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HEAD AND NECK

Assessment of the relationship between polysomnography parameters and plasma malondialdehyde levels in patients with obstructive sleep apnea

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Abstract

Purpose The relationship of plasma malondialdehyde (MDA) with major parameters of PSG was investigated to find out if there was a correlation between these variables.

Methods Polysomnograms were done on a total of 37 adults who do not have a history of any systemic illness, smoking, and supplement use. Plasma MDA measurements and their relationship with PSG parameters were analyzed.

Results The mean MDA concentrations in patients with lower AHI values were also lower than those in the patients with higher AHI ($p < 0.001$). Higher predominance of apnea in patients with similar AHI values, longer mean apnea durations, O_2 saturation dips to < 90%, and higher ODI values predicted higher plasma MDA concentrations.

Conclusions Higher oxidative stress measurements predicted more severe clinical picture. These findings show that oxidative stress measurement with MDA may provide a simple tool to screen patients for OSA and help select them for PSG study appropriately, if indicated

Keywords Obstructive sleep apnea · Oxidative stress · Polysomnography · Malondialdehyde

Introduction

Obstructive sleep apnea (OSA) is a disease that causes medical, social, and economical burden on the society at least as much as cardiovascular diseases and stroke; and has a prevalence between 9 and 38%, depending on the methodology of measurement [1]. Sleep questionnaires have unacceptably high rates of false-negativity [2] and polysomnography (PSG) is still the golden standard in the screening and diagnosis of OSA. However, new screening tests with promising potentials such as measurement of oxidative stress markers are emerging on the horizon. Oxidative stress is the byproduct of many chemical reactions taking place in the cells and its amount depends on the balance between the level of the oxidant molecules and the activity of the antioxidant enzyme systems. It has been found in the pathogenesis of many disease processes such as cardiovascular and cerebrovascular diseases, cancer, and even Parkinson's disease [3–6]. There are vast amounts of evidence that the OSA increases the level of oxidative stress. In this study, the relationship of plasma MDA values not only with apnea–hypopnea index (AHI), but also with some other major parameters of PSG

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was investigated so as to find out if there was a correlation among these variables.

Materials and methods

Patient population

A total of 37 patients (23 males and 14 females) aged between 18 and 65 who referred to a tertiary referral center ENT Department with the complaints of daytime sleepiness, snore, witnessed apnea episodes, and chronic fatigue with the suspicion of OSA between December 2016 and December 2017 were enrolled in the study. History of upper and lower airway disease, airway surgery, smoking, systemic illnesses (endocrine, cardiovascular, renal and liver diseases), obesity (body mass index [BMI] ≥ 35), and use of vitamin and/or herbal supplements were the exclusion criteria from the study.

Polysomnography

The study comprised of nasal and oral airflow measurement, oximetry, sleeping position assessment, respiration detection from both chest and abdomen, electrocardiography and electroencephalography, submental and right anterior tibial electromyography with the Embla S4500® (Natus Medical, Ontario Canada) device. The sleep parameters were analyzed with the Embla REMLogic® software. The AASM (American Academy of Sleep Medicine) 2013 criteria were used for the scoring of sleeping events. Patients were segregated into groups according to apnea–hypopnea index (AHI) values with 5/h and 15/h thresholds, mean and maximum apnea durations, mean and minimum O_2 saturations, duration of sleep time with less than 90% O_2 saturation, and oxygen desaturation index (ODI) values.

Plasma malondialdehyde (MDA) measurement

Blood samples were drawn in the morning after the PSG study and transferred rapidly to the laboratory and after extraction, each plasma sample was stored in − 80 °C. For MDA analysis, the lipid peroxidation assay kit of Abcam® (Cambridge, UK) was used. Fluorometric method was selected and the results were reported as nanomol/milliliter (nmol/ml) units.

Study budget, ethical committee approval, and statistical analysis

The budget for the purchase of the assay kit was provided by Hacettepe University Scientific Research Projects Coordination Unit. The study and the methodology were independently reviewed and approved by Hacettepe University Non-Interventional Clinical Studies Ethical Committee with GO 16/22-32 reference number. All patients were given verbal and written information regarding the nature of the study and informed consent was taken. IBM's Statistical Package for Social Sciences (SPSS) software version 16.0 was used for the statistical analysis. Normal distribution was assessed with the Kolmogorov–Smirnov Test. Student's *t* test was used to test the significance of difference between the groups and the Pearson's Correlation Coefficient was determined for the correlations. For 95% confidence interval (CI), p values < 0.05 were considered significant.

