SCIENTIFIC INVESTIGATIONS

Plasma C-Reactive Protein in Nonobese Children With Obstructive Sleep Apnea Before and After Adenotonsillectomy

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Study Objective: Sleep-disordered breathing (SDB) is a prevalent condition in children and is associated with increased cardiovascular morbidity. Circulating levels of C-reactive protein (CRP), a proinflammatory protein, are associated with increased risk for atherosclerosis. Plasma CRP levels in snoring children have yielded conflicting results, such that it remains unclear whether OSA is mechanistically involved in such elevations of CRP.

Methods: Consecutive nonobese children with polysomnographically demonstrated obstructive sleep apnea underwent blood draws in the morning after their corresponding sleep studies on 2 occasions, namely at diagnosis of obstructive sleep apnea and 10 to 14 weeks after adenotonsillectomy. High-sensitivity CRP serum concentrations were determined within 2 to 3 hours after collection, using a particle-enhanced turbidimetric immunoassay technique.

Results: Twenty children with obstructive sleep apnea (mean age 7.3 ± 1.9 years; 55% boys; relative body mass index: 88% ± 12.0%) with a mean apnea-hypopnea index at diagnosis of 15.6 ± 2.9 events per hour of total sleep time and nadir SaO_2 of 82.3% ± 2.5% were included. Mean initial CRP levels at obstructive sleep apnea diagnosis were 0.67 ± 0.21 mg/dL and decreased to 0.23 ± 0.07 mg/dL after adenotonsillectomy (p < .05), along with significant decreases in measured apnea-hypopnea index (2.2 ± 0.8 events/h of total sleep time ; p < .01) and improved oxygenation (mean nadir SaO_2 values: 88.6% ± 1.9%; p < .01).

Conclusions: Obstructive sleep apnea is frequently associated with increases in CRP levels that are reversible upon treatment. Thus, obstructive sleep apnea induces a systemic inflammatory response in children, which, if left untreated, may potentially lead to cardiovascular morbidity.

Keywords: C-reactive protein, sleep-disordered breathing, children, inflammation, obstructive sleep apnea

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Sleep-disordered breathing (SDB) is a frequent disorder in children, with an estimated prevalence of 2% to 3%,[1-2] which has been associated with increased risk for cardiovascular morbidities. Nocturnal elevations of systemic blood pressure and sustained diurnal hypertension[3,4] and severity-dependent changes in left-ventricular geometry and function[5] have all been recently reported in children with SDB and have been ascribed to the concurrent presence of sustained sympathetic activation[6-8] and platelet-leukocyte-endothelial interactions leading to initiation and propagation of atherogenesis-related processes.[9-11]

C-reactive protein (CRP), an important circulating marker of inflammation, is now considered as a reliable marker for subsequent cardiovascular morbidity.[12-14] This protein, which is synthesized in the liver in response to upstream inflammatory signaling pathways involving interleukin-6, has also been shown to directly participate in atheromatous lesion formation.[15] In a previous study, we showed that plasma CRP levels were increased in children with SDB, compared with controls, and that CRP concentrations were significantly correlated with disease-severity measures, such as hypoxemia and sleep fragmentation.[16] Furthermore, we found that these correlations were still valid after adjusting for the degree of obesity.[16] Similar to our findings, Larkin and colleagues further reported elevated plasma CRP levels in adolescents with SDB who were free of any known cardiovascular disease, thereby suggesting that SDB in children may apportion an additional risk for cardiovascular morbidity beyond that imposed by the presence of obesity.[17] Notwithstanding such findings, normal levels of plasma CRP have also been reported in Greek children with SDB,[18] suggesting that the causal relationship between CRP elevations and SDB may not be always operational. Therefore, in order to better understand the contribution of SDB to the inflammatory process ultimately leading to increased cardiovascular risk, we examined the plasma levels of CRP in children with SDB before and following treatment.

