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/m^2), polysomnographic study, spirometry, laboratory examination for estimation of arterial blood gases, and serum levels of MMP-2 and MMP-9 measurements using ELISA.

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

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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-a. 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.

The included patients were classified into two groups:
(1) Cases: it consisted of 20 obese patients (BMI>30) who were diagnosed with OSA on the basis of both clinical and polysomnographic criteria (AHI≥5 events/h).
(2) Controls: it consisted of 10 obese (BMI>30) healthy individuals free from any known diseases without OSA (Apnoea Hypopnea Index (AHI)<5 events/h).

All study participants were subjected to the following:
(1) Thorough history taking including:
   (a) Snoring.
   (b) Excessive daytime sleepiness.
   (c) Morning headache.

Epworth sleepiness scale (ESS):

This scale was then used to choose the most appropriate number for each situation:
0: would never doze.
1: slight chance of dozing.
2: moderate chance of dozing.
3: high chance of dozing.

Interpretations of ESS [4]:
   (a) Supernormal: ESS 0–5.
   (b) Normal: ESS 5–10.

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(c) Sleepy: ESS 10–15.
(d) Very sleepy: ESS 15–20.
(e) Dangerously sleepy: ESS>20.

(2) Thorough clinical examination.
(3) Anthropometric measurements including:
(a) Body weight in kg and height in m.
(b) BMI (in kg/m^2).

The National Institutes of Health [5] has classified obesity according to BMI into the following:
(a) Class I obesity: includes patients with BMI 30.0–34.9.
(b) Class II obesity: includes patients with BMI 35.0–39.9.
(c) Class III obesity: includes patients with BMI>40.0.

(4) Polysomnographic study: in sleep laboratory unit in the Chest Department, Cairo University Hospital, patients underwent 8 h sleep/night polysomnographic study using SOMNO (SMNO medics Gm bH Franconian wine region of Randersacker, Germany) Screen Plus (cardiorespiratory screening) with detailed analysis of the recorded data including:
(a) Pulse oximetry.
(b) A microphone applied to detect snoring.
(c) ECG lead.
(d) Oronasal airflow using thermal sensors and nasal pressure transducer.
(e) Chest and abdominal movements recording using two separate belts to detect the effort.
(f) Leg movements are recorded by anterior tibialis electromyogram.

From recording of sleep study, we detected the following:
(a) Apnea.
(b) Hyperpnea.
(c) AHI.
(d) Snoring index.
(e) Arrhythmia index.

The severity of sleep-related obstructive breathing events were rated as follows:
(a) Mild: 5–15 events/h.
(b) Moderate: 15–30 events/h.
(c) Severe: >30 events/h [6].

(5) Spirometry (Forced Vital capacity (FVC)%, Forced Expiratory Volume 1st Second (FEV_1)%, FEV_1/FVC, Expiratory Forced Flow (FEF)25–75%): spirometry was performed using Sensor-medics Vmax series, 2130 Spirometer, (Sensor Medics Corporation 22705 Savi Ranch Parkway Yorba Linda, 92887-4645 California, USA) V6200 Autobox, 6200DL.
(6) Arterial blood gases: pH, PO_2, PCO_2, HCO_3, and O_2 sat% were determined.
(7) 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.
(8) 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).
(9) 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		Total
	Cases	Controls	
Sex			
Females			
Count (% within group)	7 (35.0)	6 (60.0)	13 (43.3)
Males			
Count (% within group)	13 (65.0)	4 (40.0)	17 (56.7)
Total			
Count (% within group)	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/m^2)
Cases	
N	20
Mean	43.45
SD	9.24
Control	
N	10
Mean	45.12
SD	4.09
P-value	0.234

Table 10 shows that the mean value of MMP-2 among cases was 220.49 ± 161.74, whereas among controls the mean value was 157.16 ± 70.23, and the difference was statistically insignificant.

For MMP-9 the mean value among cases was 169.57 ± 135.22, whereas among controls the mean value was 87.29 ± 43.01. The difference was statistically significant.

Table 4 Statistical comparison between cases and controls with respect to daytime sleepiness

	Group		Total
	Cases	Controls	
Daytime sleepiness			
Yes			
Count (% within group)	20 (100.0)	5 (50)	25 (83.3)
No			
Count (% within group)	0 (0.0)	5 (50)	5 (16.7)
Total			
Count (% within group)	20 (100.0)	10 (100.0)	30 (100.0)
P-value	0.002		

Table 5 Statistical comparison between cases and controls with respect to Epworth sleepiness scale

Groups	ESS
Cases	
N	20
Mean	19.80
SD	5.24
Control	
N	10
Mean	7.60
SD	2.07
P-value	0.000

ESS, epworth sleepiness scale.