Results

The patient population consisted of 23 males and 14 females (age interval: 18 and 65) with the mean ages being 44.2, and 41.6, respectively. The BMI values were between 22.1 and 35 kg/m^2. The minimum and maximum AHI values were 1.2/h and 138.5/h, respectively. There were six patients in the AHI < 5/h group (simple snorers or patients complaining of fatigue because of the reasons other than OSA), and 31 patients in the AHI ≥ 5/h group (OSA patients). Plasma MDA values were between 0.258 nmol/ml and 1.331 nmol/l (mean: 0.856 [± 0.259] nmol/ml). The summary of these values is presented in Table 1.

It was hypothesized that the severity of the PSG parameters would correlate with the plasma concentrations of MDA, hence these correlations were analyzed. The results were as follows:

- *AHI and MDA concentrations* When the groups were separated as non-OSA patients (AHI < 5/h) and OSA patients (AHI ≥ 5/h), the mean MDA concentration in the non-OSA group was 0.615 (± 0.237) nmol/ml and the mean MDA concentration in the OSA group was 0.903 (± 0.213) nmol/ml. The difference of the mean MDA concentrations between the groups reached statistical significance ($p = 0.005$, $t = -2.972$). Similarly, when the study population separated into two groups as the cutoff value of AHI was taken as 15/h; the AHI < 15/h group consisted of non-OSA subjects with vague complaints of tiredness, simple snorers, and mild OSA patients (AHI ≥ 5/h but < 15/h). The other group consisted of the moderately severe (15/h ≤ AHI < 30/h) and severe OSA patients (AHI ≥ 30/h). The mean MDA concentration of the group with the lower AHI values was 0.678 (± 0.159) nmol/ml and the mean concentration of the more severe group was 0.992 (± 0.198) nmol/ml. This difference also reached statistical significance ($p < 0.001$, $t = -5.182$). This indicated that the oxidative stress values were increased in the more severe OSA population.

Table 1 Demographic, polysomnographic parameters and measured metabolite concentrations in patients

	Minimum	Maximum	Mean ± std deviation
Age	18	65	43.3 ± 13.9
Body mass index (BMI)	22–1	35.0	28.9 ± 4.4
Apnea–hypopnea index (AHI)	1–2	138.5	34.8 ± 34.9
Mean apnea duration (s)	0.0	49.7	15.9 ± 10.2
Max. apnea duration (s)	0.0	123.1	33.2 ± 28.7
Sleep duration with < 90% O_2 saturation (min)	0.0	304.2	35.9 ± 71.2
Mean O_2 saturation	83.7	98.0	93.5 ± 3.1
Minimum O_2 saturation	60.0	96.0	84.2 ± 9.7
Oxygen desaturation index (ODI)	0.0	115.3	26.9 ± 31.9
Plasma MDA concentration (nmol/ml)	0.258	23–8	0.856 ± 0.259

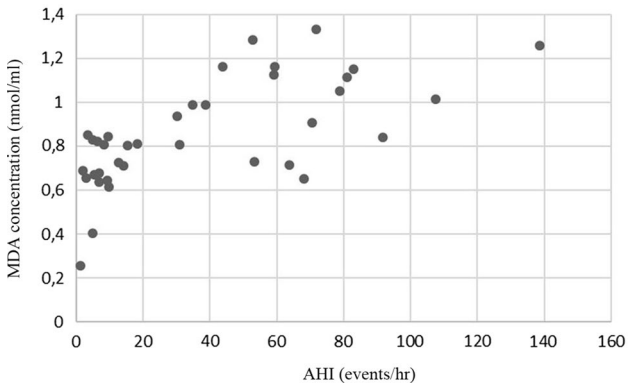

Fig. 1 Patients' malondialdehyde (MDA) concentrations plotted against apnea-hypopnea index (AHI) values

When the patients were separated as "apnea-predominant patients" (AI > HI) and "hypopnea-predominant patients" (HI > AI); the patients who experienced more apneic episodes than hypopneic episodes had mean MDA concentration of 1.042 (± 0.205) nmol/ml. The concentration was 0.743 (± 0.183) nmol/ml in the group vice versa. This difference reached statistical significance ($p < 0.001$, $t = -4.625$). AHI and MDA values were positively correlated ($r = 0.662$, $p < 0.001$). The plasma MDA concentrations corresponding to the AHI values of the patients were plotted on a graph in the Fig. 1.

