

Research Article

Relationship between Plasma Homocysteine and Obstructive Sleep Apneasin Children: A Preliminary Study

Marco Zaffanello,¹ Emma Gasperi,¹ Giuseppe Lippi,² Laura Tenero,¹ Michele Piazza,¹ Luca Sacchetto,³ Giorgio Piacentini¹

¹Department of Surgical Sciences, Dentistry, Gynecology and Pediatrics, Pediatric Division, University of Verona, Verona, Italy

²Department of Biochemistry, University of Verona, Verona, Italy

³Department of Surgical Sciences, Dentistry, Gynecology and Pediatrics, Otorhinolaryngology Section, University of Verona, Verona, Italy

Abstract

Objectives: Plasma homocysteine concentration is increased adult patient swith obstructive sleep apneas, whilst it is still unknown if its plasma levels may also be enhanced in obstructive sleep apneain 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, in clusing plasma homocysteine, folate and vitamin B12.

Results: Twenty children were finally included in ourstudy. 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

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

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 in clude nutritional deficiencies (low folate in the diet) and genetic disorders, especially involving amutation in the methylenetetrahydrofolate reductase (*MTHFR*) and methionine synthase reductase (*MTRR*) genes. In particular, plasma Hcyconcentration is inversely correlated with dietary intake of folic acid and with the serum levels of vitamins B6 and B12.^[10, 11]

Address for correspondence: Marco Zaffanello, MD. Cerrahi Bilimler, Dis Hekimligi, Jinekoloji ve Pediatri Anabilim Dalı, Çocuk Bolumu, Verona Universitesi, Verona, Italy

Phone: +939 045 812 71 21 **E-mail:** marco.zaffanello@univr.it

Submitted Date: April 14, 2018 **Accepted Date:** April 26, 2018 **Available Online Date:** May 04, 2018

©Copyright 2018 by Eurasian Journal of Medicine and Oncology - Available online at www.ejmo.org



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.^[12] A high serum concentration of Hcy is also toxic to the blood vessels wall.^[11,13-15] 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.^[16]

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 $>10 \mu\text{mol/L}$.^[17]

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_2 ; digital pulse oximetry), heart rate (HR; finger probe), electrocardiogram (ECG; three chest electrodes), body position (mercury sensor) and tracheal sounds (microphone).^[8] This method has been extensively described elsewhere.^[18] 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.^[19-21]

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_2). A further correlation analysis was performed between laboratory (plasma Hcy, folate and vitamin B12) and polygraphic data (AHI, ODI, and minimum SpO_2), 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_2) 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
Demographic and clinical characteristics		
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^2)	17.7 ± 4.2	12.9-29.7
Sleep respiratory results		
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_2 (%)	97.5 ± 1.3	94-99
Min SatO_2 (%)	89.4 ± 7.1	69-95
Snoring (%)	2.9 ± 5.2	0-20
Laboratory exams		
Haemoglobin (g/L)	127 ± 14	110-150
Platelets count (μl)	333.800 ± 73.800	245.000-481.000
Homocysteine ($\mu\text{mol/L}$)	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_2 : Peripheral oxygen saturation.

were $<10 \mu\text{mol/L}$ in 17 children (age range, 1.6-12.6 years), whilst plasma Hcy levels were $>10 \mu\text{mol/L}$ 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_2) were entered as independent variables showed that plasma Hcy concentration 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 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_2 ($p=0.704$).

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_2) 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.^[22] High plasma Hcy levels have been

