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Oxidative and Carbonyl Stress in Pregnant Women with Obstructive Sleep Apnea

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Abstract

Purpose—Pregnant women are particularly susceptible to sleep disordered breathing.

Obstructive sleep apnea (OSA) in pregnancy is associated with poor pregnancy and fetal outcomes. Oxidative stress caused by intermittent hypoxemia and re-oxygenation may impact pregnancy health. We hypothesize that pregnant women with OSA have a pronounced oxidative stress profile.

Methods—A case-control study was performed to study oxidative stress markers in the serum of pregnant women with or without OSA. Patients with OSA were identified between 2003–2009. Contemporaneous controls were pregnant subjects without apnea, gasping or snoring around the time of delivery. Serum markers of oxidative and carbonyl stress were measured by spectrophotometric/fluorometric methods. Multiple linear regression analysis was used with a model including age, body mass index at delivery, history of diabetes and gestational age.

Results—Serum samples from 23 OSA cases and 41 controls were identified. Advanced oxidation protein products (AOPP), a marker for oxidative stress, and advanced glycation end

products (AGEs), a marker for carbonyl stress, were significantly lower in women with OSA than in controls (p-value < 0.0001). Total antioxidant capacity (TAC) was higher in women with OSA in comparison to controls (p-value < 0.0001). The difference in AGEs remained significant even after adjusting for confounders.

Conclusion—Contrary to our hypothesis the results of this study suggest that pregnant women with OSA have higher antioxidant capacity and lower oxidative and carbonyl stress markers compared to controls, suggesting a possible protective effect of intermittent hypoxia. Whether OSA in pregnancy impacts oxidative stress differently than OSA in the general population remains to be confirmed.

Keywords

Oxidative stress; carbonyl stress; obstructive sleep apnea; sleep disordered breathing; pregnancy

Introduction

Sleep related breathing disorders are a spectrum of disorders that consist of airflow limitation, hypoxemia and / or arousals. Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder, with obesity being its strongest risk factor. OSA has been shown to be associated with a variety of adverse outcomes such as diabetes mellitus type 2 [1] cardiovascular complications including hypertension [2], stroke [3] and a higher all-cause mortality [4, 5].

Pregnant women are particularly susceptible to sleep disordered breathing, with multiple large studies showing snoring to occur in up to a third of pregnant women in late gestation [6, 7]. In a small cohort study of 150 pregnant women with obesity, the prevalence of OSA was 15% percent [8]. OSA in pregnancy is associated with an increased risk of preeclampsia, gestational diabetes [6, 9] and reduced birth weight [10]. One of the proposed pathophysiologic mechanisms for OSA-associated consequences is oxidative stress due to intermittent hypoxemia and re-oxygenation [11].

Oxidative stress arises from the imbalance between the high production of reactive oxygen species (ROS) and low antioxidant defense. It leads to damage of lipids, proteins, and DNA. Oxidative stress is closely related to carbonyl stress since many products of lipid peroxidation are carbonyls that further react with amines and induce structural as well as functional changes to proteins. Some evidence suggests that carbonyl products, rather than the initial oxidative insult, are the culprit in causing cellular damage [12]. Oxidative stress has been recognized as the crucial mechanism of several disorders including cardiovascular disease, diabetes and pregnancy complications [13], as well as obstructive sleep apnea [14, 15]. The link between obstructive sleep apnea and oxidative stress is thought to be related to intermittent hypoxemia [11]. The severity of OSA, in particular the oxygen desaturation index, has been found to be independently associated with oxidative stress [16]. Other evidence that oxygen desaturation and tissue hypoxia are the hallmark of injury in OSA in pregnancy is suggested by a recent study by the investigators that identified a high expression of a tissue marker of cellular hypoxia, carbonic anhydrase IX, in placental tissue of women with OSA during pregnancy compared to controls [17]. Animal experiments have

shown that antioxidants could be beneficial in OSA confirming the role of oxidative stress in this sleep-related disorder [18]. On the other hand, intermittent hypoxia has also been suggested to have some protective effects against some cardiovascular complications [19] and may even have some therapeutic benefit in patients with COPD as it may improve ventilation [20]. Given the complicated hormonal and physiological milieu of pregnancy, which includes a higher inflammatory state, it is unclear how OSA would impact oxidative stress.

