Plasma C-Reactive Protein Levels Among Children With Sleep-Disordered Breathing

Riva Tauman, MD; Anna Ivanenko, MD, PhD; Louise M. O’Brien, PhD; and David Gozal, MD

ABSTRACT. Introduction. Levels of C-reactive protein (CRP), an important serum marker of inflammation with major implications for cardiovascular morbidity and atherogenesis, are elevated among adult patients with sleep-disordered breathing (SDB). We hypothesized that elevated CRP plasma levels would also be present among children with SDB.

Methods. Eighty-one children (mean age: 9.3 ± 3.7 years) underwent polysomnographic evaluations. Samples for plasma CRP level and lipid profile determinations were drawn the next morning.

Results. Because plasma CRP levels were not normally distributed in this cohort, logarithmic transformation was applied. Log plasma CRP levels were significantly higher in the SDB group (obstructive apnea/hypopnea index [AHI] of ≥5), compared with the mild SDB group (AHI of ≥1 and <5) and the control group (AHI of <1). Significant positive correlations were found between log CRP levels and AHI \( r = .53 \) and arousal index \( r = .28 \), whereas an inverse correlation was found between the lowest nocturnal arterial oxygen saturation and log CRP levels \( r = -.47 \). These correlations persisted after exclusion of outliers. Moreover, 94% of the children with elevated log CRP levels reported excessive daytime sleepiness and/or learning problems, compared with 62% of the children with normal log CRP levels.

Conclusions. Plasma CRP levels were increased among some children with SDB and were correlated with AHI, arterial oxygen saturation nadir, and arousal index measures. These changes were particularly prominent among children who were sleepy or presented with neurobehavioral complaints. The intermittent hypoxemia and sleep fragmentation of SDB may underlie inflammatory responses, the magnitude of which may ultimately lead to the cardiovascular, cognitive, and behavioral morbidities of SDB. Pediatrics 2004;113:e564–e569. URL: http://www.pediatrics.org/cgi/content/full/113/6/e564; sleep-disordered breathing, C-reactive protein, atherogenesis.

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leep-disordered breathing (SDB) is characterized by repeated events of partial or complete upper airway obstruction during sleep, resulting in disruption of normal ventilation, hypoxemia, and sleep fragmentation. SDB is associated with neurobehavioral and cardiovascular morbidities.1–7 The increased prevalence of hypertension and atherogenesis among SDB patients has been ascribed to sympathetic activation6–10 and to endothelial dysfunction,11–13 most likely resulting from initiation and propagation of inflammatory responses within the microvasculature.

C-reactive protein (CRP), an important serum marker of inflammation, has emerged as one of the most powerful independent predictors of risk for future cardiovascular morbidity.14–16 In addition, recent evidence suggests that CRP may directly participate in atheromatous lesion formation through leukocyte activation and endothelial dysfunction.17–20 CRP has also been found to be independently associated with insulin resistance21–23 and low levels of the fat-derived cardioprotective hormone adiponectin,24 supporting the association between inflammation, atherogenesis, and insulin resistance.

Among adult patients with SDB, plasma CRP levels are elevated and are correlated with the severity of the disease.25 Moreover, plasma CRP levels are decreased after treatment with continuous positive airway pressure,26 which suggests that SDB leads to inflammatory responses that ultimately promote cardiovascular complications. Although substantial evidence of the cardiovascular morbidity of SDB among children has been accumulated in the past decade, no studies thus far have examined the levels of CRP among children with SDB. In this study, we assessed plasma CRP levels among a large cohort of children and also examined whether CRP levels were associated with some of the typical clinical complaints that usually lead to referral for evaluation of snoring.

METHODS

Consecutive snoring children who were being evaluated for the presence of SDB were enrolled in the study. Exclusion criteria included the presence of genetic disorders, cerebral palsy, neuromuscular diseases, or any systemic diseases or acute infectious processes. All parents completed a detailed, routine, intake, clinical questionnaire that inquired, among many other questions, about the presence of daytime sleepiness and learning and behavioral problems of their child (see “Appendix”). Daytime sleepiness was considered present if a positive answer was obtained for either of the following 2 questions: “Is your child sleepy during

Abbreviations: SDB, sleep-disordered breathing; CRP, C-reactive protein; BMI, body mass index; HDL, high-density lipoprotein; TST, total sleep time; AHI, obstructive apnea/hypopnea index; SPO2, arterial oxygen saturation.