- *Mean apnea duration and MDA concentrations* The median value of the mean apnea durations of the study population was 15.9 s. The 16 patients who had shorter mean apnea durations had mean plasma MDA value of 0.698 (± 0.178) nmol/ml, whereas the remaining 21 patients had mean plasma MDA value of 0.976 (± 0.210) nmol/ml. This observation reached statistical significance ($p < 0.001$, $t = -4.257$). Mean apnea duration correlated positively with MDA values ($r = 0.507$, $p = 0.001$).
- *The effect of the decrease of plasma O_2saturations to less than 90% on plasma MDA concentrations* Depending on the patients' respiratory and circulatory reserves, apneic episodes might lead to the decrease of plasma O_2 saturations below the 90% saturation value, hence the increased oxidative stress. Indeed, the mean plasma MDA value was 0.944 (± 0.207) nmol/ml in patients who experienced O_2 saturations < 90%, in contrast to the MDA value of 0.647 (± 0.177) nmol/ml in patients who did not ($p < 0.001$, $t = 4.158$). There was a positive correlation between the measurements ($r = 0.338$, $p = 0.041$).
- *ODI and MDA concentrations* The patients who had lesser ODI value than the mean ODI of 9.1/h had mean plasma MDA concentration of 0.683 (± 0.162) nmol/ml. In the group with higher values, the mean plasma MDA concentration was 0.988 (± 0.162) nmol/ml. This difference has also reached statistical significance ($p < 0.001$, $t = -4.936$) with a positive correlation ($r = 0.584$, $p < 0.001$).
- Plasma MDA concentrations corresponding to various PSG parameter subgroups are presented in Table 2.

Discussion

There has been an interest to find novel molecular markers to detect OSA and grade its severity in recent decades. Among those were inflammatory markers like interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-∝), c-reactive protein (CRP), ICAM-1, VCAM-1, etc. [7]. However, there is insufficient evidence in the literature to support their widespread use as screening tools. Systematic studies emphasize that testing a single molecule fails to diagnose the patients and only use of two or more molecular tests simultaneously has enough predictive value and diagnostic capability [8]. Besides their cost, there are many disease processes that have the potential to confound the interpretations based on these measurements. Another popular approach to evaluate the OSA and its effects on patients' well-being is the measurement of oxidative stress. Many molecules like

Table 2 Plasma MDA concentrations corresponding to various PSG parameter subgroups

Variables	Subgroups	Numbers	Mean ± std. deviation	*p* value (95% CI)
Presence of OSA	Absent (AHI < 5/h)	6	0.615 ± 0.237	0.005
	Present (AHI ≥ 5/h)	31	0.903 ± 0.213	
AHI	AHI < 15/h	16	0.678 ± 0.159	< 0.001
	AHI ≥ 15/h	21	0.992 ± 0.198	
Mean apnea duration (s) (median: 15.9)	< 15.9 s	16	0.698 ± 0.178	< 0.001
	≥ 15.9 s	21	0.976 ± 0.210	
Presence of SaO_2 < 90%	Present	26	0.944 ± 0.207	< 0.001
	Absent	11	0.647 ± 0.177	
ODI (median: 9.1/h)	ODI < 9.1/h	16	0.683 ± 0.162	< 0.001
	ODI ≥ 9.1/h	21	0.988 ± 0.203	

thiobarbituric acid-reacting substances (TBARS), 8-isoprostane, 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), etc. were investigated to measure the oxidative stress in OSA patients [9–11].

MDA is a lipid pre-oxidation product that is increased in prolonged oxidative stress conditions. There are two main measurement protocols for this molecule in the literature that are used predominantly: measurement of TBARS and high-performance liquid chromatography (HPLC). Measurement of TBARS, detects MDA and some other lipid peroxidation products which react with thiobarbituric acid. HPLC allows more specific measurement of MDA. In this study, a commercial testing kit available for measurement of MDA levels based on lipid peroxidase assay was used.

Like many new molecular markers investigated, there are conflicting reports on the levels of MDA in OSA patients. Some studies found meaningful correlations of this molecule and other oxidative stress markers with the severity of OSA, whereas others failed to do so. In addition to many methodological discrepancies between studies, many studies only investigated AHI as the sole PSG parameter. Barcelo et al. found that the plasma TBARS concentrations were increased significantly in 14 severe male OSA patients compared with the 13 healthy male subjects. However, they failed to show the decrease in the TBARS values after 1 year of continuous positive airway pressure (CPAP) therapy [12]. Jordan et al. [11] detected showed that the plasma MDA concentrations were positively correlated with the duration of sleeping time with less than 85% and 90% O_2 saturations, but there was no correlation with AHI values. Vatansever et al. [13] compared 24 healthy male subjects with 25 OSA patients and found higher levels of MDA in the moderate and severe OSA group than the healthy subjects. Celec et al. reported decreased plasma TBARS concentrations after CPAP therapy. However, salivary TBARS concentrations were not decreased significantly [14]. Other teams also detected increased TBARS values in OSA patients compared with healthy subjects, and revealed that the CPAP therapy significantly decreased these values in patients with high AHI values [15].