Several methods can be used for the quantification of oxidative and carbonyl stress. Markers for oxidative stress include advanced oxidation protein products (AOPP) as a measure of protein damage. Carbonyl stress can be measured by advanced glycation end products (AGEs) or fructosamine concentrations. To counter oxidative/carbonyl stress, the body absorbs exogenous or produces endogenous antioxidants, which can be measured as total antioxidant capacity (TAC).

We hypothesized that pregnant women with sleep apnea have alterations in oxidative and carbonyl stress markers compared to pregnant controls. This study aims to evaluate the changes of oxidative and carbonyl stress markers as well as antioxidants in pregnant women with OSA.

Materials and Methods

Study population

A case-control study was performed using serum of pregnant women with or without OSA. The Institutional Review Boards for Human Studies at Rhode Island Hospital and Women and Infants Hospital of Rhode Island in Providence, RI, approved the study. Pregnant women with OSA were identified from 2003–2009 if they had OSA documented by the International Classification of Diseases-9 codes and had a vaginal or cesarean delivery. Medical records were reviewed to confirm documentation of OSA history, an available polysomnography study at the institution, or a documented prescription for CPAP for OSA if polysomnography was performed at an outside institution and was not available for review. The timing of the diagnosis of OSA in relation to pregnancy and gestational age could not consistently be ascertained. As previously described [21, 22], only subjects with OSA who had undergone prenatal screening during pregnancy and had stored residual serum samples were included in this study.

Contemporaneous control subjects were identified from a database of English-speaking pregnant women recruited from the same population as the controls from 2006–2008. These women were screened for sleep disordered breathing around the time of delivery using the multivariable apnea prediction index [23] regarding snoring, gasping, and witnessed apneas in a cross sectional study [6]. Answers were scored from 0 (never) to 4 (always). Participants who scored zero on all three questions and had available residual second trimester serum samples were identified as controls and included in the study. This study excluded subjects who had fetal demise during the index pregnancy.

Biochemical analyses

Markers of oxidative stress (AOPP), carbonyl stress (AGEs, fructosamine) and antioxidant status (TAC) were measured in the serum of OSA patients and controls. Serum was stored at -80°C before analyses. For the detection and quantification of the particular biochemical markers established spectrophotometric/fluorometric methods were used that were previously described in detail elsewhere [18]. For AOPP measurements, samples were incubated with glacial acetic acid and concentrations were calculated from absorbance values at 340 nm. Samples were diluted in phosphate-buffered saline for AGEs measurements. Samples were incubated with 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and hydrogen peroxide in acetate buffer for TAC measurements. AGEs were measured with excitation at 370 nm, emission at 440 nm. TAC measurements were conducted at 660 nm. AOPP and AGEs were normalized to total proteins measured using bicinchoninic acid. For all methods the intra-assay coefficients of variation were below 10%. The sensitivity of the assays varies, but these methods have been successfully used for salivary analysis with concentrations lower by an order of magnitude [24]. Three fresh pregnancy samples were frozen and thawed up to 4 times to verify sample stability and assay reproducibility in the study samples.

During the analysis of oxidative and carbonyl stress markers investigators were blinded to the OSA or control status.

Statistical analyses

Statistical analysis was conducted using the SAS© software, version 9.3 (SAS Institute Inc., Cary, NC, USA). Descriptive analyses were performed with mean, median, minimum, maximum and standard deviation, based on data distribution. Fisher's exact test and Student's t-tests were used for bivariate analysis. Multivariable linear regression analysis was used to compare the markers between controls and cases. Models included in the regression analyses included age, body mass index, gestational age and a diagnosis of diabetes mellitus.

Results

Clinical Characteristics

Fifty-five cases were identified, of which 23 had residual serum available for biomarker testing. Among the 23 cases, four had documented CPAP prescription. A total of one thousand control subjects were initially recruited, of which 321 had scored zero for snoring, gasping, and witnessed apneas. Of the 321, 41 controls had residual serum for testing. The mean age of the cases and controls were 29.2 ± 5.9 and 26.5 ± 6.4 years, respectively (Table 1). BMI was significantly higher in the cases than in the controls.

