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SHORT COMMUNICATION

Urinary F_2-isoprostane metabolite levels in children with sleep-disordered breathing

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Abstract Oxidant stress-related mechanisms have been proposed as a major contributor to the increased prevalence of cardiovascular morbidity in adult patients with sleep-disordered breathing. Isoprostanes provide a reliable biomarker of oxidant injury in vivo. The purpose of the present study was to examine the hypothesis that oxidant stress, as evidenced by increased levels of F_2-isoprostane metabolites (IsoP-m) in urine, is present in children with a spectrum of sleep-disordered breathing. Assays were performed on urinary samples obtained from each of 47 pediatric patients immediately upon awakening after standard overnight polysomnography. Of the subjects, 15% had mild, 9% had moderate, and 6% had severe sleep-disordered breathing. After controlling for correlations between BMI and IsoP-m and SpO2 values, IsoP-m values were unrelated to any polysomnographic measures. The absence of increased levels of urinary F_2-isoprostane metabolites in children with sleep-disordered breathing suggests that oxidative stress is not a significant feature of pediatric sleep-disordered breathing.

Keywords Oxidant stress · Sleep-disordered breathing · Pediatric · Isoprostane

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Introduction

Oxidant stress-related mechanisms were proposed as a major contributor to cardiovascular morbidity in adult patients with sleep-disordered breathing (SDB) [1, 2], and there is evidence of their improvement after continuous positive airway pressure (CPAP) treatment [3–6].

The cumulative findings suggest that intermittent hypoxia associated with SDB may activate a cascade of oxidant stress reactions which targets lipid-related compounds, and thereby favoring the onset and acceleration of atherogenesis. Furthermore, episodic hypoxia in a rodent model of SDB [7] leads to increased oxidant stress and is mechanistically associated with neurobehavioral deficits [8–10].

Studies have also questioned the relationship between oxidant stress and SDB in adults. Wali et al. found no oxidative stress differences between normal controls and those with obstructive sleep apnea (OSA) with no changes after treatment with CPAP [11]. Although it was a split-night study, Svatikova et al. found no change in markers of oxidative stress and lipid peroxidation compared to controls [12].

F_2-isoprostanes are specific products of lipid peroxidation and their metabolites were validated for the assessment of oxidative stress in vivo [13, 14], specifically within the pediatric population [15]. F_2-isoprostanes are a family of

Prostaglandin (PG) $F_{2\alpha}$ isomers initially described as products of noncyclooxygenase oxidative modifications of arachidonic acid that have resulted from free-radical attack of cell membrane phospholipids or circulating LDLs [16, 17] In contrast to the classic prostaglandins, which are formed through the action of PGH synthase isozymes from free arachidonic acid [18], F_2-isoprostanes are formed in situ from the fatty acid backbone esterified in membrane phospholipids. Isoprostanes maybe formed by either of two routes of peroxidation [19, 20], an endoperoxide mechanism or a dioxetane/endoperoxide mechanism. After their formation, isoprostanes are released from the membrane phospholipids in response to cellular activation, presumably through a phospholipase-mediated mechanism, and circulate in plasma. They may circulate as free form or as esters in phospholipids in plasma. The factors that regulate the release of endogenous isoprostanes from cell membranes and interconversion between the free and esterified forms are at present poorly understood. Of relevance to the present study, isoprostanes are excreted in the urine, and provide a reliable, noninvasive measure of global oxidative stress [21, 22]. The purpose of the present study was to examine the hypothesis that oxidant stress, as evidenced by increased urinary F_2-isoprostane metabolites (IsoP-m) [13], is present in children with SDB and may provide a noninvasive early screening method for early detection of SDB-related damage.

An emerging body of work suggests that 'primary' snoring in children causes sleep fragmentation sufficient to impair daytime functioning [23], executive function [24], verbal and global IQ, selective and sustained attention, memory [25], attention, [26] and mental development [27]. Thus, we included children with a broad spectrum of SDB severity.

Materials and methods

Research participants were recruited during clinical evaluation for suspected SDB and among participants in a larger community-based study for standard overnight polysomnographic (PSG) evaluation and provided a urinary sample the following morning. The study was approved by university and hospital Institutional Review Boards, parental consent and child assent, when appropriate.

