Original Article

Oxidative Stress and Obstructive Sleep Apnoea Syndrome

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ABSTRACT

Rationale. Even though oxidative stress has been proposed as an underlying mechanism for the symptoms in patients with obstructive sleep apnoea syndrome (OSAS), little information is available on the effects of anti-oxidant treatment on their improvement.

Objectives. To observe the effects of anti-oxidant treatment on polysomnographic parameters and oxidative stress markers in OSAS patients.

Methods. Polysomnography (PSG) was performed on 20 male patients. They were administered continuous positive airway pressure (CPAP) therapy for two nights followed by oral intake of vitamin C (100 IU BD) and vitamin E (400 IU BD) for 45 days and a repeat PSG was done. Ten healthy normal subjects underwent the same protocol excepting the CPAP therapy.

Results. In OSAS patients, plasma lipid peroxidation increased significantly and whole blood reduced glutathione decreased significantly. The CPAP therapy as well as anti-oxidant treatment reduced the lipid peroxidation and restored the reduced glutathione concentrations. After anti-oxidant intake, OSAS patients slept better with decrease in Epworth sleepiness score and the number of apnoeic episodes. They spent more time in stages 3 and 4 of sleep. The optimum pressure of CPAP device was significantly lowered also.

Conclusions. Oxidative stress contributes to sleep behaviour in OSAS patients, and anti-oxidant intake improves the quality of sleep in them. **[Indian J Chest Dis Allied Sci 2009;51:217-224]**

Key words: Anti-oxidants, Polysomnography, Sleep apnoea, Stress.

INTRODUCTION

Obstructive sleep apnoea syndrome (OSAS) is characterised by excessive day-time sleepiness, snoring, repeated upper airway obstruction during sleep and hypoxaemia.[1] Due to excessive day-time sleeping, there is absenteeism from work, diminished work output, motor vehicle accidents while driving, etc.[2-5] Even though it is recognised that these individuals are prone to several cardiovascular disorders, notably myocardial infarction, pulmonary hypertension and ventricular arrhythmias,[6,7] till date the exact mechanism behind the disturbed sleep and the observed symptoms remains elusive.[8] There is overwhelming evidence that obstructive sleep apnoea is becoming a major contributor to morbidity and mortality not only in developed countries[9] but in developing countries, like India also.[9-10] Even though several risk factors have been identified,[9] application of nasal continuous positive airway pressure (CPAP) is the usual method of choice for treating these patients.

During apnoeic episodes, the oxygen saturation falls and when the breathing resumes, there is re-oxygenation. The recurrent hypoxia-re-oxygenation cycles may result in oxidative stress in OSAS patients which may be a contributing factor for the symptoms observed in these patients.[11-13] Even though there are few studies which refute the role played by oxidative stress,[14,15] there are several other studies which support it. For instance Barcelo *et al*[16, 17] reported that thiobarbituric acid-reactive substance (TBARS) formation is higher and anti-oxidant status is lower in OSAS patients. Similar findings have been obtained by other investigators,[18-21] who further showed that CPAP treatment reduces the oxidative stress parameters. In most of these studies, the CPAP treatment has been given for periods ranging from 3 to 12 months which may be bothersome to the patients. There is negligible information in the literature on the effects of short-term CPAP treatment on oxidative stress. More importantly, no study has addressed the issue whether treatment with anti-oxidants has a beneficial eftect upon the poly-somnographic measurements and the observed symptoms. Even though such a possibility was mentioned, it has never been tested.[11] Hence, the overall goal of the present study was to assess whether anti-oxidants improve the sleep quality in

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OSAS patients. We tested this possibility in OSAS patients by CPAP treatment for two nights followed by anti-oxidant (vitamin C and vitamin E) therapy for 45 days and then repeated protocols with CPAP treatment. Our results demonstrate that anti-oxidant treatment is a useful therapy in reducing the severity of sleep disorders in OSAS patients.

MATERIAL AND METHODS

The present study was conducted strictly in accordance with the ethical guidelines for biomedical research on human subjects by Central Ethics Committee on Human Research, Indian Council of Medical Research – 2000 and those contained in "Declaration of Helsinky" and was approaved by the Ethics Committee of the Institute.

