

## Research Article

# Relationship between Plasma Homocysteine and Obstructive Sleep Apneas in Children: A Preliminary Study

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### Abstract

**Objectives:** Plasma homocysteine concentration is increased adult patient with obstructive sleep apneas, whilst it is still unknown if its plasma levels may also be enhanced in obstructive sleep apnea in childhood. We investigated the possible relationship between plasma homocysteine and obstructive sleep apnea in children.

**Methods:** A total number of 199 children were studied between July 2016 and June 2017. All children underwent an overnight respiratory polygraph study. Laboratory data were retrospectively reviewed, including plasma homocysteine, folate and vitamin B12.

**Results:** Twenty children were finally included in our study. Linear regression analysis showed that plasma homocysteine was positively correlated with age ( $\beta=2.930$ ;  $p=0.010$ ), and inversely correlated with folate ( $\beta=-4.830$ ;  $p<0.001$ ), vitamin B12 ( $\beta=-3.844$ ;  $p=0.002$ ) and haemoglobin ( $\beta=-3.503$ ;  $p=0.003$ ). No significant correlation was found with platelet count ( $p=0.578$ ) and apnea-hypopnea index ( $p=0.272$ ). After age adjustment, homocysteine levels remained inversely correlated with folate, but no association was found with sleep respiratory parameters.

**Conclusion:** This retrospective study suggests that serum homocysteine levels is not increased in children with obstructive sleep apnea, whilst, a folate-enriched diet seems advisable in children with obstructive sleep apnea to counteract oxidative stress. Further controlled studies are needed.

**Keywords:** homocysteine; obstructive sleep apnea; overnight respiratory polygraph; sleep disordered breathing

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Obstructive sleep apnea (OSA) is characterized by recurrent partial or complete airflow interruption and by oxygen desaturation during the sleep.<sup>[1,2]</sup> The diagnosis of obstructive sleep apnea syndrome (OSAS) is usually performed with polysomnography.<sup>[3,4]</sup> In children, hyperplasia of tonsils and adenoids<sup>[5]</sup> along with obesity<sup>[3]</sup> and craniofacial disharmony<sup>[6,7]</sup> are important causes of high airway narrowing. Notably, oxidative stress increased blood pressure and arterial stiffness are the most severe complications of OSAS.<sup>[8,9]</sup>

Homocysteine (Hcy), sulphur-containing amino acid, is an

essential metabolite of the homocysteine-methionine cycle, which mediates methylation and plays a crucial role in maintaining the biochemical balance. The leading causes of increased Hcy include nutritional deficiencies (low folate in the diet) and genetic disorders, especially involving a mutation in the methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (*MTRR*) genes. In particular, plasma Hcy concentration is inversely correlated with dietary intake of folic acid and with the serum levels of vitamins B6 and B12.<sup>[10,11]</sup>

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The impaired intake of folic acid, and the ensuing low concentration of folate in serum are frequently associated with systemic complications, which may also include abnormal DNA methylation.<sup>[12]</sup> A high serum concentration of Hcy is also toxic to the blood vessels wall.<sup>[11,13-15]</sup> Although reliable evidence has been provided that plasma Hcy levels may be lower in children than in the adulthood, a small percentage of adolescents children display increased values of this non-protein amino acid, thus enhancing their risk of future cardiovascular events.<sup>[16]</sup>

Since an increased serum concentration of Hcy contributes to enhancing the oxidative stress in children with OSAS, this preliminary study was aimed to explore the association between plasma Hcy concentration and OSAS, as well as their possible dietary management.

## Methods

### Study Population

A total number of 199 children underwent overnight polygraph study aimed to investigate the sleep disordered breathing (SDB) in our Department, between September 2016 and August 2017. All children have been referred from a dedicated outpatient facility belonging to a large paediatric pneumology service. Children with neurological, skeletal and muscular disorders were excluded (n. 31). The results of laboratory tests were retrospectively reviewed to collect data about haemoglobin, platelet count, as well as the plasma concentrations of folate, Hcy and vitamin B12. Hyperhomocysteinemia was defined as a plasma Hcy concentration >10 µmol/L.<sup>[17]</sup>

The present study was based on pre-existing clinical data. The study was performed in accordance with the Declaration of Helsinki (<http://www.wma.net/en/30publications/10policies/b3/index.html>) and under the terms of relevant local legislation.

