The role of matrix metalloproteinases 2 and 9 in obstructive sleep apnea
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Background Obstructive sleep apnea (OSA) is a common condition that is characterized by intermittent and recurrent pauses in respiration results in multiple cycles of hypoxia/reoxygenation with an increased production of reactive oxygen species.

Aim The aim of the study was to evaluate serum levels of matrix metalloproteinase (MMP) 2 and MMP-9 as markers of oxidative stress in obese patients with OSA.

Patients and methods Study was performed on 30 obese patients who had been referred to the Chest Department of Kasr Al-Aini Hospital for clinical suspicion of OSA to perform polysomnography. They were classified into two groups: cases group that consisted of 20 obese patients who were diagnosed as OSA and controls group that consisted of 10 obese individuals, without OSA. The two groups were subjected to the following: complete history taking, clinical examination, Epworth sleepiness scale, BMI (in kg/m²), polysomnographic study, spirometry, laboratory examination for estimation of arterial blood gases, and serum levels of MMP-2 and MMP-9 measurements using ELISA.

Introduction In recent years, obstructive sleep apnea (OSA) has emerged as a major public health problem because of its profound impact on patients’ health and quality of life [1].

Obesity is one of the most important risk factors for sleep-disordered breathing [2].

Production of matrix metalloproteinase (MMP) 9 is stimulated by hypoxia and by several cytokines, such as IL-6 and TNF-α. These cytokines are increased and hypoxia is induced by apnea and hypopnea during sleep in patients with OSA [3].

Aim The aim of the study was to evaluate serum levels of MMP-2 and MMP-9 as markers of oxidative stress in obese patients with OSA.

Patients and methods The present study was conducted during the period from May 2011 to May 2012; it included 30 obese patients who had been referred to the Chest Department of Kasr Al-Aini Hospital for clinical suspicion of OSA to perform polysomnography.

Results In a comparison between cases and controls regarding serum levels of MMP-9, it was found that the mean value of MMP-9 among cases was 169.7 ± 135.22, which was higher than in controls as the mean value was 87.29 ± 34.01, and the difference was statistically significant. MMP-2 also was higher in cases than in controls. However, the differences were statistically insignificant.

Conclusion MMP-9 could be used as a marker of oxidative stress in OSA. Egypt J Broncho 2014 8:10–16 © 2014 Egyptian Journal of Bronchology.

Keywords: matrix metalloproteinase 9, obesity, obstructive sleep apnea, oxidative stress

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Measurement of serum levels of MMP-2 and 9:
(a) All participants went to sleep at 9:00 p.m. and were awakened at 5:00 a.m.; samples of peripheral venous blood were collected at 5:00 a.m. and were stored at -80°C until assay.

Quantitation of human MMP-2 levels in serum:
(a) Human MMP-2 levels were measured in serum using Quantikine ELISA Kit (catalog no. DMP2F0; R&D System Inc., Minneapolis, Minnesota, USA).

Quantitation of human MMP-9 levels in serum:
Quantitation of MMP-9 levels in serum was performed using Quantikine ELISA Kit (catalog no. DMP900; R&D System Inc.).

Results
The results are shown in Tables 1–13.

| Table 1 Sex distribution: statistical comparison between cases and controls |
|-----------------------------|-----------------------------|
| Group            | Cases | Controls | Total |
| Sex             |       |          |       |
| Females         | 7 (35.0) | 6 (60.0)  | 13 (43.3) |
| Males           | 13 (65.0) | 4 (40.0)  | 17 (56.7) |
| Total           | 20 (100.0) | 10 (100.0) | 30 (100.0) |
| P-value         | 0.255 |          |       |

| Table 2 Age distribution: statistical comparison between cases and controls |
|-----------------------------|-----------------------------|
| Groups          | Age (years) |
| Cases           |       |
| N               | 20     |
| Mean            | 51.40  |
| SD              | 11.682 |
| Control         |       |
| N               | 10     |
| Mean            | 43.30  |
| SD              | 14.960 |
| P-value         | 0.118  |

| Table 3 Statistical comparison between cases and controls with respect to BMI |
|-----------------------------|-----------------------------|
| Groups          | BMI (kg/m²) |
| Cases           |       |
| N               | 20     |
| Mean            | 43.45  |
| SD              | 9.24   |
| Control         |       |
| N               | 10     |
| Mean            | 45.12  |
| SD              | 4.09   |
| P-value         | 0.234  |
mean value was 87.29 ± 43.01. The difference was statistically significant.

For MMP-9 the mean value among cases was 169.57 ± 135.22, whereas among controls the mean value was 157.16 ± 70.23, and the difference was statistically insignificant.

