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ORIGINAL ARTICLE

# Role of cytokines in children with obstructive sleep apnea

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***Abstract:***

*To explore the inflammatory and anti-inflammatory factors in children with and without obstructive sleep apnea (OSA) and observe the effects of these factors after treatment in children with OSA. Methods: A total of 142 children were enrolled in this study. Based on overnight polysomnographic evaluation and physical examination, they were divided into two groups; OSA group (47) and control group (95). OSA was diagnosed if obstructive apnea index (OAI) >1. According to apnea-hypoapnea index (AHI), OSA children were further divided into mild (AHI≥1 and≤5), moderate (AHI>5 and≤10), and severe (AHI>10) groups. A blood sample was collected for analysis of interleukin 6 (IL-6), intracellular adhesion molecule 1 (ICAM-1), and interleukin 10 (IL-10). Results: Serum levels of IL-6 and ICAM-1 were significantly higher and serum level of IL-10 was significantly lower in OSA group compared to control group. Of 47 OSA children, 43 received treatment (38 underwent adenotonsillectomy (T&A); 4 received inhaled nasal corticosteroid; and 1 received noninvasive positive pressure ventilation (NIPPV); 7 lost during follow-ups, remaining 36 were followed up after three months. There was substantial decrease in IL-6 and ICAM-1 and increase in IL-10 after treatment (P<0.01). The difference was statistically significant (P<0.05), independent of age, gender, and body mass index (BMI). Conclusion: Up-regulation of pro-inflammatory cytokines have been well established in childhood OSA, but down-regulation of anti-inflammatory cytokine IL-10 and its up-regulation following treatment is a new finding; all of which in turn could promote the onset and progression of atherogenesis in children. As this inflammatory response is reversible, early recognition and treatment of OSA children would be beneficial in decreasing the risks.*

***Keywords***: *Obstructive sleep apnea, cytokines, interleukins*

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## INTRODUCTION

Obstructive sleep apnea (OSA) is a sleep disorder characterized by repeated episodes of upper airway occlusion during sleep that is associated with daytime behavioral changes and abnormalities in cardiovascular function. This may be due to rise in pro-inflammatory processes occurring with the microvasculature leading to sympathetic activation and endothelial dysfunction [1,2,4,8]. OSA has been recognized as the important and single most risk factors for cardiovascular disease in adults. Circulating pro-inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor α (TNF- α), intercellular adhesion molecule 1 (ICAM-1), high sensitivity C-reactive protein (hs-CRP) and E-Selectin have been shown to be elevated in adult subjects with OSA independent of obesity [3, 21]. It has been confirmed that OSA also contribute to childhood cardiovascular morbidity

with an estimation as high as 10.3% [5,6,9,11,12].

A few studies have investigated the associations between circulating inflammatory cytokines concentration and childhood OSA. It is possible that the mechanisms linking OSA to cardiovascular morbidity in childhood not only involve pro-inflammatory processes but also involve anti-inflammatory processes. It is possible that with up-regulation of pro-inflammatory cytokine, such as IL-6, there might have been down regulation of anti-inflammatory cytokine IL-10. IL-10 is a pleiotropic cytokine produced by Th2-type T cells, B cells, monocytes, and macrophages that inhibits a broad array of pro-inflammatory immune responses including those of the vessel wall. IL-10 has been shown to play a key role in atherosclerosis in mice. To verify this, mice that lack IL-10 and fed on atherosclerotic diet were found to have early atherosclerotic vascular injury compared to those with systemic excessive IL-10 gene expression. Based on this, we hypothesized that subjects with OSA not only up-regulate the pro-inflammatory cytokines (IL-6, IL-8, TNF-α, etc.) but also will down-regulate anti-inflammatory factor such as IL-10 resulting in cardiovascular diseases [12,13,16,17,29,30].

We assumed that like adults, the children have same type of mechanisms. We therefore aimed to evaluate the associations of childhood OSA and circulating concentrations of the pro-inflammatory cytokines (IL-6, ICAM-1) and anti-inflammatory cytokine IL-10 and assess the effects of OSA treatment on their levels. Gozal et al. also found that there was increase in the level of pro-inflammatory cytokine IL-6 and decrease in anti-inflammatory cytokine IL-10 in children with OSA and returned to control levels after treatment [27]. To support that finding, we aim at exploring the pro-inflammatory and anti-inflammatory cytokines levels and study the impact to these factors after treatment in children with OSA in our hospital setting.