The prevalence of co-morbidities such as diabetes (all types) was significantly higher in the group with OSA but prevalence of preeclampsia was similar in both groups. Gestational age at birth was smaller in women with OSA but the difference did not reach statistical significance (Table 1).

Women without available serum samples were compared to women with available serum samples who were included in the study for demographics, comorbidities and pregnancy outcomes. There were no significant differences observed between the two groups in age, weight and body mass index at sampling, history of pre-gestational diabetes and chronic hypertension, development of gestational diabetes or hypertension during the index pregnancy as well as gestational age at delivery and mean birth weight (data not shown).

Median apnea hypopnea index (AHI) was 15.4 events per hour with an interquartile range of 22.6 events per hour for those with polysomnography data. The majority of women had mild disease (46%), defined as having an apnea hypopnea index (AHI) 5–14.9 events per hour, 23% had moderate disease, (AHI 15–29.9 events per hour) and 31% had severe disease (AHI 30 events per hour). Median nadir oxygen saturation was 84%, interquartile range 7%. Median percent time spent at oxygen saturation below 90% was 3.65%, interquartile range 11.1%).

Biochemical markers

Measurements were reproducible and stable over 4 freeze thaw cycles in 3 samples for all assays (mean recovery 96% AGES, 87% fructosamine, 116% AOPP, 100% TAC).

Markers of oxidative stress were compared between the group with OSA and controls. AOPP concentrations were significantly lower in women with OSA compared to controls, (5.2 $\mu\text{mol/g}$ versus 6.0 $\mu\text{mol/g}$, respectively, $p=0.04$) (Figure 1). Carbonyl stress also appeared to be significantly lower in pregnant women with OSA compared to controls. AGE levels were 19432 AU/g in cases compared to 27344 AU/g in controls ($p<0.0001$) and fructosamine levels were 7.9 mmol/l in pregnant women with OSA compared to 9.6 mmol/l in controls ($p<0.05$). Antioxidant status measured as TAC was significantly higher (618.2 vs. 586.4 $\mu\text{mol/L}$, $p<0.0001$) in patients with OSA when compared to controls.

Using multivariable logistic regression analysis, with a model that included age, body mass index (BMI), diabetes and gestational age at sampling, the association between OSA status and oxidative stress measured by AOPP was no longer significant. In contrast, the association of OSA with reduced carbonyl stress as measured by AGEs remained significant after adjusting for the above co-variables ($p<0.01$) (Table 2). Antioxidant capacity was no longer significant after adjusting for covariates.

Figure 2 shows the relationship between the model adjusted for age and diabetes, and obesity. Antioxidant capacity decreased with increasing BMI while carbonyl stress measured by AGEs increased with rising BMI.

Discussion

As sleep disordered breathing and OSA have been linked to adverse pregnancy outcomes such as preeclampsia and gestational diabetes, oxidative stress related to repetitive episodes of hypoxia and re-oxygenation has been proposed as one of the hypothetical mechanisms underlying this association [25, 26]. Based on this study, pregnant women with OSA have higher levels of anti-oxidant capacity, and lower levels of oxidative and carbonyl stress

markers in the second trimester when compared to pregnant women at low risk for OSA and sleep disordered breathing at a similar stage in pregnancy. Oxidative stress markers were no longer significantly lower in women with OSA once adjusted for covariates. The direction of these markers in OSA is opposite of the observed effect of BMI (Figure 2) and the expected effect of pregnancy complications. To our knowledge, this is the first study that has evaluated oxidative or carbonyl stress in pregnant women with OSA.

Both pregnancy and OSA may impact oxidative and carbonyl stress [27–31]. AOPP concentrations increase in the first and second trimester of healthy pregnancies compared to non-pregnant women [32]. AOPP reaches higher concentrations in mild and severe preeclampsia [33], as well as in gestational diabetes [34] compared to healthy pregnant controls. Similarly, studies measuring advanced glycation end products showed that women with pre-gestational diabetes had higher levels of AGEs compared to normoglycemic controls and women with gestational diabetes [35–37]. The advanced glycation end product system was also implicated in the pathogenesis of preeclampsia in some studies [38]. TAC levels increase significantly throughout pregnancy [39], are highest in labor at 37 to 41 weeks [40]- the period of greatest stress in pregnancy-, and lower in pregnancies complicated by pre-gestational diabetes and preeclampsia [41, 13]. Though a higher prevalence of these comorbidities would have been expected to increase the oxidative and carbonyl stress markers and lower antioxidant capacity in the OSA group, AOPP and AGEs levels were observed to be lower and TAC higher in the OSA group, despite these comorbidities. These findings may suggest that although OSA and pregnancy complications coexist, and oxidative stress likely plays a role in these complications in general, OSA does not appear to contribute to these complications via oxidative stress. In addition, as our entire sample – both cases and controls- consisted of pregnant women, it is unlikely that the findings in our study are influenced by the pregnant state or pregnancy stage since we adjusted levels for gestational age at sampling.