The following criteria were employed in selection of subjects for the current study; (a) excessive day-time sleepiness; (b) loud snoring and (c) body mass index (BMI) >25 kg/m^2; and (d) they should not be consuming alcohol or taking psychotropic drugs. Out of 8918 patients attending the Out-patient Department of Vallabhbhai Patel Chest Institute, over a period of 20 months, we could get 70 such patients. However, only 30 agreed for undergoing over night polysomnography (PSG). Since there was no evidence of sleep apnoea in four and one patient had concealed the usage of anti-depressants, they were excluded from the study. Four patients did not report for the follow-up. Even though 21 patients stayed for the full duration of the study, there were some doubts about the compliance *vis-a-vis* the oral intake of anti-oxidant medication in one patient. Consequently, results from 20 patients are presented in this article.

Study Plan

These patients were given a sleep questionnaire for filling and their day-time sleepiness was confirmed further from the Epworth Sleepiness Scale (ESS)[22] before subjecting them to PSG. These patients slept in the sleep laboratory one night prior to sleep study (for acclimatisation to new environment). Diagnostic sleep study was done the next night. The diagnosis of OSAS was based on stnadard PSG (Rembrandt version 5.0.3, Medcare, Netherland) during a full night stay in the sleep laboratory. Polysomnographic studies included recording of electro-encephalogram, electro-oculogram, electromyogram, oronasal flow, thoracoabdominal movements, electrocardiogram, limb movements, body position and arterial oxygen saturation, and were analysed manually by skilled staff, according to the standard criteria. Airflow was registered with a thermistor, respiratory efforts, with strain strips and transcutaneous saturation (SaO_2) monitored continuously using a pulse oximeter. Apnoea was defined as complete cessation of airflow for >10 sec and was classified either as obstructive, central or mixed, based on the presence or absence of the respiratory efforts. Hypopnoea was defined as a reduction of ≥50% in oronasal flow for >10 sec or ≥30% reduction in oronasal flow with >4% decrease in SaO_2 or an arousal.[23] Apnoea-hypopnoea index (AHI) was calculated for each patient using the formula[24] given below:

$$AHI = \frac{Number\ of\ apnoeas + Number\ of\ hypopnoeas}{Total\ sleep\ time}$$

After diagnosis, titration of CPAP, using a mask and apparatus (Respironics, PA) was done the following night. The CPAP, at the prescribed pressure, was given for two subsequent nights. These patients were then put on anti-oxidants (vitamin E 400 IU BD and vitamin C 100 IU BD) for 45 days. After 45 days, the patients were again assessed by the same sleep questionnaire and ESS. They were also independently assessed by a second examiner. They slept in the sleep laboratory the same night (for acclimatisation to new environment). The next night, a repeat sleep study was performed. This time, split night sleep study was done (diagnostic, titration and CPAP done on the same night).

Venous blood samples were collected in ethylene dia tetraacetic acid (EDTA) vials and plain vials early in the morning (6 AM) before the patient got out of the bed. Plasma was separated for estimation of lipid peroxidation. Whole blood was lysed for estimation of total reduced glutathione. Serum was separated from blood samples in plain vials for estimation of triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and cholesterol. Three collections of venous blood samples were done totally - one before sleep study (Baseline), second, after two nights of CPAP and third, after treatment with anti-oxidants. All the samples collected were stored at -80 ^oC before subjecting them to biochemical analysis. The measurements were done in triplicate for concordance in values.

Along with PSG the following routine investigations were done: (a) haemoglobin; (b) total leukocyte count (TLC); (c) differential leukocyte count (DLC); (d) fasting blood sugar (FBS); (e) chest radiograph (postero-anterior view); (f) electrocardiogram (ECG); (g) pulmonary function tests including forced expiratory volume at the end of 1st second (FEV_1), forced vital capacity (FVC); and FEV_1/FVC ratio; (h) thyroid function tests as and when required; (i) arterial blood gases (ABG) analysis; and (g) computed tomography (CT) of the paranasal sinuses as and when required.

Ten healthy male subjects were also included in this study as control subjects. These control subjects did not have loud snoring and excessive day-time

sleepiness and they also underwent the routine investigations mentioned above. None of them was a smoker.

The body mass index (BMI) was calculated in all subjects using the following formula: weight in kg/(height in meters).[2] The circumference of the neck was measured at the cricothyroid membrane level. All the measurements were performed by the same observer. A questionnaire prepared at our institute was completed by the subjects and Epworth sleepiness score was completed with the help of the subjects. The nasal septum, nasal mucosa, soft palate, uvula, tonsils, tongue, and chin abnormalities were assessed semi-quantitatively. All subjects were examined by the same observer.

