Respiratory Medicine (2014) 108, 1229–1233

Available online at www.sciencedirect.com

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

The association between exhaled nitric oxide and sleep apnea: The role of BMI

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Received 25 November 2013; accepted 18 May 2014
Available online 12 June 2014

KEYWORDS
Exhaled no;
Sleep apnea;
Obesity

Summary
Background: Obstructive sleep apnea syndrome is associated with airway inflammation. Measurement of exhaled nitric oxide is a non-invasive method for evaluation of airway diseases. It seems that obesity is an exacerbating factor for airway inflammation. We aimed to evaluate the changes of exhaled nitric oxide after sleep in patients suffering from OSA regarding BMI.
Method: In 54 patients referred for polysomnography, exhaled nitric oxide measurements were performed before and after sleep. Subjects were divided into three categories: normal, obese with sleep apnea and non-obese, based on polysomnographic recordings and BMI.
Results: 47 subjects had abnormal apnea/hypopnea index (AHI mean = 39.7) and 7 were normal regarding AHI (AHI mean = 3.0). BMI was significantly correlated to AHI, number of desaturations and hypoxia. Among those with apnea, 31 subjects were obese and 16 were non-obese. Exhaled nitric oxide levels in normal and OSA subjects showed no significant change, but a significant increase was found in obese patients with apnea (14.7 pre-sleep mean, 20.0 post-sleep mean).
Conclusions: Obesity is an effective factor in the inflammation of airways in patients with obstructive apnea.

Introduction

Obstructive sleep apnea (OSA) syndrome is characterized by an incomplete or complete and intermittent airflow obstruction during sleep which is associated with respiratory effort. The obstruction is usually associated with upper airway obstruction and causes such complaints as

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http://dx.doi.org/10.1016/j.rmed.2014.05.010
0954-6111/

snoring and apnea, which are often reported by the patient's bed partner. This sleep is not refreshing and may lead to daytime sleepiness [1]. OSA is a risk factor for cardiovascular disorders and is associated with increased morbidity and mortality [2]. Periods of decreased oxygen saturation in OSA patients leads to oxidative stress and free radical formation and release of inflammatory mediators [3]. This inflammation and oxidative stress can be detected and monitored using non-invasive methods such as induced sputum, exhaled breath condensate and exhaled gases.

One of the first exhaled gases used for the evaluation of patients with apnea was nitric oxide. The non-invasive technique of fractional exhaled NO (FENO) makes it an ideal test for evaluation of patients with upper airway diseases [4].

By now there are few studies with controversial results for the evaluation of this index among patients with apnea. One of the first studies in this regard was conducted by Olopade et al. which showed a significant increase in NO after sleep among patients with apnea [5], but Agusti et al. didn't find a significant difference between patients with apnea and normal subjects in terms of NO concentration [6]. The prevalence of OSA in general population is 4% in men and 2% in middle-aged women [7], which reaches to 20%–40% in individuals with body mass index (BMI) higher than 30 kg/m^2 [8]. Although the relationship between obesity and systemic inflammation is obviously known [9–11], the relationship between obesity and airway inflammation has not been clearly understood among patients suffering from OSA.

In this study we aimed to evaluate the changes of exhaled nitric oxide after sleep in patients suffering from OSA regarding BMI.

Materials and methods

Study subjects

Study subjects included 54 patients referred to sleep clinic of Shahid Sadoughi University of Medical Sciences for night-time polysomnography. Exclusion criteria included severe respiratory infection in the last 6 weeks, treatment with oral or inhalational corticosteroids, consuming drugs releasing nitric oxide in the body (e.g. isosorbide dinitrate) and using leukotriene modifiers (e.g. zafirlukast) and cigarette smokers (those who smoked at least 0.5 pack-years were considered as smokers, but ex-smokers were included in the study if they have quitted smoking more than 1 year ago).