OSA is a condition known to be associated with an enhanced oxidative stress and reduced antioxidant capacity in the non-pregnant population [42]. Hypoxia/re-oxygenation injury that is characteristic of OSA leads to an increased production of reactive oxygen species [16, 43], as demonstrated by Dyugovskaya et al. [44] and increased carbonyl stress as demonstrated by higher AGEs levels that correlate with the severity of OSA [45, 46]. On the other hand, possible benefits of intermittent hypoxia through increased sensitivity to hypoxia, increased hematopoiesis, alveolar ventilation, diffusion capacity, and alterations in the autonomic nervous [47] system have been described. Protection against ischemia reperfusion injury has also been described [48]. Intermittent hypoxia is believed to trigger antioxidant defense mechanisms and help stabilize cellular membranes. This may in fact suggest that OSA in pregnancy may have a protective effect with a favorable oxidative stress profile where intermittent hypoxia may result in short episodes of stress similar to labor, causing the body to respond with increased antioxidant capacity. However, it is also possible that an upregulation of the counter regulatory mechanisms of the anti-oxidant pathways as a response to augmented oxidative stress occurs in response to obstructive events and therefore counteracts the oxidative stress pathways.

Another possible explanation is that the oxidative and carbonyl system may need a long duration of an insult to be appropriately activated. As some women in our sample were diagnosed with OSA during pregnancy and the uncertainty regarding whether the condition predated pregnancy, it is possible that they may not have had the insult for long enough duration of time. Though this hypothesis may explain why levels were not elevated in women with OSA and comorbidities, it would not explain why levels were in fact lower than those without OSA, and lower concentrations of these stress markers may, in fact, support the alternative possibility of a protective effect of intermittent hypoxia. However, it is also possible that unknown confounders may have altered the levels of antioxidants in controls in our sample.

This study findings need to be interpreted in the context of several limitations, however, including small sample size of cases and controls with available residual serum. As control subjects were obtained from a convenience sample that was screened for sleep disordered breathing and had residual serum available for testing, we did not have polysomnography data in this group to definitely exclude the diagnosis of OSA which could have resulted in the null findings. Furthermore, given the retrospective nature of the study, we were unable to ascertain adherence and compliance with therapy in patients whose records indicated that therapy has in fact been prescribed (n=4). It is also possible that other subjects had been on CPAP but therapy was not documented in the obstetric record. Prior studies by the investigators have shown that continuous positive airway pressure therapy for one month reduces levels of the same oxidative and carbonyl stress markers without impacting total antioxidant capacity [18]. However, in a recent randomized controlled trial designed as an intent-to-treat trial by Paz y Mar et al.[49], there was no significant reduction in oxidative stress at two months measured by urinary F2 isoprostane and leukocyte oxidant generating enzyme myeloperoxidase among participants treated with CPAP as compared to those treated with sham CPAP. However, morning arterial stiffness measures using pulse wave velocity were significantly reduced by the use of CPAP in this study. The authors of the study concluded that improvements in cardiovascular outcomes in response to CPAP therapy may not be mediated by oxidative pathways.

It is also possible that women who are more symptomatic and reporting daytime sleepiness may have a more pronounced inflammatory and oxidative profile compared to women who are not. However, data on daytime sleepiness were not available in the cases for analysis in this study. Furthermore, previous studies of salivary oxidative stress markers have shown some circadian variation in salivary levels of stress markers [24] with levels of oxidative and carbonyl stress markers being higher in the morning than in the evening. It is possible that potential circadian variation [50] in these levels may have impacted our results.