Biochemical Estimation

Total glutathione concentration was estimated in venous blood samples by the method of Griffith,[25] using Ellmans reagent. Whole blood was lysed by the addition of 6% acetic acid and total glutathione was immediately precipitated by the addition of 10% 5-sulphosalicylic acid. After centrifugation at 4 °C, the supernatant was kept at –80 °C. The standard assay mixture contained 700µL of 0.3 mM/L nicotinamide adenine dinucleotide phosphate (NADPH), 100µL of 6mM/L 5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB), 5µL sample, 95µL sodium-EDTA buffer (100mM/L, pH 7.5). All the reagents were made in sodium phosphate (125 mM/L)-EDTA (6.3 mM/L) buffer (pH 7.5). To start the reaction, 100 µL of glutathione reductase (15 U/mL) was added and the A_{412} was monitored for 3 minutes.

Lipid peroxide levels in the plasma were estimated in venous blood samples. The TBARS assay was carried out by precipitation of lipid peroxides in phosphotungstic acid-sulfuric acid system,[26] malondialdehyde levels were determined by reaction with thiobarbituric acid (TBA).[27] The assay mixture contained 200 µL of distilled water, 200 µL of plasma, 50 µL of butylated hydroxyl toluene (BHT) (11 mg/10mL ethanol) and 400 µL of orthophosphoric acid (OPA) (1.115 OPA in upto 50 mL distilled water). To the assay mixture, 50 µL of TBA (160 mg/10mL) of 0.1M sodium hydroxide) was added and incubated in boiling water bath for 45 minutes. The eppendorfs were ice cooled and colour was extracted with 1000 µL of butanol. After centrifugation at 10000 rpm for 5 minutes, absorption of the supernatant was read at 535 nm.

Triglycerides, and cholesterol were estimated on the early morning fasting blood sample using standardised Bayer diagnostic kits. The LDL and very low density lipoprotein (VLDL) were derived from these values using the formula, LDL=(total cholesterol)–(HDL cholesterol)–(TG)/5; VLDL=TG/5. Serum creatinine was estimated using standardised Bayer diagnostic kit.

Statistical Analysis

All the data were expressed as mean±SEM. Paired 't' test with two-tail significance was used to compare the changes in study parameters in the same patient before and after the treatment. Unpaired 't' test was used to compare the baseline data in the control subjects and the patients. The tests were considered significant if they yielded $p<0.05$.

RESULTS

Three of the 20 patients who completed the study had hypertension and their arterial blood pressure was under control on anti-hypertensive medication. Two patients with type 2 diabetes mellitus were on oral hypoglycemic drugs and their fasting blood glucose (FBS) levels were under control (90±3.4 mg/dL). Six patients were found to have deviated nasal septum and mild to moderate nasal obstruction on CT. Any chest disease in all the 20 patients was ruled out by physical examination, chest radiograph pulmonary function testing, ABG, TLC, DLC and if needed CT. Cardiac disease was ruled out by history, auscultation, ECG, and chest radiography.

The mean age of the OSAS patients and control subjects was 44±2.4 years and 30.7±1.2 years, respectively. The BMI and neck circumference along with systolic and diastolic blood pressure, pulse rate, haemoglobin and FBS values of the control subjects and OSAS patients are given in table 1. The baseline body weight, BMI, neck circumference, systolic and diastolic blood pressure were significantly higher in patients than those in control subjects (Table 1). The baseline pulse rate in OSAS patients was similar to that in control subjects (Table 1). Oral intake of anti-oxidants did not change any of these parameters in both the control subjects as well as the OSAS patients (Table 1).

Polysomnographic Parameters

The ESS, PSG measurements and the titrated pressure of CPAP treatment are shown in table 2. The baseline ESS was significantly higher in OSAS patients than that in control subjects ($p<0.001$, Table 2). The ESS decreased significantly in OSAS patients after treatment with anti-oxidants ($p<0.001$, Table 2), even though it still remained higher than that in the control subjects; whereas it had no effect in control subjects (Table 2).