Study design

This was a cross-sectional study on patients referring to sleep clinic of Shahid Sadoughi University of medical sciences for night-time polysomnography. Neck and abdominal circumferences were measured by a tape meter. Height was measured using a stadiometer to the nearest 0.5 cm. Weight was measured by a digital weight scale (Laica, Italy, accuracy: $\pm$100 g). BMI was calculated by dividing weight in kilograms by the square height in meters. Subjects were divided into two groups based on their BMI: BMI $\leq$30 as obese and BMI $<$30 as non-obese. The study was approved by ethics committee of Shahid Sadoughi University of Medical Sciences. An informed consent was obtained from all participants.

FENO measurement

FENO was measured before and after night-time polysomnography (10 PM, and 6 AM) by a portable electrochemistry-based device (NObreath®, Bedfont Scientific Ltd., UK). All tests were performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines [12]. Results were reported in part per billion (ppb). Subjects were asked to perform expiration for 10 s with a constant flow (50 ml/s) and pressure (10 CmH_2O) in a sitting position. Measurements were repeated until we obtained at least two acceptable maneuvers with FENO differences less than 4 ppb. The mean of two tests was reported as the final result. The measurements were repeated after work shift (8 h). The subjects were asked to avoid heavy exercise such as stair climbing and heavy lifting, large meals and smoking (for recreational smokers with less than 0.5 pack-year of smoking) one hour before test.

Lung function assessment

Spirometry was performed with spirolab III device (MIR, Italy). Forced vital capacity (FVC), forced expiratory volume after 1 s (FEV_1), peak expiratory flow (PEF), and forced expiratory flow between 25% and 75% of FVC ($FEF_{25-75\%}$) were measured according to ATS/ERS guidelines [13]. All tests were performed under standardized conditions at body temperature and ambient pressure saturated with water vapor, with the subject in a sitting position and using a nose clip. All subjects performed spirometry under similar conditions. The highest of three technically acceptable recordings was used.

Polysomnography

Night-time polysomnography was performed for all subjects (device: SOMNOscreen plus TM PSG, SOMNOmedics, Germany). Sleep stages were defined using electroencephalograms (channels: C4-A2 and O2-A1), electrooculograms, and chin electromyogram. Respiratory events were detected by a thermistor and a nasal prong. Respiratory movements were detected by thoracic and abdominal bands. O_2 saturation was assessed by a pulse-oximeter. The test was scored by an occupational medicine specialist blinded to the study according to American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events [14]. Apnea/hypopnea index (AHI) $<$5 was considered normal, and those with AHI $\geq$5 with obstructive and mixed apneas were considered as cases of obstructive sleep apnea syndrome (OSA). Hypoxia was defined as O_2 saturation $<$90%. Total time of hypoxia and number of desaturations during sleep were also measured.

Statistical analysis

Data analysis was done using Windows IBM SPSS Statistics version 19 (IBM Corp., North Castle, NY). Quantitative data were expressed by mean ± standard deviation (SD) for normally distributed and mean (10–90 percentiles) for non-parametric ones. Kolmogorov–Smirnov test was used to test normality of data. Polysomnographic and exhaled nitric oxide data were not normally distributed. Student's *t*-test and non-parametric tests including Wilcoxon signed-rank and Mann–Whiney *U* tests were used for analysis of data. Correlation between parameters was tested by Pearson's and Spearman correlation tests. Level of significance was set at $P < 0.05$.

Results

Anthropometry and spirometry

Among all subjects, 42 were males and 12 were females. Among those with OSA, 31 subjects (66%) were obese and 16 (34%) were non-obese. There was one ex-smoker and one recreational smoker. Mean anthropometric measurements were significantly different between obese and non-obese subjects ($P < 0.001$). Mean spirometric parameters were not significantly different between two groups. Among spirometric parameters only FVC was significantly correlated with BMI ($P = 0.018$, $r = -0.423$). Table 1 shows anthropometric and spirometric parameters in the study population.