### Overnight Respiratory Polygraphy

The overnight respiratory polygraph study (SOMNO screen™PSG, SOMNOmedics GmbH, Randersacker, Germany) was based on continuous monitoring of nasal airflow, chest and abdominal respiratory movements (thoracic and abdominal belts), arterial oxygen saturation (SaO<sub>2</sub>; digital pulse oximetry), heart rate (HR; finger probe), electrocardiogram (ECG; three chest electrodes), body position (mercury sensor) and tracheal sounds (microphone).<sup>[8]</sup> This method has been extensively described elsewhere.<sup>[18]</sup> The sleep analyses (DOMINO® software, Somnomedics v.2.6.0) of valid recording session was manually performed. Obstructive respiratory events were scored as previously reported.<sup>[19-21]</sup>

## Statistical Analysis

Statistical analysis was performed using SPSS Statistics 22.0® software for Windows. The demographic, clinical characteristics and sleep respiratory polygraphic results were shown as mean, standard deviation (SD) and minimum/maximum values. A linear regression analysis was carried out to explore the association between plasma Hcy (entered as the dependent variable) and the laboratory (haemoglobin, platelet count, plasma folate, vitamin B12), demographic (age) and nocturnal polygraphic data (AHI, ODI, and minimum SpO<sub>2</sub>). A further correlation analysis was performed between laboratory (plasma Hcy, folate and vitamin B12) and polygraphic data (AHI, ODI, and minimum SpO<sub>2</sub>), with adjustment for the age of the children. The value of statistical significance was set at p<0.05.

## Results

Overall, complete records (i.e., entailing both laboratory and polygraphic data) were available for 20/199 children (13 females and 7 males; mean age, 5.7±3.5 years). Demographic (sex, age), clinical (body growth) and overnight polygraph data (AHI, ODI, minimum SpO<sub>2</sub>) of our study population are shown in Table 1. The plasma Hcy levels

**Table 1.** Summary of the demographic, clinical characteristics, in-laboratory overnight polygraph study and laboratory findings of the 20 children enrolled in the study

	Mean±SD	Min-max
<b>Demographic and clinical characteristics</b>		
Totals (males, %)	20 (35)	-
Age (years)	5.7±3.5	1.6-12.6
Weight (kg)	21.6±13.1	8-52
Height (cm)	107±25	70-155
BMI (kg/cm <sup>2</sup> )	17.7±4.2	12.9-29.7
<b>Sleep respiratory results</b>		
eTST (h)	9.1±1.2	6-11
AHI (n/h)	5.6±7.6	0-30
ODI (n/h)	4.5±8.6	0-34
Mean SatO <sub>2</sub> (%)	97.5±1.3	94-99
Min SatO <sub>2</sub> (%)	89.4±7.1	69-95
Snoring (%)	2.9±5.2	0-20
<b>Laboratory exams</b>		
Haemoglobin (g/L)	127±14	110-150
Platelets count (µl)	333.800±73.800	245.000-481.000
Homocysteine (µmol/L)	7.2±2.4	2.4-11.3
Vitamin B12 (pmol/L)	493±192	230-1080
Folate (nmol/L)	20.4±10.4	6-42.3

AHI: Apnea hypopnea index; BMI: Body mass index; eTST: Estimated total sleep time; ODI: Oxygen desaturation index; SD: Standard deviation; SatO<sub>2</sub>: Peripheral oxygen saturation.

were <10 µmol/L in 17 children (age range, 1.6-12.6 years), whilst plasma Hcy levels were >10 µmol/L in the remaining 3 children.

The linear regression analysis, in which plasma Hcy concentration was entered as a dependent variable and age, folate, vitamin B12, haemoglobin, platelet and AHI (or ODI or minimum SatO<sub>2</sub>) were entered as independent variables showed that plasma Hcy concentration was positively correlated with age (β=2.930; p=0.010), and inversely correlated with folate (β=-4.830; p<0.001), vitamin B12 (β=-3.844; p=0.002) and haemoglobin (β=-3.503; p=0.003). No correlation was found between plasma Hcy levels and platelets (p=0.578), AHI (p=0.272), ODI (p=0.333) (Fig. 1) or minimum SatO<sub>2</sub> (p=0.704).

When the linear correlation analysis was adjusted for the age of the children (Table 2), no significant association was found between laboratory (Hcy, folate, vitamin B12, haemoglobin, and platelet) and sleep respiratory polygraphic parameters (AHI, ODI, minimum SpO<sub>2</sub>) except between plasma Hcy and folate (-0.559; p=0.013). Notably, no significant correlation could also be observed between plasma Hct and vitamin B12 (-0.412; p=0.080).

**Discussion**

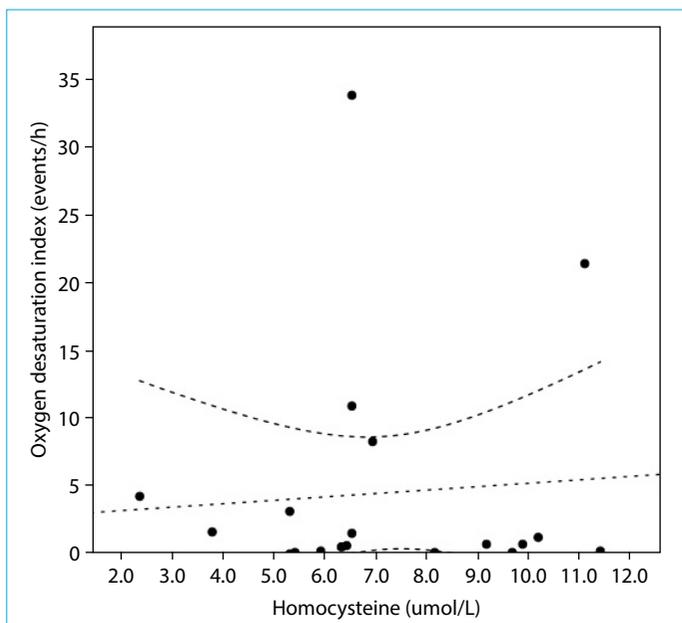
Oxidative stress and systemic inflammation are important causes of vascular endothelial dysfunction. In particular, oxidative stress in patients with OSA was proven to be an essential player of impaired endothelial function in the paediatric age.<sup>[22]</sup> High plasma Hcy levels have been