Sleep Study

Repeat PSG measurements after the anti-oxidant therapy for 45 days were possible in 14 patients only, as the rest refused to undertake it as they felt that

Table 1. Anthropometric parameters, blood pressure, pulse rate, fasting blood sugar and haemoglobin in control subjects and OSAS patients

Parameters	Control Subjects		OSAS Patients		
	Baseline	After Anti-oxidant	Baseline	After CPAP	After Anti-oxidant
Body weight (kg)	69.8±2.2	69.3±2.5	93.2±4.2*	93.2±4.2	93.3±3.9
Body mass index (kg/m^2)	24.5±0.7	24.4±0.7	32.9±1.2*	32.9±1.2	32.9±1.1
Neck circumference (cm)	35.0±0.4	35.0±0.4	41.5±0.6*	41.6±0.6	41.6±0.6
Systolic blood pressure (mmHg)	124.2±1.3	115.2±7.1	133.0±2.1**	126.7±6.2	127.0±4.2
Diastolic blood pressure (mmHg)	79.6±0.6	79.2±0.8	89.1±1.2**	87.4±1.3	86.4±1.1
Pulse rate (per min)	71.2±0.4	71.0±1.0	73.6±1.3	73.2±1.2	73.6±1.2
Fasting blood sugar (mg/dL)	90.0±2.5	92.0±2.3	98.4±2.1	98.2±1.8	99.4±1.3
Haemoglobin (g/dL)	14.8±1.1	14.8±1.1	14.4±1.2	14.4±1.2	14.4±1.2

*=p<0.001, **=p<0.01, compared to baseline values in control subjects
OSAS=Obstructive sleep apnoea syndrome; CPAP=Continuous positive airway pressure

Table 2. Polysomnography parameters in control subjects (n=10) and OSAS patients (n=14). Epoworth sleepiness score was derived from 20 OSAS patients

Parameters	Control Subjects		OSAS Patients	
	Baseline	After Anti-oxidant	Baseline	After Anti-oxidant
CPAP (cm H_2O)	-	-	9.6±3.1	8.3±0.9*
Stage 3+4 as SPT (%)	22.2±1.1	23.6±2.5	17.1±2.1	28.2±4.1**
REM as SPT (%)	23.3±1.4	23.7±1.4	9.1±2.4†	11.9±3.1
Apnoea-hypopnoea index (per hr)	2.3±0.5	3.2±0.6	61.0±8.9†	54.7±8.7**
Apnoeic episodes	7.1±1.5	12.3±1.9	108.8±18.4	88.7±16.4**
Epworth sleepiness score	2.3±0.4	1.6±0.4	15.3±1.2	6.4±0.9***

*=p<0.05, **=p<0.01, ***=p<0.001, after anti-oxidants *versus* baseline of OSAS patients
†=p<0.001, compared to baseline of control subjects
OSAS=Obstructive sleep apnoea syndrome; SPT=Sleep percentage time; REM=Rapid eye movement; PAP=Continuous positive airway pressure

(disturbed) sleep was no longer affecting their day-to-day activities. The results from these 14 patients are presented in table 2. The data has been time matched for split night and full night study. The duration of diagnostic part of split night study was noted and data of same duration was analysed from full night study for comparison.

Stage 3 + Stage 4 and rapid eye movement (REM) as sleep percentage time (SPT)

The time spent in stages 3 and 4 was relatively lower in OSAS patients compared to the control subjects and anti-oxidant treatment significantly improved the duration of sleep stages 3 and 4 (Table 2). The patients reported having a more refreshing sleep. Seven patients who wre suffering morning headache, found it to be relieved after treatment with anti-oxidants.

Apnoea

The number of (baseline) apnoeic episodes were significantly higher in OSAS patients than that in control subjects (p<0.001, Table). Anti-oxidant treatment significantly reduced the frequency of apnoeas (p<0.01, Table 2). This decrease was associated with less number of awakenings at night.

Apnoea-Hypopnea Index (AHI)

The baseline AHI was significantly higher in OSAS patients than that in control subjects (p<0.001, Table 2). The AHI decreased significantly in OSAS patients after treatment with anti-oxidants (p<0.01, Table 2).

Nasal Continuous Positive Airway Pressure (CPAP)

There was a significant drop in the optimal pressure of CPAP after treatment with anti-oxidants compared to baseline (p<0.05, Table 2). Indeed, in one patient who required an optimal pressure of 6.4 cmH_2O, there was no requirement of the CPAP device after treatment with anti-oxidants.