Other therapy modalities also seem to have an effect on oxidative stress measures similar to CPAP therapy. In a study by Itzhaki et al. plasma TBARS values decreased significantly after 1 year treatment of oral appliance for OSA [16]. Bakan et al. found decreased mean serum MDA values in 29 male and 20 female OSA patients after uvulopalatopharyngoplasty (UPPP) [17]. Vuralkan et al. [18] found decreased mean serum MDA concentrations after 6 months in 25 patients who had undergone uvulopalatal flap procedure. Dal-Fabbro et al. compared mandibular advancement appliance, CPAP, and placebo oral appliance in 29 moderate or severe OSA patients and compared basal and 1-month treatment PSG and oxidative stress parameters. In patients who received actual treatment, there were improvements in Epworth Sleepiness Scale and AHI, but lipid peroxidation values were not improved significantly [19]. Cho et al. evaluated 20 healthy children with 22 children who had sleep-disordered breathing (SDB) who underwent adenotonsillectomy. Urinary MDA values were not different statistically in the control group (2.66 ± 1.07 µmol/g creatinine), preoperative patient group (2.27 ± 1.07 µmol/g creatinine), and the postoperative patient group (2.03 ± 0.68 µmol/g creatinine) [20].

As it can be seen, however, there is conflicting reports in the literature regarding the effect of interventions on MDA concentrations. Furthermore, the statistical comparison of MDA levels in OSA patients was mostly done based on limited number of PSG parameters such as only AHI or ODI. To better define the picture of increased oxidative stress in OSA we aimed to measure the effect of other parameters such as mean apnea time, presence of O_2 saturation < 90%, ODI, and predominance of apnea in this study. When the patients were separated into two different groups based on AHI level of 5/h, the patients with higher AHI values had significantly higher MDA levels. As the patients with AHI levels less than 5/h are not OSA patients, by definition, only simple snorers or patients with

nonspecific causes of fatigue or unremitting sleep other than OSA were included in this group with lower MDA values. This observation underscored the possible role of plasma MDA measurement as a screening tool in the population. Once the normal range of values of MDA is more objectively determined in a larger healthy population in future studies, measurement of plasma MDA levels may provide an important pre-screening tool for the patients' enrollment in the more expensive and cumbersome PSG test. The higher mean MDA concentrations in moderately severe and severe OSA groups, also have possible implications, as this test could be an objective success criteria for post-OSA intervention results in patients. Currently, many publications report the decrease in AHI to < 20/h or to the 50% of the pre-treatment values as success criteria. However, simply comparing AHI would be misleading in some patients who have quality-of-life improvements documented in sleepiness questionnaires, which is in stark contrast to unchanged or even increased AHI values. Detection of improvements in oxidative stress levels, could provide relief to both patients and clinicians. The patients with higher apnea dominance had higher MDA concentrations and this also emphasizes that simply reporting AHI is misleading as oxidative stress could be different in patients with similar AHI levels. This observation also stresses the importance of interpreting the PSG results comprehensively. The investigators of this study recommend considering measurement of oxidative stress values also as a supplementary test as it provided a more direct, cellular-level insight into the end-results of OSA in our study.

Finally, the major weakness of this study to stress out is the low number of patients included. This situation decreases our chance to generalize our results on a population-wide basis as the results might not represent the whole community. However, most of the published studies on this subject have low numbers of patients enrolled and there is not a widely accepted population-wide reference range for plasma MDA levels. Hence, we believe that the data from our study would provide a basis for a larger, more generalizable studies by providing a stepping stone on this active area of research.

Conclusion

MDA values are increased in patients who had higher AHI, higher apnea dominance compared with hypopnea, higher mean apnea time, higher ODI values and in patients who experienced blood oxygen saturation dips to < 90% during sleep time.

Acknowledgements Informed consent was obtained from all individual participants included in the study. All of the authors declare no conflicts of interest.

Compliance with ethical standards

Conflict of interest The authors disclose no conflicts of interest.

Informed consent All patients were given verbal and written information regarding the nature of the study and informed consent was taken.

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