Table 11 shows a positive correlation between AHI, ESS, BMI, and MMP-2 and MMP-9, but it was found to be statistically insignificant.

Table 12 shows positive significant correlation between MMP-2 and MMP-9 among patients with mild Obstructive Sleep Apnoea Syndrome (OSAS). In addition, there was a positive correlation between AHI, ESS, BMI, and MMP-2 and MMP-9, but it was found to be statistically insignificant.

Table 13 shows positive significant correlation between MMP-2 and MMP-9 among patients with moderate to severe OSAS. In addition, there was a positive correlation between AHI, ESS, BMI, and MMP-2 and MMP-9, but it was found to be statistically insignificant.

Discussion

In recent years, OSA has emerged as a major public health problem because of its profound impact on patients’ health and quality of life [1].

Obesity is one of the most important risk factors for sleep-disordered breathing [2].

In addition, Nagayoshi et al. [7] reported that several research studies had repeatedly and consistently confirmed that OSA is more common in male patients than in female patients and that male-to-female ratio is estimated to be ∼2 : 1 in the general population, supporting our result.

In the present study, the mean age of patients was 51.40 ± 11.68 years, which was higher than that of controls (mean age 43.30 ± 14.96 years) (Table 2). The difference between the two groups was statistically insignificant.

This is in agreement with the study by Peppard et al. [8] who reported an increase in the prevalence of OSA with age, which could not be explained by other risk factors such as obesity.
Reduced pharyngeal size, changes in upper airway muscle function, and respiratory instability. Among postmenopausal women, the reductions in the circulating levels of sex hormones and pharyngeal lengthening contribute to increased risk for OSA [9].

In the current study, the BMI mean value among patients was 43.45 ± 9.24, denoting that most of our patients were considered class III obesity (Table 3). This is in agreement with the study by Shelton et al. [10] who stated that obesity is believed to predispose to OSA because of mass loading to the upper airway of the neck.

Several mechanisms have been proposed to account for the increasing prevalence of OSA with increasing age, such as changes in pharyngeal mechanics, reduced pharyngeal size, changes in upper airway muscle function, and respiratory instability. Among postmenopausal women, the reductions in the circulating levels of sex hormones and pharyngeal lengthening contribute to increased risk for OSA [9].

In the current study, the BMI mean value among patients was 43.45 ± 9.24, denoting that most of our patients were considered class III obesity (Table 3).

This is in agreement with the study by Shelton et al. [10] who stated that obesity is believed to predispose to OSA because of mass loading to the upper airway of the neck.

In addition, Strohi and Redline [11] stated that excess body weight is a major risk factor for snoring and sleep-related breathing disorders.
sleep apnea and that 70% of patients with OSAS are overweight.

However, the mean value of BMI among controls was 45.12 ± 4.09, which was higher than in patients, but it did not reach any statistical significance (Table 3).

ESS is a validated tool for the systematic assessment of impaired daytime alertness. ESS is applied frequently because of its simplicity in routine practice, especially to describe sleepiness of patients with OSA. ESS was constructed to measure the patient’s ability to remain awake or the propensity to doze off in typical daily situations [4].

In this study, it was found that all 20 (100%) patients had daytime sleepiness compared with five (50%) controls who had daytime sleepiness and five (50%) controls who had not (Table 4).

The mean value of ESS among patients was 19.80 ± 5.24 compared with controls who had the mean value of 7.60 ± 2.07. This increase in daytime sleepiness and ESS among patients compared with controls was statistically highly significant (Table 5).

This is in agreement with the study by Banamah [12] who studied 27 patients with OSA and 26 obese individuals without OSA as a control group; he found highly significant increase in ESS among patients with OSA in comparison with controls.

In the current study, we found that, among patients 18 (90%) had snoring, whereas among controls nine (90%) had snoring, with no statistically significant difference (Table 10).

This is in agreement with the study by Gottlieb et al. [13]; they found that snoring has poor predictive value for OSA owing to a high prevalence in the general population.

However, Viner et al. [14] stated that snoring is a hallmark of OSA, and in its absence the diagnosis of OSA is unlikely.

Morning headache is a less common manifestation of sleep apnea. If reported, one must consider the possibility of hypercapnia secondary to obesity hypoventilation syndrome [15].

In the present study it was found that among patients 13 (65%) had morning headache and among controls four (40%) had morning headache. This difference was not statistically significant (Table 6).

This is not in agreement with the study by Kiely et al. [16] who stated that patients with OSA often report morning headache. However, McNicholas and Bonsignore [17] stated that no systematic study has been undertaken to analyze whether morning headache has the potential to predict the presence or absence of OSA. Therefore, the utility of this symptom in the objective clinical assessment of patients with suspected OSA remains uncertain.

