Endothelial function and carotid intima media thickness

INTRODUCTION

With increasing urbanization worldwide, particularly in developing countries where a large proportion of the world's population resides, a new set of diseases are being identified. Obstructive sleep apnea (OSA) is one such disease. The incontrovertible data linking OSA to cardiovascular morbidity and mortality (1–4) has further put the spotlight on this condition in recent times. It has been hypothesized that abnormal endothelial function may be an important mediator responsible for the increase in cardiovascular and renal morbidity in OSA patients (1, 5). Chronic intermittent hypoxia and sleep fragmentation are two of the most important mechanisms resulting in abnormal endothelial function in OSA (6).

Flow mediated dilatation is a useful non-invasive method for the assessment of macro-vascular endothelial function (7). Several studies in OSA patients have investigated endothelial function in macro-vasculature using flow mediated dilatation of brachial artery (8, 9). A few studies have also attempted to measure endothelial function in the micro-vasculature in OSA patients (10, 11). These studies have utilized techniques such as venous occlusive plethysmography, laser doppler flowmetry and more recently, peripheral arterial tonometry. Assessment of endothelial function in OSA patients, allows us to address the larger problem of increased cardiovascular morbidity in this population at an incipient stage.

A useful marker of subclinical cardiovascular disease is carotid intima media thickness (CIMT) (12). Assessment of CIMT using carotid artery ultrasound, offers a non-invasive method to detect atherosclerosis in its inchoative

stages (13). Previous studies have demonstrated an increased CIMT in OSA patients (14). Additionally, an improvement in CIMT has been observed with continuous positive airway pressure therapy (CPAP) therapy in OSA patients (15).

In this study, we attempted to investigate endothelial function and carotid intima media thickness in OSA patients and compare it with non OSA controls.

MATERIALS AND METHODS

Study Population

The present study was conducted over a one year and four months period between November 2013 and March 2015. Study participants included subjects of either gender, older than 18 years of age. The study group included 20 subjects with an apnea hypopnea index (AHI) ≥ 15 events/hr and excessive daytime sleepiness (ESS score > 10), who were naïve to CPAP therapy. Subjects with diabetes mellitus (FBS ≥ 126mg/dL and/or HbA1c > 6. 5%), hypertension (mean of three BP measurements taken at 1 min intervals > 140/90 mmHg or those using antihypertensive medication), dyslipidemia (total serum cholesterol > 200 mg/dL and/or LDL cholesterol > 130 mg/dL by immunocolorimetric assay), hypothyroidism (serum TSH > 5. 50 mIU/L by chemiluminescence) and proteinuria (urine ACR > 300 mg/g of creatinine) were excluded from the study. The control group consisted of 20 non OSA subjects, with an AHI < 5 events/hr who met inclusion and exclusion criteria.

The study protocol was approved by the institutional ethics committee, All India Institute of Medical Sciences, New Delhi. Written informed consent was taken from all participants in the study.

Polysomnography

Prior to polysomnography, all subjects underwent a detailed anthropometry including a body composition analysis (TANITA TBF-410, TANITA Corp., Tokyo, Japan). All study participants underwent an overnight in-laboratory supervised polysomnography (SOMNOscreen plus, SOMNOmedics, Randersacker, Germany) at the polysomnography laboratory of the Department of Medicine at AIIMS. The study was supervised by a trained laboratory technician. Polysomnography included electroencephalography, electrooculography, electrocardiography, surface electrodes for sub mental and tibialis anterior electromyography, pulse oximetry, pressure transducers for nasal and oral air flow and piezo electric strain gauges for chest and abdominal movements (16). The scoring of the sleep study was done by trained personnel using the scoring criteria of American Academy of Sleep Medicine (17). Apnea was scored in case of an air flow reduction of ≥ 90% lasting for ≥ 10 seconds. Hypopnea was scored in case of an air flow reduction of ≥ 30% lasting for ≥ 10 seconds and accompanied by either a ≥ 3% arterial oxygen desaturation or an arousal. The apnea hypopnea index (AHI) was determined as the total number of apneas and hypopneas per hour of sleep. The oxygen desaturation index (ODI) was determined as the number of arterial oxygen desaturations ≥ 3% per hour of sleep.

Endothelial function

Macrovascular endothelial function was assessed by flow mediated dilatation (FMD) of the brachial artery, performed by an experienced radiologist. Flow mediated dilatation was performed in the morning after at least 8 hours of sleep. Brachial artery FMD was measured using a 9 MHz transducer (LOGIQ e, GE Healthcare, Milwaukee, USA) with the patient lying comfortably in the supine position, 1cm above the antecubital fossa in the right arm. B mode scan sections were obtained in the antecubital fossa in the longitudinal plane (18). A baseline diameter was recorded initially. Subsequently, the brachial artery was occluded by inflating the sphygmomanometer cuff to 50mm Hg above the systolic blood pressure for 5 minutes. The cuff was deflated rapidly after 5 minutes and the brachial artery diameter was again recorded one minute after the deflation of the cuff (19). Flow mediated dilatation (FMD) was calculated and expressed as the percentage change in the brachial artery diameter.