Oxidative Stress Parameters

Lipid Peroxidation (LPO)

The baseline LPO levels were significantly higher in OSAS patients compared to control subjects (p<0.001, Figure 1, Table 3). The CPAP therapy, as well as anti-oxidant treatment significantly reduced LPO levels (p<0.01, Figure 1, Table 3). Anti-oxidant intake did not affect the LPO levels in control subjects (p>0.05, Figure 1, Table 3).

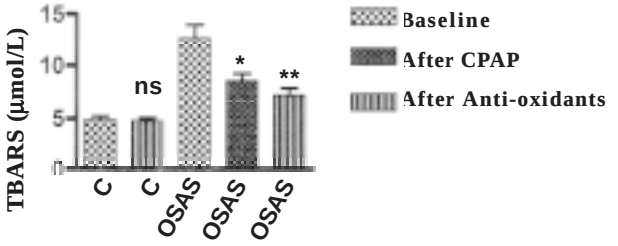

Figure 1. Lipid peroxidation levels in control subjects (C) and OSAS patients.

***=p<0.05, **=p<0.01, compared to baseline values in OSAS patients**

OSAS=Obstructive sleep apnoea syndrome; TBARS= Thiobarbiteric acid reactive substance; ns=Not significant; CPAP=Continuous positive airway pressure

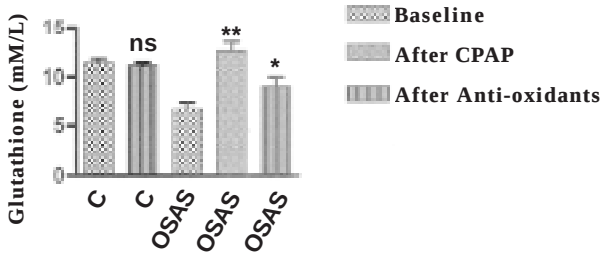

Figure 2. Total reduced glutathione levels in control subjects (C) and OSAS patients.

***=p<0.05, **=p<0.01, compared to baseline values in OSAS patients**

OSAS=Obstructive sleep apnoea syndrome; ns=Not significant; CPAP=Continuous positive airway pressure

Measurement of Anti-oxidant Capacity

Total reduced glutathione. The baseline reduced glutathione levels were significantly lower in OSAS patients compared to control subjects ($p<0.001$, Figure 2, Table 3). The CPAP treatment increased it significantly ($p<0.01$, Figure 2, Table 3). Anti-oxidant treatment improved reduced glutathione levels significantly ($p<0.05$, Figure 2, Table 3).

Lipid Profile

Lipid profile in control subjects and OSAS patients is shown in table 3. The baseline LDL levels were significantly higher and HDL levels were significantly lower in OSAS patients than the control subjects (Table 3). They remained the same even after CPAP treatment and after intake of anti-oxidants.

Qualitative Responses

Anti-oxidant intake improved the quality of life in all the 20 patients with OSAS. After anti-oxidant intake, four subjects who used to sleep while driving and had hired drivers due to this problem before, are driving on their own, an advocate who used to sleep in the court room has been participating actively in the court proceedings and eight others, who used to sleep while talking to someone, reported back saying that this problem was no longer troubling them or the frequency of falling asleep was very less compared to that before treatment. These and the remaining seven patients used to fall asleep while reading and watching television. This behaviour disappeared totally in all of them after treatment with anti-oxidants. All 20 patients reported that their quality of life had improved.

DISCUSSION

A major finding of the present study was that OSAS patients exhibit oxidative stress and more importantly, like CPAP therapy, anti-oxidant treatment improves the sleep behaviour and reduces the frequency of apnoeas.