METHODS

Consecutive nonobese children diagnosed with SDB were enrolled in the study and were studied twice, namely at the time of SDB diagnosis and 10 to 14 weeks after undergoing curative adenotonsillectomy. Exclusion criteria were the presence of genetic disorders, cerebral palsy, neuromuscular diseases, or any under-

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lying systemic diseases or acute infectious processes. Blood was drawn the morning after the child underwent a standard polysomnographic evaluation in the sleep laboratory at the Kosair Children's Hospital. Plasma CRP was measured within 2 to 3 hours after collection using the Flex reagent Cartridge (Date Behring, Newark, DE), which is based on a particle-enhanced turbidimetric immunoassay technique. This method has a detection level of 0.05 mg/dL and exhibits linear behavior up to 255 mg/dL, with intraassay and interassay coefficients of variability of 9% and 18%, respectively. Plasma was obtained from the blood sample and was stored at -80°C until assayed.

A standard overnight multichannel polysomnographic evaluation was performed in the sleep laboratory on 2 different occasions, specifically during the initial diagnostic study and 10 to 14 weeks after undergoing adenotonsillectomy. Children were studied for up to 12 hours in a quiet darkened room with an ambient temperature of 24°C and in the company of 1 of their parents. No drugs were used to induce sleep. The following parameters were measured: chest and abdominal wall movement by respiratory impedance or inductance plethysmography; heart rate by electrocardiogram; air flow by a sidestream end-tidal capnograph, which also provided breath-by-breath assessment of end-tidal CO_2 levels (PetCO$_2$; BCI SC-300, Menomonee Falls, WI); nasal pressure catheter; and an oronasal thermistor. Arterial oxygen saturation (SpO$_2$) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc., Hayward, CA), with simultaneous recording of the pulse waveform. The bilateral electrooculogram, 8 channels of electroencephalogram, chin and anterior tibial electromyograms, and analog output from a body-position sensor (Braebon Medical Corporation, NY) were also monitored. All measures were digitized using a commercially available polysomnography system (Rembrandt, MedCare diagnostics, Amsterdam). Tracheal sound was monitored with a microphone sensor (Sleepmate, VA), and a digital time-synchronized video recording was performed.

All overnight sleep studies were scored in a blinded fashion without a priori knowledge of the patient's identity or treatment status. Sleep architecture was assessed by standard techniques.[19] The proportion of time spent in each sleep stage was expressed as percentage of total sleep time (TST). The apnea index was defined as the number of apneas per hour of TST. Central, obstructive, and mixed apneic events were counted. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement for duration of at least 2 breaths.[20-22] Hypopneas were defined as a decrease in oronasal flow of at least 50%, with a corresponding decrease in SpO$_2$ of 4% or greater and/or an arousal.[22] The obstructive apnea-hypopnea index (AHI) was defined as the number of apnea and hypopneas per hour of TST. Children with an initial AHI of 5 or more events per hour of TST were considered to have SDB and were referred for surgical removal of enlarged tonsils and adenoids. Complete resolution of SDB after surgery was defined as a postsurgical AHI < 1 per hour of TST.

The mean oxygen saturation, as measured by pulse oximetry (SpO$_2$) in the presence of a pulse waveform signal void of motion artifact, and the nadir SpO$_2$ were recorded. Since criteria for arousals have not yet been developed for children, arousals were defined as recommended by the American Sleep Disorders Association Task Force report[23] using the 3-second rule and/or the presence of movement arousal.[24]

Height and weight were obtained from each child. Body mass

Table 1—Demographic and Polysomnographic Characteristics of 20 Children with Sleep-Disordered Breathing Before and Following Adenotonsillectomy

