammatory disease, pregnancy or possible pregnancy, lack of informed consent, and patients judged by the investigator to be ineligible for inclusion in the study.

Patients suspected of having OSA were recruited and underwent home sleep test using the LS-100 device (Fukuda Denshi Co. Ltd., Tokyo, Japan) with a respiratory airflow and oxygen saturation monitor. Patients suspected of having moderate or severe OSA [apnea–hypopnea index (AHI) >15] were evaluated using full-night polysomnography (PSG) followed by blood sampling and endothelial function tests the next morning. Newly diagnosed patients who required CPAP therapy with an AHI of >20 events per hour were treated for 3 months. All experimental evaluations were repeated 3 months after CPAP intervention in each case.

Measurement of FMD

The degree of endothelial dysfunction of the brachial artery was evaluated using a FMD test, as described elsewhere [10]. Briefly, the use of alcohol, caffeine, or vasoactive drugs and smoking were restricted during the fasting period. All subjects rested for at least 15 min in a seated position in a quiet, dark, air-conditioned room (22–25 °C) before the FMD assessments. The test was performed in the morning after 12 h of fasting. A high-resolution linear artery transducer was coupled to a computer-assisted analysis software program (UNEX EF18G, UNEX Co., Nagoya, Japan) that used an automated edge detection system to measure the diameter of the brachial artery. A forearm cuff was then inflated for 5 min at 50 mmHg above the systolic blood pressure just prior to FMD measurement. A longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and 10 s immediately after cuff deflation. The measurements were obtained by two experienced independent technicians who were blinded to the patient data. The %FMD was then estimated as the percent change in the vessel diameter over baseline at maximum dilatation during reactive hyperemia.

PSG and definition of OSA

Full PSG was performed overnight between 9:00 pm and 6:00 am using the Somno Track Pro system or Somno Screen Plus (Somno Medics, Randersacker, Germany), using the America Academy of Sleep Medicine recommended criteria [11]. The recordings included an electroencephalogram, electrooculogram, electromyogram, electrocardiogram, thermistors for the nasal and oral airflow, thoracic and abdominal impedance belts to measure the respiratory effort, pulse oximetry to assess the oxyhemoglobin level, a tracheal microphone to detect snoring, and a sensor to evaluate changes in position during sleep performed simultaneously. The degree of OSA was determined according to the AHI, defined as the total number of episodes of obstructive apnea and hypopnea per hour of sleep. The AHI is used as one index of the presence and severity of sleep apnea and it was defined as the total number of episodes of apnea and hypopnea per hour of sleep as described previously [11]. Patients with an AHI of >20 were enrolled in the following study as candidates for CPAP therapy.

CPAP therapy

Patients with an AHI of >20 slept while attached to the automatic titration device (ResMed S9; ResMed Ltd., Sydney, Australia). After using the CPAP device for 3 months, the data for CPAP use and the mean AHI were obtained from data cards inside the CPAP machine. Adherence with the CPAP therapy was defined as use of CPAP for at least 4 h/night on 70% of the nights. The sleep stages and respiratory parameters were scored according to the standard criteria of the American Academy of Sleep Medicine [11]. At the end of the study protocol, the patients underwent repeat PSG while using the CPAP device to evaluate the precise efficacy of the CPAP therapy.

Laboratory methods

Blood samples were drawn in the morning after a 12-h overnight fast. The plasma level of the nitric oxide compounds (NO_x; NO_2^- + NO_3^-) was measured according to the Gries method (BioAssay Systems, Hayward, CA, USA) and the level of asymmetrical dimethylarginine (ADMA) was measured using ELISA (Immundiagnostik, Bensheim, Germany).

The assessments of circulating endothelial progenitor cells (EPC) using flow cytometry (FCM) were performed by researchers blinded to the clinical data. Briefly, mononuclear cells isolated from a volume of 10 mL of peripheral blood were incubated for 30 min in the dark with Peridinin Chlorophyll Protein (PerCP)-conjugated monoclonal antibodies against human CD45 (Becton Dickinson Pharmingen, San Jose, CA, USA), allophycocyanin (APC)-conjugated monoclonal antibodies against the human kinase insert domain-conjugating receptor (KDR: R&D, Minneapolis, MN, USA), phycoerythrin (PE)-conjugated monoclonal antibodies against human CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany) and fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies against human CD34 (Becton Dickinson Pharmingen). The control ligand (IgG-PE conjugate) was used to detect nonspecific associations and define the threshold for glycoprotein binding. Following incubation, the cells were lysed and washed with phosphate-buffered saline (PBS). A quantitative four-colored flow cytometric analysis was performed using a fluorescence-activated cell sorter (BD FACSCanto™ II; Becton Dickinson). Each analysis included 1×10^6 events. The assays of the EPCs in each sample were performed in duplicate, with the mean level reported. The number of cells was normalized and expressed as the number of cells per 1×10^5 mononuclear cells.