Table 6 Statistical comparison between cases and controls with respect to morning headache

	Group		Total
	Cases	Controls	
Morning headache			
Yes			
Count (% within group)	13 (65)	4 (40)	17 (56.7)
No			
Count (% within group)	7 (35)	6 (60)	13 (43.3)
Total			
Count (% within group)	20 (100.0)	10 (100.0)	30 (100.0)
P-value	0.181		

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.

Table 7 Statistical comparison between cases and controls with respect to polysomnographic data

Groups	AHI	Desaturation index	Minimal O_2 sat%	Average O_2 sat%	Duration of desaturation <90%	Snoring index
Cases						
N	20	20	20	20	20	20
Mean	23.77	39.31	67.60	88.60	37.99	220.46
SD	17.55	26.83	15.80	5.71	29.28	189.14
Controls						
N	10	10	10	10	10	10
Mean	2.25	9.51	83.5	93.80	6.73	150.58
SD	1.52	7.82	7.21	1.69	8.44	137.84
P-value	0.000	0.001	0.004	0.022	0.001	0.356

Table 8 Statistical comparison between cases and controls with respect to spirometric data

Groups	FVC% pred	FEV_1% pred	FEV_1/FVC	FEF25–75% pred
Cases				
N	20	20	20	20
Mean	66.08	83.99	56.28	40.83
SD	18.27	10.45	23.52	14.67
Controls				
N	10	10	10	10
Mean	69.50	84.50	56.60	42.60
SD	10.32	7.92	20.42	8.01
P-value	0.459	0.660	0.774	0.775

Table 9 Comparison between cases and controls with respect to arterial blood gases

Groups	pH	PO_2	PCO_2	HCO_3	O_2 sat%
Cases					
N	20	20	20	20	20
Mean	7.41	71.40	42.98	26.61	93.70
SD	0.039	14.81	9.01	5.45	4.08
Controls					
N	10	10	10	10	10
Mean	7.41	79.30	39.20	24.90	95.80
SD	0.036	12.89	4.85	2.13	2.53
P-value	0.842	0.098	0.243	0.480	0.462

Table 10 Statistical comparison between cases and controls with respect to matrix metalloproteinases 2 and 9

Groups	MMP-2	MMP-9
Cases		
N	20	20
Mean	220.49	169.57
SD	161.74	135.22
Controls		
N	10	10
Mean	157.16	87.29
SD	70.23	43.01
P-value	0.253	0.048

MMP, matrix metalloproteinase.

Table 11 Correlation between AHI, Epworth sleepiness scale, BMI, and matrix metalloproteinases 2 and 9 among cases

	MMP-2	MMP-9
AHI		
Correlation coefficient	0.011	0.030
P-value	0.965	0.900
N	20	20
ESS		
Correlation coefficient	0.141	0.114
P-value	0.554	0.633
N	20	20
BMI		
Correlation coefficient	0.375	0.338
P-value	0.104	0.145
N	20	20

ESS, epworth sleepiness scale; MMP, matrix metalloproteinase.

Table 12 Correlation between AHI, Epworth sleepiness scale, BMI, and matrix metalloproteinases 2 and 9 among cases with mild OSAS

	MMP-2	MMP-9
AHI		
Correlation coefficient	0.286	0.240
P-value	0.493	0.232
N	8	8
ESS		
Correlation coefficient	0.208	0.170
P-value	0.565	0.639
N	8	8
BMI		
Correlation coefficient	0.232	0.367
P-value	0.518	0.297
N	8	8
MMP-2		
Correlation coefficient		0.964
P-value		0.000
N		8

ESS, epworth sleepiness scale; MMP, matrix metalloproteinase.

Table 13 Correlation between AHI, Epworth sleepiness scale, BMI and matrix metalloproteinases 2 and 9 among cases with moderate to severe OSAS

	MMP-2	MMP-9
AHI		
Correlation coefficient	0.364	0.259
P-value	0.245	0.417
N	12	12
ESS		
Correlation coefficient	0.165	0.135
P-value	0.609	0.675
N	12	12
BMI		
Correlation coefficient	0.406	0.322
P-value	0.191	0.308
N	12	12
MMP-2		
Correlation coefficient		0.979
P-value		0.000
N		12

ESS, epworth sleepiness scale; MMP, matrix metalloproteinase.

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 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 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 O_2 sat% among patients compared with controls. However, average O_2 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 O_2 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 FEV_1 and FVC with the FEV_1/FVC ratio remaining unchanged. Some authors have found a restrictive model in obese patients with an increased FEV_1/FVC ratio.

In addition, Canoy *et al.* [21] reported that obese patients are prone to have reduced FEV_1, 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 PO_2 among patients was 71.40 ± 14.81, which was lower compared with controls (mean value was 79.30 ± 12.89). For O_2 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 PO_2 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-a, 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 AHI 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.

In our study, there was a positive correlation between MMP-9 and BMI but was statistically insignificant (Table 11).

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.

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