From the Kosair Children’s Hospital Research Institute, Division of Pediatric Sleep Medicine, Department of Pediatrics, University of Louisville, Louisville, Kentucky.

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Reprint requests to (D.G.) Kosair Children’s Hospital Research Institute, University of Louisville School of Medicine, 571 S Preston St, Suite 321, Louisville, KY 40202. E-mail: david.gozal@louisville.edu

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daytime?” “Does your child fall asleep at school?” Behavioral problems were considered present if a positive answer was obtained for any of the following questions. “Does your child have attention-deficit/hyperactivity disorder?” “Is your child hyperactive?” Learning problems were categorized on the basis of a positive response to 1 of the following questions. “Does your child have learning difficulties at school?” “Were there changes in your child’s school performance?” The study was approved by the institutional human study review committee, and parental consent, as well as child assent for children ≥7 years old, was obtained. All procedures were approved by the Kosair Children’s Hospital Institutional Review Board.

Blood for high-sensitivity assessments of plasma CRP levels was drawn the morning after each child underwent a standard polysomnographic evaluation in the sleep laboratory at the Kosair Children’s Hospital. Plasma CRP levels were measured with a 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 intraassy and interassay coefficients of variability of 9% and 18%, respectively. Serum levels of lipids, including total cholesterol, high-density lipoprotein (HDL) cholesterol, calculated low-density lipoprotein cholesterol, and triglycerides, were also assessed with Flex reagent cartridges (Date Behring, Newark, DE).

A standard, night-time, polysomnographic evaluation was performed in the sleep laboratory. Children were studied for up to 12 hours in a quiet darkened room with an ambient temperature of 24°C, in the company of 1 of their parents. No drugs were used to induce sleep. The following parameters were measured: heart rate and abdominal and thoracic movements associated with respiratory impedance or inductance plethysmography, heart rate assessed by electrocardiography, and air flow monitored by side-stream end-tidal capnography, which also provided breath-by-breath assessments of end-tidal carbon dioxide levels (BCI SC-300; Menomonee Falls, WI), and a thermistor. Arterial oxygen saturation (SaO2) was assessed by pulse oximetry (Nellcor N 100; Nellcor Inc, Hayward, CA), with simultaneous recording of the pulse waveform. Bilateral electrooculograms, 8 channels of the electroencephalogram, chin and anterior tibial electromyograms, and analog output from a body-position sensor (Brabron Medical Corp, Ogdenburg, NY) were also monitored. All measures were digitized with a commercially available polysomnographic system (Rembrandt; MedCare Diagnostics, Amsterdam, The Netherlands). Tracheal sounds were monitored with a microphone sensor (Sleepmate, Midlothian, VA), and a digital, time-synchronized video recording was obtained.

Sleep architecture was assessed by standard techniques.27 The proportion of time spent in each sleep stage was expressed as percentage of total sleep time (TST). Awakenings were defined as sustained arousal lasting for ≥15 seconds. The apnea index was defined as the number of episodes of apnea per hour of TST. Central, obstructive, and mixed apneic events were counted. Obstructive apnea was defined as the absence of airflow with continued chest and abdominal movements for the duration of at least 2 breaths.28,29 Hypopnea was defined as a decrease in nasal flow of ≥50% with a corresponding decrease in SaO2 of ≥4% and/or arousal.29 The obstructive apnea/hypopnea index (AHI) was defined as the number of episodes of apnea and hypopnea per hour of TST. Children with AHI values of ≥1 episode per hour of TST but <5 episodes per hour of TST were considered to have mild SDB, whereas children with AHI values of ≥5 episodes/hour of TST were considered to have moderate SDB. Control children were defined as nonsnoring children with AHI values of <1 episode per hour of TST.