In the present study, there was statistically highly significant increase in AHI, desaturation index, duration of desaturation less than 90%, and minimal O2 sat% among patients compared with controls. However, average O2 sat% was lower in patients than in controls, and this reduction was statistically significant (Table 7).

This is in agreement with results obtained by Kaynak et al. [18] who demonstrated that the minimal oxygen saturation point was statistically lower in patients with OSA than in controls.

In addition, Nakagawa et al. [19] studied 93 patients with OSA and 18 controls; they found that there was statistically significant difference between patients and controls in AHI, desaturation index, and duration of O2 desaturation less than 90%.

The mean value of FEF25–75% pred among patients was 56.28 ± 23.52, and among controls the mean value was 56.60 ± 20.42. The difference between patients and controls regarding all spirometric data was statistically insignificant. This may be attributed to the fact that both groups were obese and they were matched regarding BMI (Table 8).

This is in agreement with the study by Biring et al. [20] who found that obesity leads to limitations in airflow, with reduction in both FEV1 and FVC with the FEV1/FVC ratio remaining unchanged. Some authors have found a restrictive model in obese patients with an increased FEV1/FVC ratio.

In addition, Canoy et al. [21] reported that obese patients are prone to have reduced FEV1, FVC, and total lung capacity in lung function tests and they usually present with restrictive lung patterns, and they found that the possible mechanisms for the abnormal lung function tests in obese patients were reduced chest wall compliance and increased peripheral airway resistance.

Comparison between patients and controls regarding arterial blood gases revealed that the mean value of PO2 among patients was 71.40 ± 14.81, which was lower compared with controls (mean value was 79.30 ± 12.89). For O2 sat%, the mean value among patients was 93.70 ± 2.53, which was also lower as compared with controls (95.80 ± 2.53). The difference in PO2 and
O$_2$ sat% may be related to age differences between the two groups (Table 9).

This is in agreement with the study by Zhang et al. [22]; they demonstrated that aging is associated with both hypoxia and increases in reactive oxygen species in aging men.

Several studies indicated that OSAS-induced hypoxic stress activates the production of inflammatory mediators by monocytes such as MMP-9 and TNF-α, and this phenomenon may contribute to the development of atherosclerosis. Therefore, it is suggested that Continuous Positive Airway Pressure (CPAP) treatment could play a role in the prevention of atherosclerosis in OSAS patients [23].

In a comparison between patients and controls regarding serum levels of MMP-9, it was found that the mean value of MMP-9 among patients was 169.57 ± 135.22, which was higher than in controls, as mean value was 87.29 ± 43.01, and the difference was statistically significant (Table 10).

These results are in agreement with those of Toshiyuki et al. [24] who demonstrated that serum levels of MMP-9 were significantly higher in all patients with OSAS than in obese controls and that the serum levels of MMP-9 were significantly higher in patients with moderate to severe OSAS than in patients with mild OSAS.

This result is in agreement with the result of Jin et al. [25] who reported that serum concentration of MMP-9 was significantly higher in patients with OSAS than in obese controls. Levels of MMP-9 were also significantly higher in moderate to severe OSAS than in the mild OSAS group or obese controls.

It was found to be in agreement also with the study by Shinji et al. [23] who reported that, in OSAS patients, the production of MMP-9 by monocytes was significantly elevated after sleep in the early morning than in controls, and was decreased after long-term CPAP treatment. In addition, the production of MMP-9 by monocytes is attributed to OSAS-induced hypoxic stress.

In the present study, there was a positive correlation between AH1 and MMP-9, but it was statistically insignificant and this may be explained by the small number of patients.

Jin et al. [25] reported that serum concentration of MMP-9 was positively correlated with BMI in OSAS patients.

This is not in agreement with the study by Toshiyuki et al. [24] who stated that in patients with OSAS the levels and activity of MMP-9 were positively correlated with BMI.

In the present study, MMP-2 also was higher in patients than in controls. However, the differences were statistically insignificant.

**Conclusion**

Obesity is considered a major risk for OSA, and it is associated with local adipose tissue hypoxia and adipose tissue dysfunction.

OSA is associated with chronic intermittent hypoxia resulting in hypoxia, oxidative stress, and production of oxygen free radicals.

The current study highlights the effect of OSA on levels of certain oxidative markers.

It was concluded that serum MMP-9 was significantly higher in obese patients with OSA than in obese individuals without OSA.