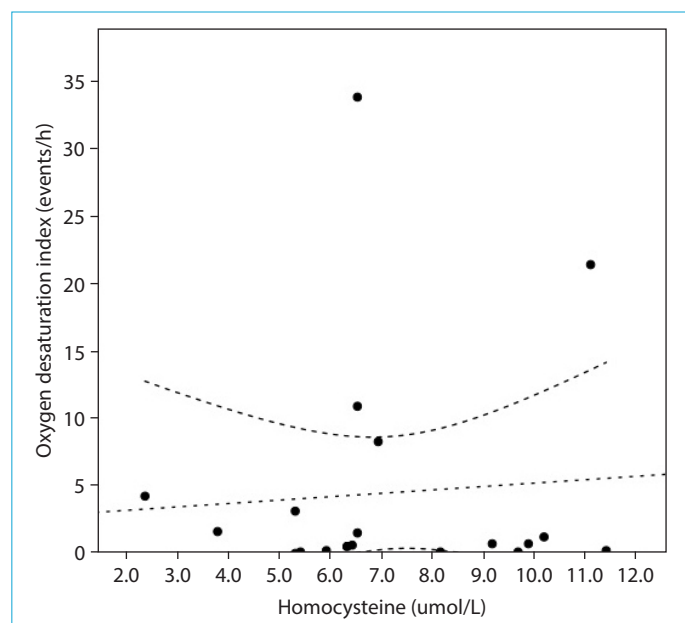


Figure 1. Plotted distribution (mean and 95% C.I.) between serum homocysteine levels ($\mu\text{mol/L}$) and oxygen desaturation index (events/h) in 20 children referred to our ambulatory for sleep disordered breathing.

frequently associated with endothelial dysfunction in the adulthood,^[23] thus promoting or even directly triggering the generation of highly toxic compounds (e.g., cysteine adducts) and oxidative stress.^[24] Folate deficiency, often resulting from insufficient intake of vegetables, leads to vitamin B12 deficiency^[25] and compensatory increase of Hcy, both condition currently regarded as significant risk factors for cardiovascular disease.^[17, 26] 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.^[27] reported that children with plasma Hcy levels above the upper reference limits ($>10 \mu\text{mol/L}$) 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, $p<0.001$).^[25]

In our child population, plasma Hcy concentration was also found to be significantly and positively correlated with age. Notably, Thakre et al.^[28] 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.^[29] The results of our preliminary do not support the existence of a significant correlation between plasma Hcy (along with folate and vi-

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 ₂ 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 ($\mu\text{mol/l}$)	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.

References

1. Wilhelm CP, deShazo RD, Tamanna S, Ullah MI, Skipworth LB. The nose, upper airway, and obstructive sleep apnea. *Ann Allergy Asthma Immunol* 2015;115:96–102. [CrossRef]
2. Zaffanello M, Piacentini G, Gasperi E, Maffei C, Fava C, Bonafini S, et al. Snoring in a cohort of obese children: Association with palate position and nocturnal desaturations. *J Pediatr Neonat Individual Med* 2016;5:e050134.
3. Zaffanello M, Piacentini G, Pietrobelli A, Fava C, Lippi G, Maffei C, et al. Ambulatory clinical parameters and sleep respiratory events in a group of obese children unselected for respiratory problems. *World J Pediatr* 2017;13:577–83. [CrossRef]
4. Aurora RN, Zak RS, Karipott A, Lamm CI, Morgenthaler TI, Auerbach SH, et al; American Academy of Sleep Medicine. Practice parameters for the respiratory indications for polysomnography in children. *Sleep* 2011;34:379–88. [CrossRef]
5. Grime C, Tan HL. Sleep Disordered Breathing in Children. *Indian J Pediatr* 2015;82:945–55. [CrossRef]
6. Tawfik KO, Sedaghat AR, Ishman SL. Trends in Inpatient Pediatric Polysomnography for Laryngomalacia and Craniofacial Anomalies. *Ann Otol Rhinol Laryngol* 2016;125:82–9. [CrossRef]
7. Zaffanello M, Cantalupo G, Piacentini G, Gasperi E, Nosetti L, Cavarzere P, et al. Sleep disordered breathing in children with achondroplasia. *World J Pediatr* 2017;13:8–14. [CrossRef]
8. Tagetti A, Bonafini S, Zaffanello M, Benetti MV, Vedove FD, Gasperi E, et al. Sleep-disordered breathing is associated with blood pressure and carotid arterial stiffness in obese children. *J Hypertens* 2017;35:125–31. [CrossRef]
9. Kontos A, van den Heuvel C, Pamula Y, Martin J, Lushington K, Baumert M, et al. Delayed brachial artery dilation response and increased resting blood flow velocity in young children with mild sleep-disordered breathing. *Sleep Med* 2015;16:1451–6.
10. Goyette P, Sumner JS, Milos R, Duncan AM, Rosenblatt DS, Matthews RG, et al. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994;7:195–200. [CrossRef]
11. Liew SC, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. *Eur J Med Genet* 2015;58:1–10.
12. Weiner AS, Boyarskikh UA, Voronina EN, Mishukova OV, Filipenko ML. Methylenetetrahydrofolate reductase C677T and methionine synthase A2756G polymorphisms influence on leukocyte genomic DNA methylation level. *Gene* 2014;533:168–72. [CrossRef]
13. Cacciapuoti F. Lowering homocysteine levels with folic acid and B-vitamins do not reduce early atherosclerosis, but could interfere with cognitive decline and Alzheimer's disease. *J Thromb Thrombolysis* 2013;36:258–62. [CrossRef]
14. Xie Y, Feng H, Peng S, Xiao J, Zhang J. Association of plasma homocysteine, vitamin B12 and folate levels with cognitive function in Parkinson's disease: A meta-analysis. *Neurosci Lett* 2017;636:190–5. [CrossRef]
15. Hainsworth AH, Yeo NE, Weekman EM, Wilcock DM. Homocysteine, hyperhomocysteinemia and vascular contributions to cognitive impairment and dementia (VCID). *Biochim Biophys Acta* 2016;1862:1008–17. [CrossRef]
16. Osganian SK, Stampfer MJ, Spiegelman D, Rimm E, Cutler JA, Feldman HA, et al. Distribution of and factors associated with serum homocysteine levels in children: Child and Adolescent Trial for Cardiovascular Health. *JAMA* 1999;281:1189–96. [CrossRef]
17. Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM. Total homocysteine and cardiovascular disease. *J Intern Med* 1999;246:425–54. [CrossRef]
18. Zaffanello M, Gasperi E, Tenero L, Piazza M, Pietrobelli A, Sacchetto L, et al. Sleep-Disordered Breathing in Children with