Conclusion

In summary, unlike the general population, pregnant women with OSA have lower levels of carbonyl stress markers compared to pregnant controls. Though these findings suggest a possible protective effect of intermittent hypoxia, results from our study would need to be confirmed in a prospective sample of incident OSA with standardization of sampling time and prior to initiation of therapy.

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Appendix

These data was presented at the American Thoracic Society International Conference in San Francisco, CA, May 14–18, 2016.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants prospectively recruited into the study. Waiver of consent was obtained for the retrospective component of the study. Both were IRB approved.

Conflicts of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. GB received research equipment support from Respiroics for a federally funded study; however Respiroics had no role in the current study.

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Fig. 1.a

Fig. 1.b

Fig. 1.c

Fig. 1.d

Figure 1.

Oxidative (AOPP, Fig. 1a), carbonyl (AGEs, Fig. 1.b and Fructosamine, Fig. 1.c) stress markers and antioxidant capacity (Fig. 1.d) – unadjusted data- in pregnant women with OSA and controls.

Fig. 1.a $p=0.04$; x: outliers. AOPP: advanced oxidation protein products; μmol : micromoles; OSA: obstructive sleep apnea.

Fig. 1.b $p<0.001$; x: outliers. AGEs: advanced glycation end products; OSA: obstructive sleep apnea.

Fig. 1.c $p=0.05$; x: outliers. mmol: millimoles; OSA – obstructive sleep apnea.

Fig. 1.d $p<0.0001$. TAC: total antioxidant capacity; μmol : micromoles; OSA: obstructive sleep apnea.

Fig. 2.a

Fig. 2.b

Figure 2.
 Effect of body mass index on the age and diabetes adjusted model of carbonyl stress markers
 Fig 2.a
 * $p < 0.01$; AGEs - advanced glycation end products
 Fig. 2.b
 TAC total antioxidant capacity

Table 1

Characteristics of the analytical sample

	Study Group			
	Full sample (n=64) Median (Min, Max)	Controls (n=41) Median (Min, Max)	OSA (n=23) Median (Min, Max)	P value (OSA vs. Controls)
Age (years)	26.0 (18.0, 43.0)	26.0 (21.0, 43.0)	29.2 (18.0,42.0)	0.1
BMI at delivery (kg/m ²)	30.2 (20.1, 60)	28.95 (20.1, 44.9)	44.0 (29.0, 60.0)	<0.0001
Birth weight (g)	3405 (620, 4786)	3333 (990, 4115)	3567 (620, 4786)	0.6
Gestational age at birth (weeks)	39.0 (24, 41.6)	39.0 (27.5, 41.6)	38.0 (24.0, 40.5)	0.07
Diabetes, any type [n (%)]	10 (15.6)	1 (2.4)	9 (39.1)	0.0002
Preeclampsia [n (%)]	3 (4.7)	1 (2.4)	2 (8.7)	0.29

Notes: a) n=19; b) n=60;

Abbreviations: BMI – Body mass index; kg – kilogram; m² – square meter; OSA – Obstructive sleep apnea

Table 2

Multiple linear regression analysis of factors affecting levels of oxidative (AOPP), antioxidative (TAC), and carbonyl (AGEs and fructosamine) stress markers among pregnant women.

Stress markers	Unadjusted			Adjusted ^a		
	Intercept	OSA/Non-OSA	Intercept	OSA/Non-OSA	Age	BMI at delivery
Oxidative						
AOPP [$\mu\text{mol/g}$]	6.36	-0.95 [*]	15.7252	-0.4809	0.01101	0.00442
Antioxidative						
TAC [$\mu\text{mol/L}$]	586.37	31.84	712.429	3.12838	2.19538	0.34719
Carbonyl						
AGEs [AU/g]	27344.00	-7912.32 ^{***}	26621	-8891.5 ^{**}	138.773	92.5726
Fructosamine [mmol/L]	9.51	-1.11	14.5864	-1.3108	-0.0091	-0.013

^{*} P-Value < 0.05;

^{**} P-Value < 0.01;

^{***} P-Value < 0.001

Abbreviations: AGEs – advanced glycation end products; AOPP – advanced oxidation protein products; g – grams; L – liters; TBARS – thiobarbituric acid reactive substances; OSA – Obstructive sleep apnea; SD – standard deviation; TAC – antioxidant capacity; mmol – millimoles; μmol – micromole