	Before adenotonsillectomy	After adenotonsillectomy	p value
Age, y	7.3 ± 1.9 (4-10)		
Boys, %	55		
Relative BMI, %	88 ± 18.0	92.4 ± 17.8	< .03
AHI, events/h	15.6 ± 2.9	2.2 ± 0.8	< .01
SpO$_2$ nadir, %	82.3 ± 2.5	88.6 ± 1.9	< .01
PETCO$_2$, mmHg	56.3 ± 4.8	52.8 ± 4.1	< .04
Arousal Index, events/h	16.8 ± 3.2	6.4 ± 2.1	< .01
TST, min	523.9 ± 24.7	531.6 ± 26.4	NS
Sleep efficiency, %	91.2 ± 4.5	90.3 ± 5.4	NS
Sleep stage, %			
1	13.6 ± 6.4	8.5 ± 7.5	< .05
2	47.0 ± 12.2	43.8 ± 11.9	NS
SWS	23.8 ± 8.1	26.4 ± 9.3	NS
REM	14.7 ± 5.2	17.6 ± 6.6	NS
CRP, mg/dL	0.67 ± 0.21	0.23 ± 0.07	< .05

Data are presented as mean ± SD unless otherwise indicated. BMI refers to body mass index; AHI, apnea-hypopnea index; PetCO$_2$, end-tidal CO_2, TST, total sleep time; SWS, slow-wave sleep; REM, rapid eye movement; CRP, C-reactive protein.

index (BMI) was calculated and also expressed as relative BMI (relative BMI), using the following formula: (BMI/BMI of the 50th percentile for age and sex) × 100, based on standardized percentile curves.[25] Obesity was defined as a BMI greater than the 95th percentile for sex and age and led to exclusion from the study.

Data Analysis

Data are presented as means ± SD unless otherwise indicated. All analyses were conducted using SPSS software (version 11.5; SPPS Inc., Chicago, IL). Comparisons between pretreatment and posttreatment measures were made with paired t-tests. All p values reported are 2-tailed, with statistical significance set at < .05.

RESULTS

Twenty children with SDB were included. Subject sleep characteristics are shown in the Table. In brief, their mean age was 7.3 ± 1.9 years, 55% were boys, and their mean relative BMI was 88% ± 12.0%. Their mean AHI at diagnosis (before the adenotonsillectomy) was 15.6 ± 2.9 events per hour of TST, and their nadir SaO$_2$ was 82.3% ± 2.5%; adenotonsillectomy was associated with significant improvements in respiratory disturbance (AHI post adenotonsillectomy: 2.2 ± 0.8 events per hour of TST; p < .01, and nadir SaO$_2$ post adenotonsillectomy: 88.6% ± 1.9%; p < .01), with mild, albeit significant, increases in relative BMI (92.4% ± 17.8%; p < .03)

Mean initial CRP levels at SDB diagnosis were 0.67 ± 0.21 mg/dL and decreased to 0.23 ± 0.07 mg/dL after adenotonsillectomy (Figure; p < .05). As in our previous study,[16] there was a statistically significant linear correlation between logAHI and logCRP (r^2: 0.22; p < .03). Of the 20 children studied, 15 children exhibited higher CRP levels at diagnosis, 3 had no changes in their CRP levels over time, and 2 children showed mild insignifi-
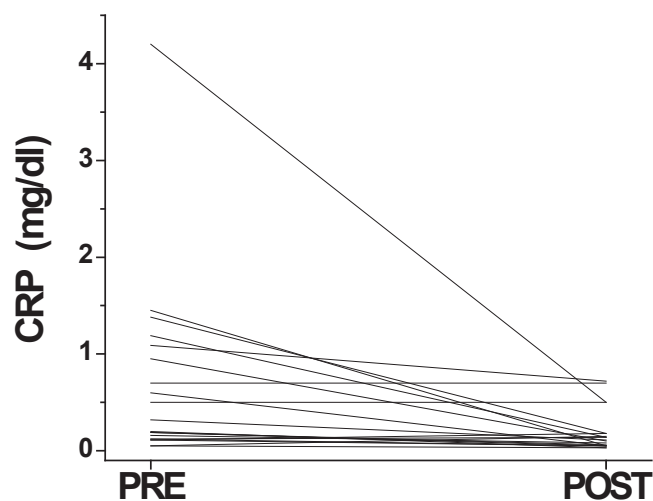
Figure 1—Individual serum C-reactive protein (CRP) levels in 20 children with sleep-disordered breathing before (Pre) and after (Post) adenotonsillectomy.

cant increases after adenotonsillectomy (from 0.12 mg/dL to 0.14 mg/dL in 1, and from 0.11 mg/dL to 0.18 mg/dL in the other.