**Methods & Materials**

Children admitted with habitual snoring problems in the First Affiliated Hospital of Xinjiang Medical University between September 2011 and November 2012 was enrolled in this study. Children with history of hypertension, diabetes mellitus, neuromuscular disorders, genetic disorders, acute upper respiratory tract infection, infectious diseases, autoimmune diseases (rheumatic fever, rheumatoid arthritis), on drugs (aspirin, ibuprofen, hormones, antibiotics), and history of adenotonsillectomy were not included in the study. The study was approved by the Clinical Research Ethics Committee of the Xinjiang Medical University and informed consent was obtained from the parents at the beginning of the assessment. The weight and standing height of the children were measured with a calibrated weight scale and stadiometer by standard anthropometric methods. Body mass index (BMI) was calculated as weight in kilogram divided by height in meter squared ($kg/m^2$).

An overnight polysomnographic (PSG) study was performed on each subject using Siesta ProFusion II PSG monitor (Compumedics Telemed PTY Ltd., Abbotsford, Australia). Obstructive apnea (OA) was defined as absence of airflow with persistent respiratory effort lasting longer than two baseline breaths, irrespective of Sao2 changes. Obstructive apnea index (OAI) was defined as the number of OAs per hour of sleep. Central apnea (CA) was defined as absence of respiratory effort associated with absence of airflow those of greater than 20 seconds with or without oxygen desaturation or arousals. Hypoapnea was defined as a reduction of 50% or more in the amplitude of the airflow signal. Apnea hypoapnea index

(AHI) was defined as the total number of apneic and hypoapneic episode per hour of sleep. Arousal was defined as an abrupt shift in electroencephalography (EEG) frequency during sleep, which may include theta, alpha and/or frequencies greater than 16Hz but not spindles with 3 to 15 seconds in duration. In rapid eye movement (REM) sleep, arousals were scored only when accompanied by concurrent increases in submental electromyography (EMG) amplitude. We defined OSA as OAI≥1.0 episode per hour of sleep. Children who had an OAI value <1.0 episode per hour of sleep were grouped as control. All subjects had fasting blood samples taken following overnight PSG. Serum IL-6, IL-10, and ICAM were measured by enzyme-linked immunosorbent assay (ELISA) (BioSource International Inc., Camarillo, California, USA). The sensitivity and inter-assay coefficients of variation of IL-6, IL-10, and ICAM were <0.1 pg/ml, 7.8%; 0.2 pg/ml, <10%; and 1.6 ng/ml, 7.8%, respectively.

Children identified to have OSA were offered adenotonsillectomy after assessment by an ENT surgeon, while others without surgical indications, and mild OSA were treated with nocturnal non-invasive positive pressure ventilation (NIPPV) and /or nasal corticosteroid therapy. Follow-up was made three months post treatment, where the children underwent second overnight polysomnographic evaluation and morning blood draw.

We used PEMS 3.1 for processing and analysis. Data were presented as mean ± standard deviation. Paired student t-test was used to compare pre and post treatment findings. Analysis of variance (ANOVA) and test independent t-test were used for comparison of PSG, IL-6, and IL-10 concentrations. All p values were two tailed with statistical significance set at <0.05.

**Results**

A total of 142 children were enrolled in this study. These included 95 control children who did not snore and had normal polysomnographic study and 47 children with polysomnographically demonstrated OSA. According to apnea-hypoapnea index (AHI), OSA children were further divided into mild (AHI≥1 and≤5), moderate (AHI>5 and≤10), and severe (AHI>10) groups.

Table 1 shows demographic characteristics of 47 OSA children with 95 matched control group. No statistical difference was exhibited in terms of age, gender, and BMI (P>0.05). Levels of serum IL-6 and ICAM-1 were significantly higher, while IL-10 were lower in OSA children compared to control group (P<0.01).
Table 2 shows polysomnographic characteristics of 47 OSA children with 95 matched control group. The difference is statistically significant (P<0.05).

Table 3 shows correlation between levels of IL-6, IL-10, and ICAM-1 with different indices. IL-6 and ICAM-1 levels were positively correlated with AHI and AI (P<0.05), negatively correlated with LSaO2 (P<0.01), and no correlation with BMI (P>0.05). IL-10 level was negatively correlated with AHI (P<0.05), positively correlated with LSaO2 (P<0.01), and no correlation with BMI and AI (P>0.05).

Table 4 shows plasma levels of IL-6, IL-10, and ICAM-1 and PSG measurement before and after treatment in 36 OSA children. Serum IL-6 and ICAM-1 levels decreased and serum IL-10 level increased after treatment. The difference was statistically significant (P<0.05). AHI and AI decreased after treatment, while LSaO2 increased, both with statistical significance (P<0.01).