Polysomnography

According to the results of polysomnography, 47 subjects (87%) had abnormal AHI (OSA). Mean AHI in the normal and OSA subjects was 3.0 (1.4–4.9) and 39.7 (8.1–70.7) events per hour, respectively. BMI was significantly correlated to AHI ($r = 0.45$, $P = 0.001$), number of desaturations ($r = 0.47$, $P < 0.001$) and hypoxia ($r = 0.46$, $P < 0.001$). We found a significant correlation between neck circumference and abdominal circumference and AHI ($r = 0.40$, $P = 0.002$; and $r = 0.36$, $P = 0.008$, respectively). Table 2 shows the results of polysomnography and exhaled nitric oxide measurements.

Exhaled nitric oxide

Mean exhaled nitric oxide in study population before and after sleep was 15.7 (3.0–33.5) ppb and 18.9 (6.5–41.5) ppb, respectively, and the difference was not statistically significant ($P = 0.292$). Obese and non-obese subjects were not significantly different regarding exhaled nitric oxide concentration before and after sleep ($P = 0.256$ and $P = 0.567$, respectively); but FENO was significantly increased after sleep in obese patients ($P = 0.002$), but not in non-obese ones ($P = 0.649$). FENO changes, before and after sleep, were not significantly correlated with polysomnography parameters. Fig. 1 compares the mean exhaled nitric oxide in three groups of subjects (Normal, Obese OSA, Non-obese OSA).

Discussion

In this study, FENO was not significantly increased after sleep among normal and OSA patients. The increase in exhaled NO after sleep was significant in obese OSA patients but not in non-obese ones. Changes of FENO before and after sleep were not significantly correlated with polysomnography parameters.

As we know, Obesity is associated with systemic oxidative stress and such comorbid conditions as hypertension, hyperlipidemia, insulin resistance and OSA; each of these conditions can also increase the oxidative stress and systematic inflammation [9,15]. Several studies on the association between FENO (as an inflammatory index) and BMI have reached to controversial results. Maniscalco et al. found that FENO level is significantly lower in patients with severe obesity than normo-weighted healthy controls which was decreased after weight reduction [16].

But two other studies conducted by Tsang et al. and de Winter-de Groot et al., found a positive correlation between BMI and FENO [17,18]. In the current study we couldn't find a significant difference in baseline FENO between obese and non-obese subjects.

Also frequent desaturations in OSA patients may lead to systemic inflammation, and local trauma due to frequent

Table 1 Anthropometric and spirometric parameters of study population.

Variable	Normal $n = 7$	Non-obese OSA $n = 16$	Obese OSA $n = 31$
Age, years	44.8 ± 7.4	50.7 ± 13.4	50.1 ± 13.4
BMI, kg m^{-2}	26.1 ± 4.4	27.4 ± 1.9[a]	35.4 ± 3.9
Neck circumference, Cm	38.7 ± 5.5	38.8 ± 3.1[a]	44.5 ± 3.2
Abdominal circumference, Cm	99.4 ± 9.2	99.1 ± 10.4[a]	117.4 ± 11.0
FVC, L	3.7 ± 1.6	3.4 ± 1.2	3.5 ± 0.9
FEV_1, L	2.9 ± 1.2	2.8 ± 1.0	2.8 ± 0.8
FEV_1/FVC, %	79.9 ± 6.0	84.4 ± 6.7	81.8 ± 8.7
$FEF_{25\%-75\%}$, L/s	2.8 ± 1.1	3.2 ± 0.9	3.1 ± 1.3

Data are presented as mean ± SD.
[a] $P < 0.05$ vs obese patients.

Table 2 Polysomnographic and exhaled nitric oxide results.

Variable, unit	Normal $n = 7$	Non-obese OSA $n = 16$	Obese OSA $n = 31$
Hypoxia, %	14.7 ± 37.5	18.1 ± 27.0	44.7 ± 35.7
Desaturations, events	4.5 ± 9.9	104.6 ± 87.4	161.1 ± 114.2
AHI,[a] events h^{-1}	3.0 (1.4—4.9)	40.1 (5.6—120.5)	39.5 (8.5—73.1)
Pre-sleep NO,[a] ppb	22.1 (5.0—58.0)	15.8 (2.0—31.0)	14.1 (3.0—31.0)
Post-sleep NO,[a] ppb	11.8 (1.0—30.0)	20.3 (5.0—45.0)	19.8* (9.0—44.0)