Microvascular endothelial function was assessed by using EndoPAT ^{TM} 2000 (Itamar Medical Ltd, Caesarea, Israel). A pair of pneumatic probes were placed over the index fingers of both hands. One arm acted as the test arm and the opposite arm acted as the control arm. The test was conducted in 3 phases. In the initial phase pulse wave tracings were obtained from both the arms for 5 minutes. In the second phase the sphygmomanometer cuff around the test arm was inflated to 50mm Hg above the systolic pressure for 5 minutes. In the last phase, pulse wave tracings were obtained from both the arms after rapid deflation of the sphygmomanometer cuff. Reactive hyperemia index (RHI) was calculated by the Endo-PAT ^{TM} 2000 software. RHI

was calculated as the ratio of the post to pre occlusion PAT amplitude of the test arm, divided by the post to pre occlusion PAT amplitude of the control arm.

CIMT was measured using a 7 MHz transducer (LOGIQ e, GE Healthcare, Milwaukee, USA), by an experienced radiologist. CIMT was measured in the far wall of the common carotid artery just proximal to the carotid bulb (20). Three readings each were taken from the right and left sides. Average of the six readings was taken as the mean CIMT.

STATISTICAL ANALYSIS

Data were expressed as mean ± SD in case of normally distributed data and median (minimum-maximum) in case of skewed data. Continuous variables were compared between the two groups using Students' ' t' test for independent samples in case of normal distribution. Wilcoxon rank test was used to compare continuous variables in case of skewed data. The Chi square test or Fisher exact test was used to compare categorical data. Correlation between variables was expressed in terms of Spearman's rank correlation coefficient. The comparison of endothelial function and CIMT between moderate OSA, severe OSA and non OSA groups was performed using one way ANOVA followed by pair wise comparison using Bonferroni correction. The p values < 0. 05 were considered significant. Statistical analysis was performed using Stata 11. 0 (College Station, Texas, USA).

RESULTS

A total of 468 subjects underwent polysomnography between November 2013 and March 2015. 362 subjects were excluded because of associated co

morbidities. 54 subjects with mild OSA (AHI ≥ 5, but < 15 events/hr) were excluded. 12 subjects refused to take part in the study. 20 moderate to severe OSA subjects (AHI ≥ 15 events/hr) constituted study group. The study group consisted of 10 subjects with moderate OSA (AHI ≥ 15, but < 30 events/hr) and 10 subjects with severe OSA (AHI > 30 events/hr). 20 non OSA subjects (AHI < 5 events/hr) constituted the control group. The baseline demographic, clinical and biochemistry data are presented in Table 1. The two groups did not differ with regard to age, gender, body mass index (BMI), waist-hip ratio (WHR), total and LDL cholesterol, triglyceride and fasting plasma glucose levels.

The polysomnographic data of two groups are presented in Table 2. As expected, the two groups differed in most polysomnographic variables. The apnea hypopnea index (AHI) (p = 0. 0001), respiratory disturbance index (RDI) (p = 0. 0001) and the oxygen desaturation index (ODI) (p = 0. 0001) were significantly higher in the OSA group. Minimum O_2 saturation was significantly lower in the OSA group (p = 0. 0001).

The baseline brachial artery diameter was not significantly different between the two groups. (mean ± SD: 4. 3 ± 0. 6 mm vs. 3. 99 ± 0. 7 mm, p = 0. 15). Flow mediated dilatation (FMD) in OSA group (mean ± SD: 8. 3 ± 2. 8 %) was significantly lower compared to non OSA group (13. 4 ± 4. 1 %), p = 0. 0001. Reactive hyperaemia index (RHI) was also significantly lower in OSA group compared to non OSA group (mean ± SD: 1. 55 ± 0. 27 vs. 2. 01 ± 0. 48; p = 0. 0007) (Table 3). FMD and RHI in the two groups are presented graphically in Figure 1. In the OSA group, FMD and RHI did not show a significant

correlation with OSA disease severity indices (AHI, ODI and minimum O_2 saturation). No correlation was observed between FMD and RHI.

Carotid intima media thickness (CIMT) was significantly higher in OSA group (mean ± SD: 0. 54 ± 0. 09 mm) compared to non OSA group (0. 48 ± 0. 08 mm), p = 0. 049. No significant correlation was observed between CIMT and OSA disease severity indices (AHI, ODI and minimum O_2 saturation) in the OSA group.

Endothelial function and CIMT were also compared between severe OSA subjects, moderate OSA subjects and non OSA controls. FMD and RHI were significantly lower in both, moderate and severe OSA groups compared to non OSA controls. These results are presented graphically in Figures 1 and 2. CIMT did not differ significantly between the three groups (Table 4).