The mean SP02, as measured by pulse oximetry in the presence of a pulse waveform signal void-of-motion artifact, and the SP02 nadir were recorded. Because criteria for arousals have not yet been developed for children, arousals were defined as recommended by the American Sleep Disorders Association Sleep Disorders Atlas Task Force report,30 using the 3-second rule and/or the presence of movement arousal.31 Arousal events were divided into 2 types, ie, spontaneous and respiratory arousals. Height and weight were recorded for each child. Body mass index (BMI) was calculated, and data were also expressed as relative BMI using the following formula: relative BMI = [BMI (BMI of the 50th percentile for age and gender) × 100 (based on standardized percentile curves).32

Data are presented as means ± SD unless otherwise indicated. Because plasma CRP levels were not normally distributed, logarithmic transformation was applied. Comparisons of demographic data and assessment of plasma CRP levels were performed using analysis of variance followed by post hoc comparisons, with P values adjusted for unequal variances when appropriate (Levene’s test for equality of variances), or χ2 analyses with Fisher’s exact test (dichotomous outcomes). Because obesity would be expected to contribute to increased CRP levels, we performed analysis of covariance with relative BMI as a covariate. Correlations of log CRP levels with arousal index, AHI, and SP02 nadir were performed by linear regression, followed by calculation of Pearson correlation coefficients. All P values reported are 2-tailed, with statistical significance set at <.05.

RESULTS

Eighty-one children (58% male), 3 to 18 years of age (mean: 9.3 ± 3.7 years), participated in the study. Of these, 32 children (19 male) were found to have SDB, 34 children (18 male) were considered to have mild SDB, and 15 children (10 male) were in the control group. Subject characteristics are presented in Table 1. There were no significant differences in age, gender, and relative BMI among the 3 groups; however, there was a trend for a higher relative BMI in the SDB group. No significant differences were observed in the serum lipid profiles for the 3 groups.

Because plasma CRP levels were not normally distributed, logarithmic transformation was applied. Log plasma CRP levels were significantly higher in the SDB group, compared with the mild SDB group and the control group (P < .0001 and P = .04, respectively) (Fig 1). Because obesity would be expected to contribute to increased CRP levels, we performed analysis of covariance with relative BMI as a covariate. Log CRP levels were found to be associated with SDB independently of relative BMI (P < .0001). Furthermore, when multiple linear regression was performed for predicting log CRP levels with AHI, relative BMI, age, and gender as covariates, AHI accounted for 26% of the variance, with relative BMI providing an additional 7% of the variance (adjusted r2 = P < .0001).

Significant correlations were found between log CRP levels and AHI for the whole group (r = .53; P < .0001) (Fig 2A), as well as between log CRP levels and arousal index (r = .28; P < .01) (Fig 2B). In contrast, a significant negative correlation was found between the SP02 nadir and log CRP levels (r = –.47; P < .0001) (Fig 2C). These correlations persisted after exclusion of outliers (r = .50 and P < .0001 for AHI; r = .24 and P = .03 for arousal index; and r = –.45 and P < .0001 for SP02 nadir). No relationship was found between other parameters of sleep disruption and log CRP levels. These findings suggest that both the severity of respiratory disturbance and that of sleep fragmentation may contribute to plasma CRP levels among snoring children.

Subsequent chart review identified a significantly higher frequency of excessive daytime sleepiness and learning problems among the children with elevated log CRP levels (defined as more than −0.52, corresponding to a laboratory value of >0.3 mg/dL), compared with children with log CRP levels of less

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TABLE 1. Demographic and Polysomnographic Characteristics of 81 Children With AHI < 1 (Control), AHI ≥ 1 and <5 (mild SDB), or AHI ≥ 5 (SDB).