These markers could be useful as prognostic factors to assess the response following CPAP treatment or Bariatric surgery; however, further studies still needed to confirm this fact.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Young T, Shohar E, Nieto F. Predictors of sleep-disordered breathing in community-dwelling adults: the sleep heart health study. Arch Intern Med 2002; 162:893–900.
2. Young T, Skatrud J, Peppard PE. Risk factors for obstructive sleep apnea in adults. JAMA 2004; 291:2013–2016.
3. Kondo S, Kubota S, Shimo T, Nishida T, Yoshimichi G, Eguchi T, et al. Connective tissue growth factor increased by hypoxia may initiate angiogenesis in collaboration with matrix metalloproteinases. Carcinogenesis 2002; 23:769–776.
4. Johns M. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. Sleep 1991; 14:540–545.
5. National Institutes of Health. The practical guide identification, evaluation and treatment of overweight and obesity in adults. Bethesda, MD: National Institutes of Health; 2000. p. 10.
6. Kapur V, Baldwin C, Resnick H. Sleepiness in patients with moderate to severe sleep-disordered breathing. Sleep 2005; 28:472–477.
7. Nagayoshi M, Yamagishi K, Tanigawa T. Risk factors for snoring among Japanese men and women: a community-based cross-sectional study. Sleep Breath 2011; 15:63–69.
8. Peppard P, Young T, Palt M, Skatrud J. Prospective study of the association between sleep-disordered breathing and hypertension. N Engl J Med 2000; 342:1376–1384.
9. Malhotra A, Huang Y, Fogel R. Aging influences on pharyngeal anatomy and physiology: the predisposition to pharyngeal collapse. Am J Med 2006; 119:9–14.
10. Shelton K, Woodson H, Gay S. Pharyngeal fat in obstructive sleep apnea. Am Rev Respir Dis 1993; 148:462–466.
11 Strohi K, Redline S. Recognition of obstructive sleep apnea. Am J Respir Crit Care Med 1996; 154:279–289.
12 Banamah A. Endothelial dysfunction in patient with obstructive sleep apnea [MSc thesis]. Cairo: Faculty of Medicine, Cairo University; 2010.
13 Gottlieb D, Yao Q, Redline S. Does snoring predict sleepiness independently of apnea and hypopnea frequency? Am J Respir Crit Care Med 2000; 162:1512–1517.
14 Viner S, Szalai J, Hoffstein V. Are history and physical examination a good screening test for sleep apnea? Ann Intern Med 1991; 115:356–359.
15 Duran J, Esnaola S, Rubio R. Obstructive sleep apnea hypopnea and related clinical features in a population based sample of subjects aged 30 to 70 yr. Am J Respir Crit Care Med 2001; 163:685–689.
16 Kiely J, McNicholas W. Respiratory disorders during sleep. Overview and historical aspects of breathing disorders during sleep. Eur Respir Mon 1999; 10:1–8.
17 McNicholas WT, Bonsignore MR, the Management Committee of EU COST ACTION B26. Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities. Eur Respir J 2008; 29:156–178.
18 Kaynak D, Goksan B, Degirmenci N. Is there a link between the severity of sleep-disordered breathing and atherosclerotic disease of carotid artery. Eur J Neurol 2003; 10:487–493.
19 Nakagawa Y, Kishida K, Kihara S, Sonoda M. Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome. Am J Physiol Endocrinol Metab 2008; 294:E778–E784.
20 Biring M, Lewis M, Liu J, Mohsenifar Z. Pulmonary physiologic changes of morbid obesity. Am J Med Sci 1999; 318:297–297.
21 Canoy D, Luben R, Welch A. Abdominal obesity and respiratory function in men and women in the EPIC-Norfolk Study, United Kingdom. Am J Epidemiol 2004; 159:1140–1149.
22 Zhang L, Ebenezer P, Dasuri K. Aging is associated with hypoxia and oxidative stress in adipose tissue: implications for adipose function. Am J Physiol Endocrinol Metab 2011; 301:E599–E607.
23 Shinji T, Yamauchi M, Fukuoka A, Makinodan K. Production of inflammatory mediators by monocytes in patients with obstructive sleep apnea syndrome. Intern Med 2009; 48:1255–1262.
24 Tazaki T, Minoguchi K, Yokoe T, Samson KT, Minoguchi H, Tanaka A. Increased levels and activity of matrix metalloproteinase-9 in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 2004; 170:1354–1359.
25 Jin YE, Hui LIU, Yuan LI, Xian LIU, Jie-Ming ZHU. Increased serum levels of C-reactive protein and matrix metalloproteinase-9 in obstructive sleep apnea syndrome. Chin Med J 2007; 120:1482–1486.