Figure shows the graphic presentation of serum plasma levels of IL-10 (a), IL-6 (b), and (ICAM)-1(C) in 47 OSA children in terms of severity. Among 47 OSA children, 43 received treatment, of which 38 underwent T&A, 4 received inhaled nasal corticosteroid therapy, 1 treated with NIPPV, and 7 children were lost during follow-up. Remaining 36 children were followed up three months post treatment.

**Table 1. Demographic Characteristics of 47 OSA Children and 95 Matched Control Children**

| | OSA Group (n=47) | Control Group(n=95) | p |
|---|---|---|---|
| **Age(Years)** | 7.0±1.4 | 7.3±1.2 | **ns** |
| **Gender(M/F)** | 22/25 | 58/37 | **ns** |
| **BMI (Kg/m2)** | 17.33±2.75 | 18.30±3.97 | **ns** |
| **IL-6 (pg/mL)** | 2.98±0.27 | 1.67±0.07 | **<0.01** |
| **(ICAM)-1(ng/mL)** | 391.7 ±115.6 | 189.8 ±106.4 | **<0.01** |
| **IL-10 (pg/mL)** | 195.2±33.6 | 458.5±102.2 | **<0.01** |

**Table 2.Polysomnographic Characteristics in 47 OSA Children and 95 Matched Control Children**

| | OSA Group (n=47) | Control Group(n=95) | p |
|---|---|---|---|
| **AHI (Frequency/h)** | 13.3±2.8 | 0.0±0.0 | **<0.0001** |
| **AI (Frequency/h)** | 4.4±0.9 | 0.0±0.0 | **<0.0001** |
| **Lowest SpO2 (%)** | 77.9±1.9 | 94.1±0.3 | **<0.0001** |
| **Arousal Index（ArI）** | 8.0±4.9 | 4±2.1 | **<0.01** |

**Table 3. Relationship Between the Levels of IL-6, IL-10, ICAM-1 with Different Indices in OSA Children**

| | IL-6 (pg/mL) | | IL-10 (pg/mL) | | (ICAM)-1 (ng/mL) | |
|---|---|---|---|---|---|---|
| | R | p | R | p | R | p |
| **Age** | -0.005 | NS | -0.088 | NS | -0.047 | NS |
| **AHI** | 0.341 | **<0.001** | -0.266 | **<0.05** | 0.28 | **<0.05** |
| **Weight** | 0.002 | NS | -0.98 | NS | -0.870 | NS |
| **BMI** | -0.121 | NS | -0.183 | NS | -0.167 | NS |
| **Lowest SaO2** | -0.19 | **<0.05** | 0.24 | **<0.05** | -0.21 | **<0.05** |
| **ArI** | 0.27 | **<0.05** | -0.159 | NS | 0.35 | **<0.001** |

**Table 4: Serum Plasma Levels of (IL-6, IL-10, ICAM-1) and PSG Measurements Before and After Treatment in 36 OSA Children**

| | Before Treatment | After Treatment | P Value |
|---|---|---|---|
| **BMI** | 18.33±3.75 | 17.86±3.50 | NS |
| **AHI (Frequency/h)** | 12.33±4.4 | 1.2±1.5 | **<0.01** |
| **ArI** | 9±3.8 | 3±1.9 | **<0.01** |
| **Lowest SaO2** | 78.8±2.4 | 88.2±2.3 | **<0.01** |
| **IL-6 (pg/mL)** | 3.08±0.3 | 2.02±0.13 | **<0.01** |
| **IL-10 (pg/mL)** | 187.2±29.63 | 476.3±86.12 | **<0.01** |
| **(ICAM)-1(ng/mL)** | 29.7±105.2 | 44.4±95.2 | **<0.01** |

