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REVIEW

Thiobarbituric acid reactive substance (TBARS) a marker of oxidative stress in obstructive sleep apnea

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Abstract *Introduction:* OSA is a common condition that is primarily characterized by intermittent and recurrent pauses in respiration results in multiple cycles of hypoxia/re-oxygenation with an increased production of reactive oxygen species (ROS).

Aim of work: Is to assess TBARS as a marker of oxidative stress in obese patients with and without OSA.

Subjects and methods: Study was performed on 51 obese subjects who had been referred to the Chest Department of Kasr Alaini Hospital with clinical suspicion of OSA in order to perform polysomnography. They were classified into two groups; Cases: consist of 33 obese patients who were diagnosed as obstructive sleep apnea (OSA) and Controls: consist of 18 obese subjects, without OSA as a control group. The two groups were subjected to polysomnograpic study and serum TBARS.

Results: There was statistically highly significant increase in Epworth sleepiness scale (ESS) among cases compared to controls. As regards the polysomnographic data, there was statistically highly significant increase in AHI, desaturation index and duration of desaturation < 90% among cases compared to control subjects. While minimal O_2 sat% and average O_2 sat% were lower in cases than in the control subjects this reduction was statistically significant. There was statistically highly significant increase in serum TBARS levels among cases as compared to controls. There was a statistically significant positive correlation between grade of obesity and serum TBARS among studied cases.

Conclusion: TBRAS could be used as a marker of oxidative stress in OSA.

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Introduction

Intermittent hypoxia in OSA can induce mitochondrial dysfunction and thereby increases oxidative stress [1].

Most of the studies regarding increased ROS and oxidative stress in patients with OSA are provided by indirect evidence mainly from circulating markers of oxidative stress [2].

Lipid peroxidation is a sensitive marker due to the high likelihood of lipids to undergo oxidation, and therefore is a highly used oxidative stress marker. Thus, increased oxidative stress in OSA was primarily shown by using various markers of lipid peroxidation in plasma and serum [3].

The lipid peroxidation biomarker TBARS was significantly increased in patients with OSA compared with control and plasma concentrations of TBARS were lowered by nCPAP treatment [4].

Subjects and methods

The present study was performed on 51 obese subjects who had been referred to the Chest Department of Kasr Alaini Hospital for clinical assessment.

The included subjects were classified into two groups:

(1) *Cases:* consist of 33 obese patients who were diagnosed as obstructive sleep apnea (OSA) based on both clinical and polysomnographic criteria (AHI $\geqslant$ 5 events/h).
(2) *Controls:* consist of 18 obese subjects, without OSA as a control group. They were clinically free from any known diseases.

Inclusion criterion:
Subjects with BMI > 30 kg/m^2.
Exclusion criteria:

(1) Known cases of D.M., hypertension.
(2) History of cardiac troubles.
(3) Presence of chest symptoms.

Study design:
The two groups were subjected to the following:

(1) Full history taking with special emphasis on key symptoms of OSA.
(2) Epworth sleepiness scale (ESS):

The patients were asked, to evaluate sleepiness (how likely are you to doze off or fall asleep in the following situations):

(1) Sitting and reading.
(2) Watching television.
(3) Sitting inactive in a public place (e.g. theater).
(4) As a car passenger for an hour without break.
(5) Lying down to rest in the afternoon.
(6) Sitting and talking to someone.
(7) Sitting quietly after lunch without alcohol.
(8) In a car, while stopping for a few minutes in traffic.

The following 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 Epworth sleepiness scale [4]:

(a) Supernormal (if ESS 0–5).
(b) Normal (if ESS 5–10).
(c) Sleepy (if ESS 10–15).
(d) Very sleepy (if ESS 15–20).
(e) Dangerously sleepy (if ESS > 20).

(3) Full clinical examination.
(4) Anthropometric measurements: All patients underwent comfort evaluation of anthropometric measures including: body weight, height, body mass index (BMI in kg/m^2), weight waist and hip circumferences, waist/hip ratio and neck circumference.