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 15) (AHI &lt; 1)</th>
<th>Mild SDB (n = 34) (1 ≤ AHI &lt; 5)</th>
<th>SDB (n = 32) (AHI ≥ 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>10.1 ± 2.9 (5–15)</td>
<td>8.9 ± 3.6 (3–17)</td>
<td>9.4 ± 4.1 (3–18)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>10:5</td>
<td>18:16</td>
<td>19:13</td>
</tr>
<tr>
<td>Relative BMI, %</td>
<td>146.8 ± 45.0</td>
<td>147.3 ± 66.5</td>
<td>175.4 ± 60.6</td>
</tr>
<tr>
<td>AHI</td>
<td>0.42 ± 0.28</td>
<td>2.1 ± 1.0*</td>
<td>14.6 ± 14.0†‡</td>
</tr>
<tr>
<td>SPO2 nadir, %</td>
<td>91.0 ± 3.7</td>
<td>88.6 ± 4.7</td>
<td>83.4 ± 7.8‡</td>
</tr>
<tr>
<td>End tidal carbon dioxide (mm Hg)</td>
<td>47.0 ± 7.1</td>
<td>45.8 ± 5.2</td>
<td>51.7 ± 9.1‡</td>
</tr>
<tr>
<td>Arousal index</td>
<td>6.6 ± 2.7</td>
<td>12.0 ± 3.6‡</td>
<td>16.2 ± 7.8‡</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>89.1 ± 7.9</td>
<td>89.9 ± 8.5</td>
<td>91.2 ± 8.4</td>
</tr>
<tr>
<td>Stage 1, %</td>
<td>8.0 ± 6.0</td>
<td>8.8 ± 7.9</td>
<td>9.0 ± 8.5</td>
</tr>
<tr>
<td>Stage 2, %</td>
<td>44.9 ± 8.2</td>
<td>45.5 ± 9.5</td>
<td>46.1 ± 9.0</td>
</tr>
<tr>
<td>Slow wave sleep, %</td>
<td>23.5 ± 6.4</td>
<td>22.4 ± 7.6</td>
<td>22.7 ± 6.6</td>
</tr>
<tr>
<td>REM sleep, %</td>
<td>23.2 ± 6.0</td>
<td>23.3 ± 6.2</td>
<td>22.2 ± 7.1</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>160.1 ± 36.7</td>
<td>172.0 ± 35.1</td>
<td>155.6 ± 35.1</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>99.8 ± 65.4</td>
<td>115.4 ± 90.7</td>
<td>101.8 ± 50.9</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>49.9 ± 16.3</td>
<td>50.9 ± 17.4</td>
<td>48.3 ± 14</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>90.3 ± 34.3</td>
<td>97.5 ± 30.4</td>
<td>86.9 ± 22.3</td>
</tr>
<tr>
<td>Log CRP</td>
<td>−0.8 ± 0.33</td>
<td>−0.91 ± 0.28</td>
<td>−0.51 ± 0.40†‡</td>
</tr>
<tr>
<td>(0.22 ± 0.27 mg/dL)</td>
<td>(0.15 ± 0.12 mg/dL)</td>
<td>(0.46 ± 0.39 mg/dL)</td>
<td></td>
</tr>
</tbody>
</table>

REM indicates rapid eye movement; LDL, low-density lipoprotein.

* P < .05, mild SDB versus control.
†‡ P < .05, SDB versus control.
‡‡ P < .05, SDB versus mild SDB.

Fig 1. Log CRP levels (mean ± SD) for 81 children with AHI of <1 (control), AHI of ≥1 and <5 (mild SDB), or AHI of ≥5 (SDB). Log CRP levels in the SDB group differed from those in the mild SDB and control groups (P < .0001 and P = .04, respectively).

than −0.52, corresponding to <0.3 mg/dL (94% vs 62%; P = .015) (Fig 3). Furthermore, for any given AHI or arousal index for which children with and without learning difficulties/sleepiness were represented, all children with elevated CRP levels except 1 reported the latter problems.

A significant positive correlation was found between log CRP levels and triglyceride levels (r = .37; P = .01). A negative correlation was found between log CRP levels and HDL levels (r = -.48; P < .0001). Multiple regression analysis confirmed AHI as the major contributing factor for elevated CRP levels (26%), with lesser contributions by relative BMI and HDL.

**DISCUSSION**

This study shows that some children with SDB have elevated plasma CRP levels and that CRP levels are correlated significantly with AHI and arousal index and correlated inversely with the SPO2 nadir, suggesting that inflammatory processes are triggered by SDB among children. Furthermore, an increased prevalence of learning problems and excessive daytime sleepiness was present among children with elevated CRP levels, even with matching for AHI or arousal index. Taken together, these findings suggest that the inflammatory processes previously shown to be elicited by SDB in a rodent model13 may also be operative among children with SDB and could play a role in the pathogenesis of the behavioral and learning deficits associated with this otherwise highly prevalent condition.