Overnight polysomnography

Standard overnight multichannel polysomnography was performed and evaluated as described in previous reports [28]. Average lights out was 21:34 (SD±32 min) and average lights on was 6:07 (SD±23 min). Research and clinical recording equipment and protocols were identical, and all PSG recordings were scored using standard techniques [29] by the same analyst blinded to the subjects.

Oxygen saturation (SpO2) was assessed as the mean total sleep time saturation (TST SpO2), SpO2 nadir, and TST SpO2 desaturation index. Indices were also calculated for number of SpO2 desaturations of <95%, <90%, <85%, <80%, <75%, <70% and <65% per hour TST. A hypoxia composite score was created by multiplying increasing weights by the time spent in decreasing SpO2 ranges during TST: $(SpO2_{96–100\%}\times 1)+...+(SpO2_{61–65\%}\times 8)$.

Mean TST PETCO2 was determined, along with the percent TST spent with PETCO2 ≥45 and ≥50 mmHg. Automated scoring of respiratory events and desaturations were scorer validated.

F_2-IsoP metabolite assay

Urinary samples were obtained immediately upon awakening the following morning, rapidly cooled and transferred under ice to storage at −80°C. The samples were processed by a blinded analyst. 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP, the predominant urinary metabolite of 15-F_{2t}-IsoP (8-*iso*-$PGF_{2\alpha}$) was measured by gas chromatography [30] and negative ion chemical ion mass spectrometry as previously described [31] and as previously validated in a pediatric population [15]. Urinary creatinine was measured by the alkaline picrate reaction using a clinical autoanalyzer. The precision and accuracy of the GC/MS assay for the isoprostane metabolite is outlined in [31]. In brief, precision was measured by analyzing six 1-ml aliquots of urine obtained in a 24 h collection from a normal volunteer. The mean of three replicate measurements of the ratio of m/z 543 (endogenous metabolite) to m/z 547 (^{18}O labeled internal standard) was found to be ±4%. Accuracy was assessed using the same urine. For this, 1.0 ng of unlabeled isoprostane metabolite was added to another four 1-ml aliquots of the urine and re-assayed. The amount of endgenous isoprostane metabolite measured in the precision experiment was subtracted from the total measured. The accuracy of the assay to measure the added 1.0 ng of the isoprostane metabolite was calculated. The accuracy was found to be 97%.

Statistical analyses

Data are presented as the means±SD unless otherwise indicated. SPSS (version 13.0) was used. A p value <0.05 was considered significant.

Results

Forty-nine consecutive children invited to participate completed the study. Sleep data were unavailable for two clinic patients due to a failed recording and insufficient sleep time (<4 h TST with no documented REM). Analyses were based on 47 subjects (40% female) who were 6.6 (SD±2.3) years (range=3.8–13.6 years) and had a BMI of 18.7 (SD±5.2; range=14.2–33.2).

Of the subjects, 76% had an OAHI <1.0/h TST, 15% had 1.0–4.9/h TST (mild SDB), 9% had >5.0/h TST (moderate SDB) and 6% (three subjects) had an OAHI >15.0/h TST (severe SDB). 34% had SpO2 nadir <90% and 10% had desaturation indices >5. 43% of the subjects with OAHI <1 had a SpO2 nadir <92.7%. 10% had composite desaturation scores >120 Table 1.

When controlled for BMI, there were no significant correlations between ng IsoP-m/mg CR and any PSG measure. Table 2. IsoP-m was normally distributed (skewness=0.134) and when split at the mean, there were no significant differences between the groups for any PSG measure. However, OAHI and SpO2 nadir were not normally distributed (skewness=3.18 and −2.26, respectively). When subjects were divided into the extreme OAHI values of OAHI=0 and OAHI >4, there was no significant difference in their IsoP-m values (F=0.05, p=0.83).

Discussion

F_2-isoprostane metabolites were unassociated with PSG measures from children with a wide range of SDB severity, despite consistent reports that pediatric SDB is a spectrum disorder with associated morbidity at even the 'normal' level of AHI <1 when snore-related arousals are present [23–27]. That 43% of the normal subjects (OAHI <1) had a SpO2 nadir value <92.7 was expected based on normative values from this population with average SpO2 nadir=92.7 (SD=4.5) [28]. Several possible interpretations of these data merit discussion.