The National Institutes of Health [5] had classified obesity according to BMI into:

Class I obesity (includes cases with BMI 30.0–34.9) – Class II obesity (includes cases with BMI 35.0–39.9) – Class III obesity (includes cases with BMI > 40.0).

(5) Polysomnographic study: (8 h per night) with the detailed analysis of the recorded data. Before the study, patients were advised to avoid tea and coffee intake or any other drugs that may have influence on the quality of sleep as sedatives, hypnotics and tranquilizers.

(1) Patients presented to the Sleep Laboratory Unit in the chest department of Cairo University Hospital 1 h before their usual bed time to get familiar and adapt with the environment. We provided them with full explanation of the nature and aim of polysomnography. The duration of polysomnographic monitoring was about eight continuous hours.
(2) Patients were connected to SOMNOscreen™ plus (Cardio-Respiratory Screening) which is computer based high technology polysomnography.

(3) Pulse oximetry applied to the index finger to detect arterial oxygen saturation (SaO_2): O_2 desaturation index (/h), number of O_2 desaturations per hour of sleep, minimal nocturnal SaO_2 (%) and time in which $SaO_2 < 90\%$ (as a percent from total sleep time).

(4) A microphone applied on the neck beside the larynx to detect snoring.

(5) Heart rhythm is monitored with a single lead ECG: to detect Arrhythmia index which is the number of cardiac arrhythmias per hour of sleep.

(6) Oronasal airflow using a thermal sensors and nasal pressure transducer.

(7) Chest and abdominal movements recorded using two separate belts to detect the effort.

(8) Leg movements are recorded via anterior tibialis electromyogram.

(9) From recording of sleep study we detect:

a. *Apnea:* defined by the cessation of airflow for a minimum of 10 s, with the oral thermistor providing the most accurate detection of an apnea. An apnea is further classified as obstructive, central or mixed based on the assessment of respiratory effort during the event [6].

b. *Hypopneas:* are generally classified only as obstructive events. Hypopneas can be scored using various definitions although most commonly they are characterized by at least a 30% reduction in airflow in association with a 3% or 4% oxygen desaturation [6].

c. A desaturation is scored when the following two parameters are met: minimum drop required is 4% (the minimum decrease in oxygen level to score a desaturation) and minimum duration required is 10 s.

d. *AHI:* refers to the number of apneas and hypopneas per hour of sleep.

e. *Snoring index:* the number of snoring events per hour of sleep.

f. *Arrhythmia index:* the number of cardiac arrhythmias per hour of sleep.

g. The severity of sleep-related obstructive breathing events will be rated as follows: mild: 5–15 events/h; moderate: 15–30 events/h; and severe: > 30 events/h [7].

6. *Thiobarbturic acid reactive substance (TBARS):* The OXItek TBARS Kit is designed to provide a standardized, reproducible assay with consistent results. Each lot of reagents is quality controlled as a kit, which includes an MDA standard [8].

Statistical analysis of the results

Data were statistically described in terms of mean ± standard deviation (±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using the Student's *t* test for independent samples in comparing normally distributed and the Mann–Whitney *U* test for independent samples when not normally distributed. For comparing categorical data, the Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between different variables was done using Pearson moment correlation equation for linear variables. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs

SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

The results are shown in Tables 1–7.

Discussion

The control group included 14 females (77.8%) and 4 (22.2%) males, while cases included 10 females (30.3%) and 23 males (69.7%) Table 1. This result is in agreement with Nakayama-Ashida et al., in 2008 who stated that OSA syndrome is more common in adult males compared to adult females [9].

Table 1 Sex distribution; Statistical comparison between cases and controls.

Sex	Group		Total
	Cases	Controls	
Sex			
Females			
Count	10	14	24
% Within Group	30.3%	77.8%	47.1%
Males			
Count	23	4	
% Within Group	69.7%	22.2%	52.9%
Total			
Count	33	18	51
% Within Group	100.0%	100.0%	100.0%

Table 2 Age distribution; statistical comparison between cases and controls.