CRP, an acute-phase reaction protein, is synthesized in the liver, and its expression is regulated by cytokines.34 CRP levels are usually stable during a 24-hour period35; the levels not only reflect the intensity of the inflammatory response but also have been shown to provide a reliable estimate of the risk of atherogenesis. Indeed, several large-scale, prospective, epidemiologic studies have shown that plasma levels of CRP are a strong independent predictor of risk for cardiovascular morbidity.15,16 Furthermore, although CRP is a nonspecific marker of inflammation, recent epidemiologic studies suggested that CRP may participate directly in atheromatous lesion formation through reduction of nitric oxide synthesis and induction of the expression of particular adhesion molecules in endothelial cells.36 In the present study, we observed higher plasma CRP levels among children with SDB, and levels were correlated with the severity of SDB. This association suggests that SDB elicits the activation of inflammatory processes and that the latter may be responsible in part for the morbidity associated with the disease. Our findings agree with those of a previous study of adult patients with SDB, in which similar correlations were found between the severity...
of disease and the degree of CRP level increases. We also found that CRP levels were correlated with the arousal index, indicating that elevated CRP levels may result from both the intermittent hypoxemia and the sleep fragmentation associated with SDB. Indeed, SDB-induced hypoxic stress has been shown to modulate levels of circulating inflammatory mediators and could lead to endothelial dysfunction through induction of cellular adhesion molecules in response to inflammatory cytokines. Plasma levels of tumor necrosis factor-α and interleukin-6 were significantly increased among patients with SDB. Elevated levels of circulating adhesion molecules such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin were also reported and correlated with the severity of SDB.

What are the potential clinical implications of these findings? The strong epidemiologic links between CRP levels and atherogenesis may indicate that the coincidence of SDB with higher CRP levels may increase the risk of atheroma formation among such children, thus promoting the development of cardiovascular morbidity later in life. CRP elevations in the context of SDB not only may provide a marker of the magnitude of the inflammatory response to SDB but also may be indicative of particular populations at increased risk for the development of long-term cardiovascular complications, which might be partially triggered or accelerated by the SDB disor-

Fig 2. A, Scatterplot of log CRP levels plotted against AHI for 81 children. B, Scatterplot of log CRP levels plotted against arousal index for 81 children. C, Scatterplot of log CRP levels plotted against SpO2 nadir for 81 children. Linear regression lines are shown and were all statistically significant (see text for details).

Fig 3. A, Scatterplot of log CRP levels plotted against AHI for 81 children. B, Scatterplot of log CRP levels plotted against arousal index for 81 children. Children with excessive daytime sleepiness and/or learning problems are indicated with open circles, whereas children without excessive daytime sleepiness and/or learning problems are indicated with closed circles. The line represents the logarithmically transformed value of 0.3 mg/dL (~0.52), which was considered the cutoff value for elevated CRP levels.
der itself. Although these speculations obviously require prospective confirmatory studies that incorporate consideration of other factors, such as a family history of SDB or cardiovascular disease, it should be emphasized that the SDB-induced elevations of CRP levels were independent of BMI. This is particularly relevant because obesity in childhood constitutes an additional risk factor for cardiovascular morbidity. In addition, CRP levels were found to be independently associated with the independent cardiovascular risk factor of insulin resistance, supporting the potential association between inflammation, insulin resistance, and atherogenesis. The potential end-organ morbidity induced by SDB through an inflammation-mediated process is intriguingly supported by the significant differences in the frequency of excessive sleepiness and learning problems among children with elevated CRP levels, compared with children with similar AHI or arousal index values who did not develop increased CRP concentrations. Of note, the findings of sleepiness and learning problems were based on subjective parental reports, which is an obvious limitation of the current study. However, objective correlates of excessive sleepiness in children, ie, the multiple sleep latency test, are very insensitive among children. Although these findings need to be corroborated with more appropriate and objective methods, rather than using parental reports alone, they raise the possibility that the magnitude of the inflammatory response may underlie components of neurocognitive and vigilance deficits associated with SDB.

No significant differences in serum lipid profiles emerged among the various SDB groups, indicating that specific alterations in lipid regulation do not seem to occur as a function of SDB severity. However, the negative correlation between HDL levels and plasma CRP levels and the positive correlation between triglyceride levels and CRP levels are in close agreement with previous reports on adults, suggesting that SDB may play a role in the regulation of HDL and triglycerides.

CONCLUSIONS

SDB among children is associated with increased plasma CRP levels, and the latter appear more frequently among children presenting with symptoms of excessive daytime sleepiness and/or learning deficits. Additional studies are needed to examine the short-term and long-term consequences of the inflammatory responses induced by SDB and their reversibility with treatment.

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