- Recurrent Wheeze/Asthma: A Single Centre Study. *Children (Basel)* 2017;4:pii:E97.
19. Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, et al; American Academy of Sleep Medicine. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 2012;8:597–619.
 20. Villa MP, Pietropaoli N, Supino MC, Vitelli O, Rabasco J, Evangelisti M, et al. Diagnosis of Pediatric Obstructive Sleep Apnea Syndrome in Settings With Limited Resources. *JAMA Otolaryngol Head Neck Surg* 2015;141:990–6. [\[CrossRef\]](#)
 21. Ross KR, Rosen CL. Sleep and respiratory physiology in children. *Clin Chest Med* 2014;35:457–67. [\[CrossRef\]](#)
 22. Loffredo L, Zicari AM, Occasi F, Perri L, Carnevale R, Angelico F, et al. Endothelial dysfunction and oxidative stress in children with sleep disordered breathing: role of NADPH oxidase. *Atherosclerosis* 2015;240:222–7. [\[CrossRef\]](#)
 23. Grochowski C, Litak J, Kamieniak P, Maciejewski R. Oxidative stress in cerebral small vessel disease. Role of reactive species. *Free Radic Res* 2017;1–13.
 24. Chambers JC, McGregor A, Jean-Marie J, Obeid OA, Kooner JS. Demonstration of rapid onset vascular endothelial dysfunction after hyperhomocysteinemia: an effect reversible with vitamin C therapy. *Circulation* 1999;99:1156–60. [\[CrossRef\]](#)
 25. Zappacosta B, Persichilli S, Iacoviello L, Di Castelnuovo A, Graziano M, Gervasoni J, et al. Folate, vitamin B12 and homocysteine status in an Italian blood donor population. *Nutr Metab Cardiovasc Dis* 2013;23:473–80. [\[CrossRef\]](#)
 26. Klee GG. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B(12) and folate. *Clin Chem* 2000;46:1277–83.
 27. Papandreou D, Mavromichalis I, Makedou A, Rousso I, Arvanitidou M. Total serum homocysteine, folate and vitamin B12 in a Greek school age population. *Clin Nutr* 2006;25:797–802.
 28. Thakre TP, Mamtani M, Ujaoney S, Kulkarni H. Association of plasma homocysteine with self-reported sleep apnea is confounded by age: results from the national health and nutrition examination survey 2005-2006. *Sleep Disord* 2012;2012:634920. [\[CrossRef\]](#)
 29. Li K, Zhang J, Qin Y, Wei YX. Association between Serum Homocysteine Level and Obstructive Sleep Apnea: A Meta-Analysis. *Biomed Res Int* 2017;2017:7234528. [\[CrossRef\]](#)