DISCUSSION

This study confirms our previous report that children with SDB often have elevated plasma CRP levels but, more importantly, demonstrates that CRP levels will decline upon resolution of SDB. Thus, SDB induces an independent effect on subclinical inflammatory processes and could play an important role in modulating the risk for cardiovascular morbidity in early life.

Substantial evidence derived from adults clearly implicates SDB as a contributing risk factor in cardiovascular diseases such as hypertension, ischemic heart disease, and cerebrovascular accidents.[26-28] Both intermittent hypoxia and sleep fragmentation have been shown to elicit inflammatory and oxidative responses that can ultimately initiate or accelerate the processes underlying atherogenesis. One of the most important confounders in these studies, however, is the presence of obesity, and pediatric patients with SDB are no exception. Indeed, recent trends in the referral population to our pediatric sleep center reveal that more than 45% of children with habitual snoring are obese (i.e., relative BMI > 95% adjusted for age and sex[29]). Obesity in childhood is a robust predictor for the development of cardiovascular consequences in adulthood and childhood and is associated with earlier emergence of insulin resistance, hypertension, and dyslipidemia, all of which constitute potential risks for the development of cardiovascular morbidity.[30-32] In this study, we specifically selected patients with SDB who were not obese to allow for a more reliable assessment of the potential role played by SDB in the putative elevation of circulating levels of CRP. Our current findings strongly support and add further validity to our previous report as well as to the study by Larkin et al,[16-17] both of which showed the presence of a severity-dependent relationship between SDB and CRP. However, while genetic and environmental differences such as physical inactivity and dietary fat and sugar content could account for the discrepant findings of Kaditis and colleagues in a cohort of Greek children with SDB,[18,33-35] the consistent declines in serum CRP upon treatment of SDB argue against such considerations.

Of note, elevated plasma levels of adhesion molecules occur in children with SDB,[36] and, remarkably analogous to adults with SDB,[37,38] they are significantly correlated with the degree of hypoxemia and sleep fragmentation. Thus, SDB appears to induce or amplify an inflammatory-response cascade that ultimately potentiates the pathophysiologic mechanisms underlying atherogenesis. If these assumptions are correct, it is somewhat reassuring to observe a decline in such inflammatory activity with effective treatment, as suggested by our current findings.

Although a major strength of our study lies in the prospective and paired study design, it would be interesting to further determine whether the concurrent presence of obesity would affect the effect size of the response to treatment. Similarly, it will be critical to determine in future studies whether SDB elicits differential responses in children born to families with a high prevalence of cardiovascular disease and whether treatment will abrogate CRP elevations in such high-risk patients. Indeed, despite a linear correlation between CRP levels and the severity of SDB (expressed as AHI) there was substantial interindividual variability in the degree of CRP changes associated with the presence of SDB, suggesting that genetic and environmental factors may account for a substantial proportion of the variance in the magnitude of the systemic inflammatory response to SDB.[39] In addition, our study population consisted of patients with relatively severe SDB, such that the magnitude of systemic inflammation ascribable to SDB in milder cases remains undefined.

In summary, children with SDB commonly display reversible increases in plasma CRP even in the absence of concurrent obesity. These findings support the hypothesis that SDB in childhood imposes an independent risk for development of subclinical inflammation and that the latter may underlie the onset and progression of atherosclerosis, particularly in risk-prone populations.

ACKNOWLEDGEMENTS

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