Group	Age (years)
Cases	
N	33
Mean	52.12
Std. deviation	11.48
Controls	
N	18
Mean	44.83
Std. deviation	12.70

Table 3 Statistical comparison between cases and controls as regards anthropometric measurements.

Group	BMI (kg/m^2)	W/H ratio	WC (cm)	NC (cm)
Cases				
N	33	33	33	33
Mean	42.82	3.75	138.61	47.03
Std. deviation	7.69	15.66	12.74	5.03
Controls				
N	18	18	18	18
Mean	44.33	0.93	132.94	44.78
Std. deviation	5.68	0.07	16.40	5.00
P-value	0.084	0.0450	0.177	0.132

Table 4 Statistical comparison between cases and controls as regards Epworth sleepiness scale (ESS).

Group	ESS
Cases	
N	33
Mean	17.61
Std. deviation	6.07
Controls	
N	18
Mean	8.78
Std. deviation	2.43
P-value	0.000[*]

[*] = statistically significant.

Table 6 Statistical comparison between cases and controls as regarding TBARS.

Group	TBARS (Umol/l)
Cases	
N	33
Mean	1.44
Std. deviation	0.54
Controls	
N	18
Mean	0.66
Std. deviation	0.17
P-value	0.000[*]

[*] = statistically significant.

In the present study the mean age of cases was 52.12 and SD ±11.48 which was higher than controls (mean age 44.83 and SD ±12.70) Table 2. This is in agreement with Peppard et al., in 2000 who reported increase in the prevalence of OSA with age that could not be explained by other risk factors such as obesity [10].

In the current study the BMI mean value among cases was 42.82 and SD ±7.69, denoting that most of our patients were considered as Class III obesity (Table 3).

This is in agreement with Shelton et al. in 1993 who stated that obesity is believed to predispose to OSA because of mass loading to the upper airway of the neck [11].

Also Strohl and Redline, in 1996 stated that excess body weight is a major risk factor for snoring and sleep apnea and that 70% of patients with OSAS are overweight [12].

However the mean value of BMI among controls was 44.33 and SD ±5.68, which was higher than cases and did not reach any statistical significance (Table 3).

Lecube et al., in 2010 found that not all subjects who are obese or have a large neck circumference suffer from sleep apnea, supporting our results [13].

As regards other anthropometric measurements we found that the mean value of W/H ratio (Waist/Hip ratio) among cases was 3.75 and SD ±15.66 which was higher than controls (mean value was 0.93 and SD ±0.07). The mean value of WC (waist circumference) among cases was 138.61 and SD ±12.74 which was higher than controls (mean value was 132.94 and SD ±16.40) and regarding NC (neck circumference) the mean value among cases was 47.03 and SD ±5.03 which was also higher than controls (mean value was 44.78 and SD ±5).

Table 7 Correlation between severity of OSA, grade of obesity and serum TBARS among cases.

	Severity of OSA	Grade of obesity
TBARS		
Pearson Correlation	0.131	0.448
P-value	0.468	0.009[*]
N	33	33

[*] = statistically significant.

The differences between cases and controls regarding these anthropometric measurements were statistically insignificant (Table 3).

Mortimore et al., in 1998 found that the predictive value of other anthropomorphic variables related to body weight, such as waist circumference, waist-to-hip ratio, is generally lower in OSA except in extreme cases, supporting our results [14].

The mean value of Epworth sleepiness scale (ESS) among cases was 17.61 and SD ±6.07 compared to controls mean value 8.78 and SD ±2.43. This increase in Epworth sleepiness scale (ESS) among cases compared to controls was statistically highly significant (Table 4).

This is in agreement with Banamah, in 2010 who studied 27 patients with OSA and 26 obese subjects without OSA as control group, he found highly significant increase in ESS among the patients with OSA in comparison to control subjects [15].

In the present study, there was statistically highly significant increase in AHI, desaturation index and duration of

Table 5 Statistical comparison between cases and controls as regarding polysomnographic data.