Polysomnographic Parameters

Even though uncomfortable, the most widely used therapeutic intervention in OSAS patients is the CPAP. After titration, when the prescribed pressure is applied, the patients sleep better as there is a great reduction in the number of apnoeic episodes, apnoea-hypopnoea index, etc. Results of the present study demonstrate that anti-oxidant treatment also

Table 3. Biochemical parameters in control subjects and OSAS patients

Parameters	Control Subjects		OSAS Patients		
	Baseline	After Anti-oxidant	Baseline	After CPAP	After Anti-oxidant
TG (mg/dL)	136.4±1.7	135.1±1.3	158.2±10.5	161.7±12.3	156.5±10.3
LDL (mg/dL)	84.3±2.4	83.0±2.2	119.5±7.8*	121.0±8.6*	116.1±6.8*
HDL (mg/dL)	44.4±1.5	44.5±1.2	40.1±1.5**	40.9±1.2**	40.7±1.1**
Cholesterol (mg/dL)	181.7±4.9	179.5±3.7	191.3±8.4	194.5±8.9	187.0±6.9
VLDL (mg/dL)	27.3±0.3	27.1±0.3	31.6±2.1	32.3±2.4	31.3±2.1
LPO (µmol/L)	4.9±0.3	4.8±0.3	12.7±1.3***	8.5±0.7†	7.1±0.7††
Total reduced glutathione (mmol/L)	11.4±0.4	11.1±0.3	6.8±0.7***	12.5±0.9††	9.1±0.9†

†=p<0.05 compared to baseline values in OSAS patients; ††=p<0.01 compard to baseline values in OSAS patients;

*=p<0.01, **=p<0.05, ***=p<0.001, compared to baseline values in control subjects

OSAS=Obstructive sleep apnoea syndrome; TG=Triglycerides; LDL=Low density lipoproteins; HDL=High density lipoproteins; VLDL=Very low density lipoproteins; LPO=Lipid peroxide

produces similar effects. Collapsible upper airways is a prominent feature in OSAS patients and the sleep state dependent reduction in upper airway dilator motor neuron activity has been considered to be responsible for the repeated episodes of airway obstruction.[28] It is proposed that the oxidative stress observed in OSAS patients inhibits the excitatory motor neuronal discharge to upper airway dilator muscles. Intake of anti-oxidants removes this inhibition and prevents the tone from falling. There are evidences to support this proposition. For instance, Jelev *et al*[29] have shown decreasing discharge of 5-hydroxy tryptamine (5-HT) projections from medullary raphe nucleus to hypoglossal nucleus, which causes more apnoeas in rapid eye movement (REM) sleep. Veasey *et al*[30] have shown that long-term intermittent hypoxia decreases responsiveness of upper airway dilator nerves to serotonergic drugs and to intrinsic neurochemical drive. They attributed this response to oxidative stress in hypoglossal region of the medulla as evident from the clear increase in isoprostane levels. It was also found that the decreased responsiveness of upper airway dilator muscle and lipid peroxidation in the medulla was largely prevented with superoxide dismutase treatment throughout long-term intermittent hypoxia.

Patients with OSAS reported that they had a comfortable sleep following anti-oxidants. The improved sleep was evident from the increase in the time spent in stages 3 and 4 of sleep and the decline in the ESS. All patients reported that they could indulge in their routine activities and day-time sleepiness was no longer a problem, as it was prior to the anti-oxidant therapy. When CPAP treatment was re-introduced after 45 days of anti-oxidant intake, there was a significant decrease in the optimal pressure requirement. These observations show that anti-oxidant treatment improves both subjective and objective parameters of sleep behaviour. Patients with OSAS spent significantly less time in REM sleep. This parameter did not improve after anti-oxidant intake. One reason for the lack of improvement may be because the observations were made during the split night study after anti-oxidant treatment, when, the patients do not have sufficient time to stay in REM sleep. Perhaps full night diagnostic study is required in these patients to demonstrate an improvement.

The mechanisms behind the observed improvements in the sleep behaviour after the anti-oxidant intake remain to be elucidated. We speculate that anti-oxidants may affect the activities of the sleep promoting or wake promoting areas of the brain[31] and decrease the day-time sleepiness. Veasey *et al*[32] have shown that long-term intermittent hypoxia (which is used as a method of inducing sleep apnoea in experimental animals) increases NADPH-oxidase gene and protein responses in wake-active brain regions, which lead to lipid peroxidation injury and pro-inflammatory response there, resulting in hypersomnolence. They have further shown that the long-term intermittent hypoxia induced hypersomnolence is prevented by both transgenic absence and pharmacologic inhibition of NADPH-oxidase by apocynm, which also conferred resistance to lipid peroxidation injury. Thus in OSAS patients, it is likely that the oxidative stress as evidenced by an increase in lipid peroxidation products injure the wake promoting cells and reduce their activity. Anti-oxidant intake will remove this inhibition, and as a consequence, there is a decrease in day-time sleepiness.