Data are presented as mean ± SD.
AHI: apnea hypopnea index, events h^{-1}: events divided by hours, Hypoxia: percent of total sleep with O_2 saturation <90%, Desaturation: number of O_2 saturation falling <90%, ppb: part per billion, *: $P < 0.05$ vs pre sleep NO.
[a] Non-parametric presented as mean (10—90 percentiles).

airway obstruction may lead to local inflammation [3]. There are several studies on non-invasive methods of assessment of airway inflammation in patients with apnea. One of the first ones was conducted by Olopade et al. They found a significant increase in oral and nasal NO after sleep in patients with moderate to severe apnea and concluded that exhaled NO can be considered as a marker of upper airway inflammation in patients with obstructive apnea [5].

The study conducted by Agusti et al. failed to show a significant difference in exhaled NO between OSA patients and normal subjects. They also couldn't find a significant relationship between the concentration of exhaled NO and AHI, BMI and O_2 saturation. These studies have not assessed obesity as an independent factor leading to airway inflammation [6]. Two studies have assessed the effect of obesity on exhaled NO. Depalo et al. assessed exhaled NO and inducible nitric oxide synthase expression (iNOS) in inflammatory cells of sputum and found that both markers were significantly higher in obese and non-obese patients with apnea. They found as well a positive relationship between exhaled NO and iNOS with AHI [19]. In another study exhaled NO and inflammatory cell profile in the induced sputum and pH in the exhaled breath condensate were compared between obese patients, with and without OSA and normal subjects. PH was significantly lower in obese patients. NO concentration and the percent of neutrophils were also higher than normal subjects, but the study didn't show a significant difference between obese patients with and without apnea regarding inflammatory markers [20]. None of these studies have assessed non-obese patients with apnea.

In the current study, the increased exhaled NO after sleep in obese patients with OSA and no significant increase in non-obese subjects with OSA and normal subjects, proposes that the obesity is an independent risk factor in the development of airway inflammation in patients with OSA. The limitations of this study were the small number of normal subjects and absence of a control group of obese non-OSA subjects. Future studies are recommended for longitudinal assessment of changes in exhaled NO among patients with apnea and after continuous positive airway pressure (CPAP) use especially in obese patients.

Conclusion

Increased exhaled nitric oxide in obese patient with apnea not in non-obese ones proposed that the obesity is a marker

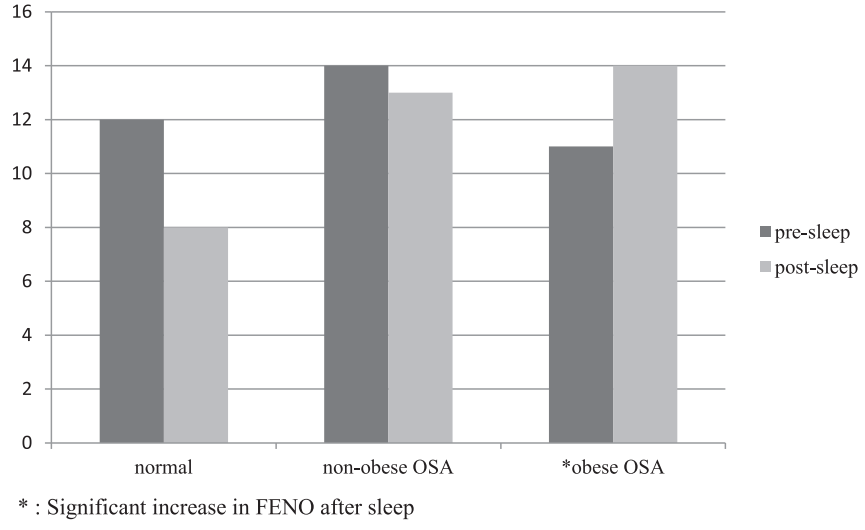

Figure 1 Comparison of the mean exhaled nitric oxide in three groups of subjects.

of airways inflammation in patients with obstructive sleep apnea.

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