Group	AHI	Desat. index	Minimal O$_2$ sat%	Average O$_2$ sat%	Duration of desat. <90%	Snoring index	Arrythmia index
Cases							
N	33	33	33	33	33	33	33
Mean	19.23	40.92	68.58	89.76	35.26	236.36	25.85
Std. deviation	13.98	26.00	17.22	5.33	31.26	148.04	23.04
Controls							
N	18	18	18	18	18	18	18
Mean	2.14	8.11	83.61	94.44	5.90	143.47	9.78
Std. deviation	1.37	9.60	7.94	1.50	9.12	134.11	13.95
P-value	0.000[*]	0.000[*]	0.001[*]	0.001[*]	0.000[*]	0.032[*]	0.003[*]

[*] = statistically significant.

desaturation <90% among cases compared to control subjects. While minimal O_2 sat% and average O_2 sat% were lower in cases than in control subjects this reduction was statistically significant (Table 5).

This is in agreement with results obtained by Kaynak et al., in 2003, who demonstrated that the minimal oxygen saturation point was statistically lower in patients with OSA than controls [16].

Also Nakagawa et al., in 2008 studied 93 patients with OSA and 18 control subjects, they found that AHI, desaturation index, and the percentage of arterial O_2 saturation from pulse oximetry (Sp_{O2}) <90% were significantly higher and the lowest Sp_{O2} was significantly lower in OSAHS patients than in the control subjects [17].

Concerning the arrhythmia index the current study revealed a higher mean value among cases (25.85 and SD ±23.04) compared to controls (9.78 and SD ±13.95) and this difference was found to be statistically significant (Table 5).

This result is in agreement with Hoffstein and Mateika, in 1994 who analyzed electrocardiographic recording in 458 patients having sleep studies and showed a 58% prevalence of arrhythmias in patients with OSA compared with 42% in non-apneic, and also they found that most arrhythmias occurr in those with AHI >40/h [18].

In the present study, there was statistically significant increase in snoring index among cases (236.36 and SD ±148.04) than control subjects (143.47 and SD ±134.11) (Table 5).

This is in agreement with Hudgel, in 1986 who stated that snoring is the primary symptom of nearly all patients with sleep apnea [19].

Also our result is matched with Viner et al., in 1991 who stated hat snoring is a hallmark of OSA [20].

In the present study, we found statistically highly significant increase in serum level of TBARS among cases (mean value was 1.44 and SD ±0.54) as compared to controls (mean value was 0.66 and SD ±0.17) Table 6.

This agreed with Lavie et al., in 2004 who found that the lipid peroxidation biomarker TBARS was significantly increased in patients with OSA compared with control. These results support the existence of an increased state of oxidative stress in OSA and its possible involvement in cardiovascular morbidity [3].

While Wali et al., in 1998 failed to identify abnormal lipid peroxidation in a small group of OSA patients [21].

In the present study a positive insignificant correlation was found between severity of OSA and TBARS Table 7.

This agreed with Lavie et al., in 2004 who studied 114 patients with OSA and 55 without OSA. Morning levels of TBARS and PD (peroxides) were measured and found to be significantly higher in OSA patients than in controls. Also the concentrations of TBARS and PD were significantly positively correlated with RDI (Respiratory Disturbance Index) [3].

There was a positive significant correlation between grade of obesity and serum TBARS among studied cases Table 7.

This is in agreement with Furukawa et al., in 2004, they studied oxidative stress in obese subjects by measuring lipid peroxidation, represented by plasma thiobarbituric acid reactive substance (TBARS), and reported that levels of TBARS were significantly correlated with BMI [22].

Conclusions

It was concluded that serum TBARS was significantly higher in obese patients with OSA than obese subjects without OSA.

This marker could be used as prognostic factor to assess the response following CPAP treatment or Bariatric surgery in patients with OSA, however further researches are still needed to confirm this fact.

Conflict of interest

None declared.

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