Oxidant Anti-oxidant Status

In OSAS, due to cyclical *hypoxia/reoxygenation*[33] there is free radical production and lipid peroxidation.[11, 33,34] Free radical production is triggered also by the leukocytes of OSA patients.[35] Additionally, sleep deprivation *per se* increases free radical production by inhibiting anti-oxidant defence mechanisms of the brain.[36]

In the present study, we determined oxidative stress by estimation of lipid peroxidation products and reduced glutathione concentrations. The former was assessed by quantification of TBARS which is a marker of lipid oxidation in man.[15] The TBARS increased significantly in OSAS patients and two nights of CPAP treatment reduced it significantly. The results are in agreement with previous investigations by other investigators,[16,17,20] except that we found even two nights of CPAP treatment to be effective in reducing TBARS. It is worth mentioning here that such a response was not evident with one night of CPAP treatment.[37] We chose a short-term CPAP treatment as our overall goal was to identify alternate strategies of interventions to improve sleep in OSAS patients. Our results indicate that continuous CPAP treatment may not be a pre-requisite for eliciting the desired effects.

The total anti-oxidant capacity, as evidenced from the reduced glutathione levels, decreased in our patients which got restored after CPAP treatment. In OSAS patients, either a no change[38] or a reduction in the anti-oxidant capacity[18, 19] has been reported. But unlike the study group of Christou *et al*[18,19] none of our patients was a smoker or had any respiratory disorder.

The conflicting results obtained by different investigators have been explained by the presence of co-morbidities and medications in OSAS patients. In their absence, it has been reported that there is no higher oxidative stress and lipid peroxidation in them.[15] The present results do not support this view. In our study also, three had hypertension and another two had type 2 diabetes mellitus. But the remaining

14 patients did not have any co-morbidity. It is true that six of them had deviated nasal septum; however, only one suffered from acute sinusitis which was corrected by prescribing medications before recruiting him to the study, Another factor which could have influenced our results is obesity, which by itself can lead to oxidative stress.[15] All our patients were clearly obese (BMl >32 kg / m^2) and in fact it was one of the inclusion criteria for choosing them for our study. While the effect of obesity *per se* may be important, it is worth noting that BMI remained constant during the entire study period of 45 days when the measurements were repeated (detailed below). A third factor which could have affected our results is the timings of sample collection for measurement of oxidant-anti-oxidant status.[15] We minimsed it by collecting the venous samples at specified timings (at 6 AM) only, in the control subjects as well as the OSAS patients.

As expected, in our study, oral intake of anti-oxidants for 45 days not only reduced the TBARS but also increased reduced glutathione concentrations. While these responses occurred, the BMI remained constant suggesting that the observed responses were independent of BMI. Since (raised) BMI is a major contributing factor for sleep apnoea,[9] it is possible that it might have contributed to cause oxidative stress.[39] Indeed such a correlation has been demonstrated in asthmatics.[40] Even though the controls chosen for the present investigation did not have a similar BMI as the patients, it has been reported that there is no association between BMI and oxidative stress in normal subjects having similar weight distribution as the study patients (asthmatics).[40]

It is pertinent to point out here that even though data was collected from control subjects, no effort was made to match their ages with OSAS patients. There is enough evidence in the literature that there is a positive and significant correlation between age and TBARS level indicating that with advancement of age there is oxidative stress.[41] In the present study, the average age of the control subjects was 30.7±1.2 years and that of the patients was 44±2.4 years. Thus, age could have contributed to the oxidative stress observed in these patients. However, it is unlikely to be a major factor. For instance, in our study, the TBARS level in OSAS patients was 12.7±1.3 µmol / L, which was 159% more than that in the control subjects (4.9±0.3 µmol / L. In a previous study,[41] it has been reported that TBARS level increased by 15% and by 20%-45%, respectively in individuals in the age groups 40-49 and >50 years compared with that in the age group 20-29 years. The main purpose of making the observations in the control subjects was to know the basal value of oxidative stress parameters, to record their sleep patterns and to evaluate whether anti-oxidant intake affects any of them.

In summary, the present study has demonstrated that oxidative stress is an underlying mechanism for the sleep disorders in OSAS patients and reducing the oxidant burden by anti-oxidant intake improves the quality of sleep in them.

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