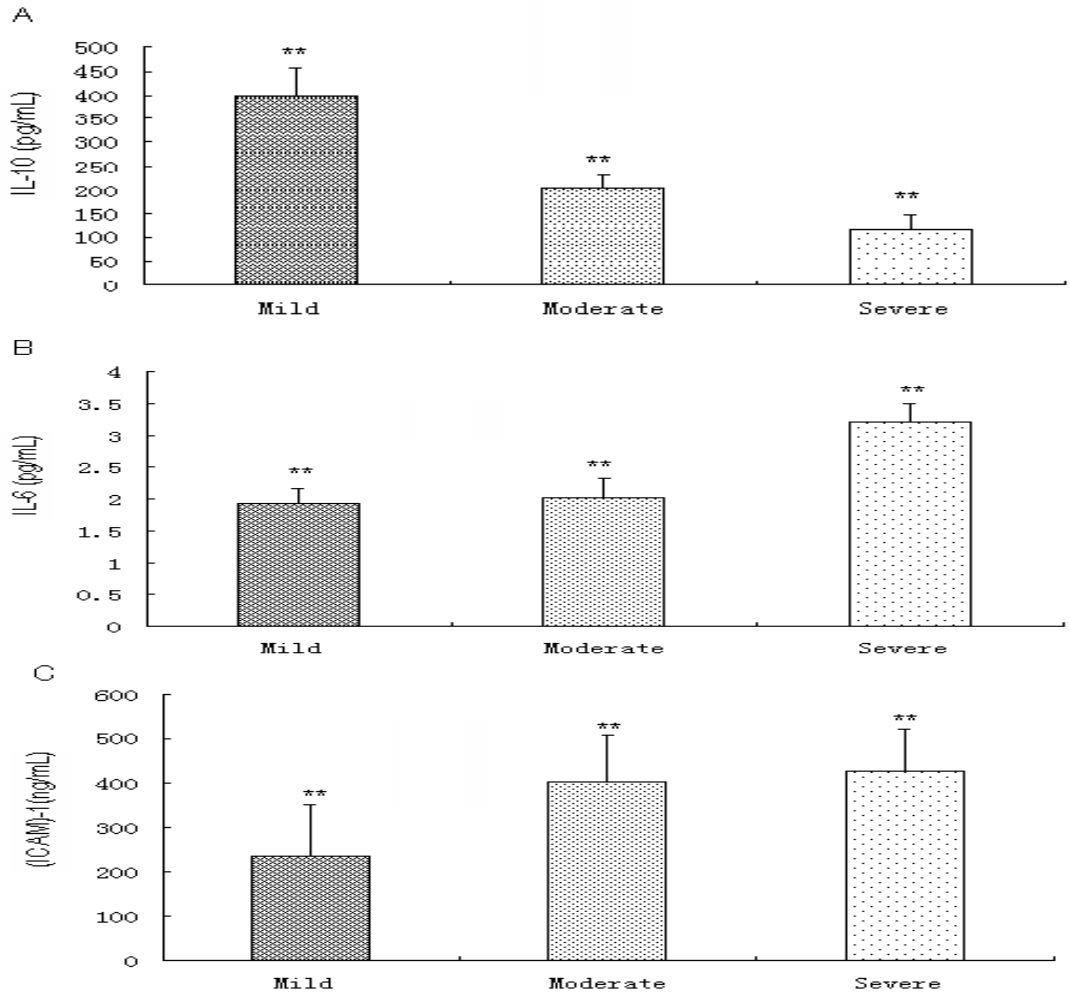


**Figure 1. Serum plasma levels of IL-10 (a), IL-6 (b), and (ICAM)-1(C) in OSA children in terms of severity.**

## Discussion

This study further confirms and supports the findings carried out by Gozal et al. that levels of IL-6 are higher and IL-10 are lower in children with OSA[27]. Our study also showed that levels of IL-6 and ICAM-1 were increased and IL-10 decreased in OSA children when compared to control group. Furthermore, there was substantial decrease in the serum levels of IL-6 and ICAM-1 and increase in IL-10 following intervention, where majority of children with OSA underwent T&A. Up-regulation of IL-10 level post treatment is also a positive finding.

Our results further support that systemic inflammation is a constitutive component and consequence of OSA in children, even in the absence of obesity and is reversible in most children upon treatment, thereby decreasing the risk of further atherosclerosis and cardiovascular disease [27]. However, the limitations of this study are; we only initially performed measurement of the IL-6, ICAM-1, and IL-10 levels for the control group and did not compare with the children with OSA post treatment. Although it is likely the treatment did result in the decrease in the pro-inflammatory cytokine response, it is not possible to exclude other factors that could have included this response. Secondly, like cytokine levels, we also did not compare the PSG values of control group with that of OSA children post treatment because of

substantial difficulties in recruiting normal healthy children without symptoms of OSA for blood taking and PSG. Lastly, the indication of T&A was truly based on the decision taken by ENT surgeon, and we did not mention the size, shape, and location of the tonsils and adenoids before T&A, an issue that may have clinical significance and needs further investigations.

## Conclusion

This study supports the hypothesis that OSA is an independent risk factor for systemic inflammation via up-regulation of pro-inflammatory cytokines and down-regulation of anti-inflammatory cytokines, all of which in turn could promote the onset and progression of atherogenesis in children. As this inflammatory response is reversible, early recognition and treatment of OSA children would be beneficial in decreasing the risks.

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