It is possible that either oxidative stress was not increased, or that such stress may have been counteracted by the subjects' intrinsic antioxidant capacity. It is also possible that the few subjects with significant SDB in our sample did not have sufficient hypoxic episodes to induce oxidative stress in which case the clinical value of oxidative stress measurements in the children is of questionable use because neurocognitive and cardiovascular consequences are found at even the low end of the SDB spectrum. It is also important to note that the overall sample size was relatively low.

It was also established that children show a differential response to intermittent hypoxia with a lower tendency to arousal from respiratory events than adults [32] and greater circulating vascular endothelial growth factor (VEGF) levels compared to adults [33]. Thus, it might be expected that children would show a heightened response to intermittent hypoxia and indeed, dose-dependent measures of cardiovascular morbidity were found with pediatric SDB [34, 35]. However, this was not found in the current study despite the fact that measurements of IsoP-m have emerged as reliable markers of oxidative stress in pediatric populations [15]. Thus, it is possible that while hypoxia-inducible factor-driven genes such as erythropoietin or VEGF would

Table 1 Polysomnography measures

	Mean	SD	Range	Pearson correlation with ng IsoP-m/mg CR	1-tailed p	Pearson correlation with BMI	*p* value	Partial correlation with ng IsoP-m/mg CR controlling for BMI	*p* value
TST (h)	7.6	0.87	6.0–9.2						
AHI	2.4	4.6	0–20.2	−0.018	0.45	0.170	0.27	−0.08	0.06
AI	1.5	3.0	0–20.2	−0.43	0.39	0.020	0.92	−0.06	0.71
OAHI	1.8	4.6	0–20.2	−0.002	0.50	0.020	0.20	−0.07	0.66
OAI	1.0	3.0	0–14.6	−0.016	0.46	0.060	0.69	−0.03	0.83
SpO2	97.2	0.99	94.3–98.8	−0.116	0.22	−0.470	0.001	0.05	0.75
SpO2 nadir	89.7	7.6	60–96.1	−0.246	0.048	−0.36	0.01	−0.12	0.45
Desturation index	1.9	4.7	0–24.7	0.065	0.33	0.25	0.10	−0.01	0.93
Hypoxia composite	106.0	15.9	100.0–182.4	0.138	0.18	0.51	0.001	−0.03	0.84
PETCO2	39.2	7.4	26.0–66.0	0.183	0.11	0.06	0.69	0.15	0.33
% TST >45mmHg	17.0	31.3	0–100.0	0.181	0.11	−0.020	0.92	0.21	0.18
% TST >55mmHg	9.6	28.1	0–99.9	0.047	0.38	−0.050	0.74	0.07	0.64

Table 2 IsoP-m values

	Mean	SD	Range	Pearson correlation with BMI	*p* value
ng IsoP-m/mg CR	29.6	11.7	2.08–54.62	0.340	0.02

be more prominently upregulated in SDB in children, the converse would occur in relation to the magnitude of oxidative stress with children exposed to episodic hypoxia developing reduced free radical and oxidative products.

Technical issues such as sample handling and processing are an unlikely explanation for our negative findings considering that incubation of urine at 37°C for 5 days did not decrease the amount of IsoP-m measured (LJR, 2005 personal communication). Neither volume of fluid intake before PSG nor nocturnal voids were monitored, but correction by simultaneous assessment of creatinine would be expected to circumvent this potential confound.

It is worth mentioning the choice to use urine samples. The foremost appeal of using urinary specimen as opposed to plasma is its non-invasive nature. This allows sample collection in a variety of clinical settings with minimum patient or caretaker discomfort. Further, plasma has to be rapidly isolated and stored at −70°C whereas urinary metabolites are stable (LJR, 2005 personal communication). Finally, urine in the bladder is naturally stored over several hours allowing the opportunity to study the cumulative production of F_2-IsoP metabolites between voids during a potential period of oxidative stress, overnight sleep-disordered breathing in the current study, as opposed to a timed plasma sample.

In summary, our findings do not support the presence of significant differences in oxidative stress as evidenced by urinary IsoP-m concentrations in a small sample of children with SDB compared to normal children.

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