frequently associated with endothelial dysfunction in the adulthood,<sup>[23]</sup> thus promoting or even directly triggering the generation of highly toxic compounds (e.g., cysteine adducts) and oxidative stress.<sup>[24]</sup> Folate deficiency, often resulting from insufficient intake of vegetables, leads to vitamin B12 deficiency<sup>[25]</sup> and compensatory increase of Hcy, both condition currently regarded as significant risk factors for cardiovascular disease.<sup>[17, 26]</sup> Nevertheless, no previous studies have been published on the potential association between plasma Hcy levels and OSAS in children to the best of our knowledge.

Papandreou et al.<sup>[27]</sup> reported that children with plasma Hcy levels above the upper reference limits (>10 micromols/L) were found to be inversely correlated with folate and vitamin B12 levels. In our limited children population, the inverse association between plasma Hcy and both folate and vitamin B12 could be confirmed, whilst an inverse correlation with haemoglobin values could also be demonstrated. Therefore, the rate of hyperhomocysteinemia was found to be much lower than that reported in an adult population of our Country (25% versus 88%; Chi-square test with Yates' correction, p<0.001).<sup>[25]</sup>

In our child population, plasma Hcy concentration was also found to be significantly and positively correlated with age. Notably, Thakre et al.<sup>[28]</sup> recently suggested that the strong positive correlation between age and plasma Hcy levels may disguise a potentially real and independent association between plasma Hcy and with self-reported sleep apnea.

A recent meta-analysis showed that adults with OSA had higher plasma Hcy level than healthy controls, and this evidence was even more straight forward in patients with moderate or severe OSA patients.<sup>[29]</sup> The results of our preliminary do not support the existence of a significant correlation between plasma Hcy (along with folate and vi-



**Figure 1.** Plotted distribution (mean and 95% C.I.) between serum homocysteine levels (µmol/L) and oxygen desaturation index (events/h) in 20 children referred to our ambulatory for sleep disordered breathing.

**Table 2.** Partial correlation analysis (adjusted for age) between blood laboratory exams and polygraphic variables of the 20 children enrolled in the study

	AHI (events/hour)	ODI (events/hour)	SatO <sub>2</sub> min (%)
Haemoglobin (g/L)	-0.009 (0.972)	0.030 (0.907)	0.356 (0.135)
Platelet (count/µl)	-0.215 (0.391)	-0.312 (0.208)	-0.010 (0.968)
Homocysteine (µmol/l)	0.145 (0.552)	0.092 (0.707)	-0.002 (0.993)
Folate (nmol/l)	0.048 (0.846)	0.034 (0.890)	0.053 (0.843)
Vitamin B12 (pmol/l)	0.013 (0.959)	0.059 (0.811)	-0.147 (0.549)

tamin B12) and different degrees of childhood OSA severity, even after adjustment for the age. Therefore, increased plasma Hcy values may only develop later in life in patients with OSAS, or may be limited to a specific subset of patients with long history of OSA.

The present study has many limitations. First, the relatively low sample size may represent a potential bias in the statistical analysis, so that these results should be confirmed in larger children populations. Then, the retrospective design of this study did not allow to perform genetic testing, so that a potential impact of mutations of both *MTHFR* and *MTRR* genes on our findings cannot be ruled out. Finally, definitive information about dietary intake of vegetables and vitamins is also lacking.

In conclusion, this preliminary study shows that plasma Hcy levels are seemingly not increased in childhood SDB. Therefore, it seems reasonable to suggest that Hcy, folate and vitamin B12 status should not be routinely assessed during the paediatric age. Nevertheless, a folate-enriched diet seems still advisable in children with obstructive sleep apnea, in order to counteract oxidative stress, whilst OSAS children reporting an insufficient intake dietary folate should be screened and eventually supplemented. Larger studies in paediatric populations are needed to corroborate these suggestions.

#### Disclosures

**Ethics Committee Approval:** The study was approved by the Local Ethics Committee.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.

**Authorship contributions:** Concept – M.Z., G.P.; Design – M.Z., G.P.; Supervision – M.Z., G.L., G.P.; Materials – L.T., E.G., M.P., L.S.; Data collection &/or processing – L.T., E.G., M.P., L.S.; Analysis and/or interpretation – M.Z., L.T., E.G., M.P., L.S.; Literature search – L.T., E.G., M.P.; Writing – M.Z., G.L., G.P.; Critical review – G.L.

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