DISCUSSION

Obstructive sleep apnea (OSA) is an increasingly prevalent condition, particularly in the developing world (21). A large body of research exists, demonstrating an association of OSA with cardiovascular and renal morbidity (1, 22). One of the earliest steps in the development of cardiovascular and renal morbidity in OSA, is impairment of endothelial function (1, 5). The mechanisms underlying impairment of endothelial function in OSA, include reduced nitric oxide (NO) availability, endothelial inflammation and endothelial oxidative stress (23). Impaired endothelial function is regarded as a key mechanism in the development of atherosclerosis (24). Carotid intima media thickness (CIMT) is an established marker of subclinical atherosclerosis (25). The assessment of endothelial function and CIMT may

best demonstrate the risk of future cardiovascular and renal disease in OSA patients. Assessment of endothelial function and CIMT in OSA, is frequently confounded by associated co morbidities such as diabetes mellitus, hypertension, dyslipidemia, hypothyroidism and proteinuria. Present study evaluated endothelial function and CIMT in moderate to severe OSA subjects and non OSA controls, after careful exclusion of diabetes mellitus, hypertension, dyslipidemia, hypothyroidism and proteinuria.

Present study has demonstrated that endothelial function is significantly impaired in moderate and severe OSA patients compared to non OSA controls. The impairment of endothelial function is observed in both, macrovascular and microvascular circulation. Present study findings are in close agreement with those of Bayram et al. (26), who demonstrated a significantly impaired FMD in normotensive subjects with moderate to severe OSA. They also observed an inverse correlation between FMD and AHI. In the study by Chung et al. (27), it was observed that FMD was significantly impaired in severe OSA compared to non OSA controls. Butt et al. used FMD and laser doppler flowmetry (LDF) to evaluate endothelial function in moderate and severe OSA subjects (28). They demonstrated an impaired FMD and cutaneous perfusion response in moderate to severe OSA subjects. Only a few studies have used peripheral arterial tonometry (PAT) to assess microvascular endothelial function in OSA (11, 29). Present study confirms the findings of Itzhaki et al. (11), who showed a significantly impaired endothelial function, as assessed by peripheral arterial tonometry (PAT), in moderate to severe OSA patients compared to non OSA subjects. Present study did not find any correlation between FMD and RHI. This lack of

correlation has been demonstrated in previous studies (30, 31). It may be possible that different mechanisms of endothelial dysfunction may be acting in different vascular beds.

CIMT is a useful marker of subclinical atherosclerosis. Present study has shown that CIMT is significantly higher in moderate and severe OSA patients compared to non OSA controls. These findings suggest a greater risk of future clinical atherosclerotic disease in moderate and severe OSA patients compared to subjects without OSA. Similar findings were observed by Tanriverdi et al. (32), who showed a significantly higher CIMT in moderate and severe OSA patients compared to non OSA controls. Present study findings are in agreement with the findings of Drager et al. (33). They demonstrated a higher CIMT in severe OSA patients compared to mild to moderate OSA and non OSA controls.

Present study does not find a significant correlation between FMD, RHI, CIMT and any of the OSA disease severity indices (AHI, ODI and minimum O_2 saturation). Perhaps the reason for this lack of correlation is a small sample size. Given the stringent inclusion and exclusion criteria, a large number of patients had to be screened prior to participation in the study.

The present study has several unique features. Present study is the first study of endothelial function in OSA from the Indian subcontinent. To our knowledge, this is also the first study of endothelial function in OSA using both, flow mediated dilatation (FMD) and peripheral arterial tonometry (PAT) in the same cohort. Most major cardiovascular risk factors were carefully excluded in cases and controls. Endothelial function was evaluated in both,

macrovascular and microvascular beds in the same cohort. We used a novel non invasive technology, peripheral arterial tonometry to evaluate endothelial function in microvasculature. Peripheral arterial tonometry is relatively easier to perform and is operator independent. Present study is the first to evaluate endothelial function in OSA, using FMD and peripheral arterial tonometry in the same cohort.

There are a few limitations to the present study. The sample size of the study was relatively small compared to previous studies of endothelial dysfunction in OSA (27, 28). This was because of stringent inclusion and exclusion criteria. A large number of patients were screened to exclude those with major cardiovascular risk factors. Second, we did not study endothelium independent vasodilatation in our study. Third, it was a cross sectional study and we did not study the impact of continuous positive airway pressure (CPAP) on measures of endothelial function and CIMT.

In conclusion, present study demonstrates an impaired endothelial function and CIMT in moderate to severe OSA patients. Impairment of endothelial function is seen in both, macrovascular as well as microvascular beds. Given the increased incidence of cardiovascular events in the OSA population (34), our findings assume importance. Non invasive tests in the form of FMD, peripheral arterial tonometry and CIMT may allow us to detect cardiovascular and renal disease in OSA population at an incipient stage.