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IRON METABOLISM DYSREGULATION AND ATHEROSCLEROTIC CHANGES IN OBSTRUCTIVE SLEEP APNOEA

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Abstract

The Syndrome of obstructive sleep apnoea (OSA) is associated with an increased risk of arterial hypertension, metabolic syndrome, diabetes mellitus type 2, brain-vascular damages and atherosclerosis.

The aim of this study is to assess the connection between iron metabolism dysregulation in OSA and atherosclerotic changes of carotid arteries.

Thirty-five OSA patients with brain-vascular atherosclerotic evidences were included in this study; changes were evaluated by carotid artery intima-media thickness (IMT) and flow-mediated dilatation (FMD). Their results were compared to an equal number of healthy volunteers. Haematological and biochemical assays were performed in all participants in the study.

Serum hepcidin levels are statistically significantly increased in OSA patients with atherosclerotic a. carotis changes (101.9 ± 10.1 µg/L) compared to the control group (18.5 ± 2.5 µg/L); $P < 0.001$. Serum homocysteine levels are significantly higher in OSA than in healthy people. Average IMT (1.22 ± 0.19) and ABI (1.71 ± 0.14) values in OSA patients with atherosclerotic a. carotis changes are increased in comparison to the control group (0.34 ± 0.07, 1.11 ± 0.06,

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resp.); $P < 0.001$. Serum vitamin B12 levels were lower in OSA patients with atherosclerotic changes in carotid arteries (71.7 ± 9.9 pmol/L) compared to the controls (449.7 ± 21.4 pmol/L); $P < 0.001$.

A connection between iron homeostasis and atherosclerotic changes was established during this study. Increased hepcidin and homocysteine concentrations might be connected to an elevated risk of cardio- and brain-vascular diseases in OSA. The use of these markers in the routine practice may be very useful for early atherosclerosis assessment in OSA.

Key words: hepcidin, atherosclerosis, obstructive sleep apnoea, homocysteine, vitamin B12

Introduction. Syndrome of obstructive sleep apnoea (OSA) is represented by symptoms caused by intermittent restriction and/or total obstruction of airflow through the upper respiratory tract during sleep. Epidemiologically increased morbidity from OSA is established [1]. Main OSA symptoms are daily drowsiness, disrupted concentration and motivation, difficulties in routine tasks performances, irritability, decreased libido, chronic fatigue, inadequate rest, restless sleep, snoring, and nocturia. During desaturation episodes, the organism is a subject of chronic stress. These changes lead to an increased frequency of occurrence of arterial hypertension, metabolic syndrome, diabetes mellitus type 2, and increased risk of brain-vascular damages [2,3]. High-sensitivity-CRP (hsCRP) is a relevant marker for inflammation in obstructive sleep apnoea patients [4].

Hepcidin-25 is an amino-peptide that regulates iron homeostasis through its duodenal absorption [5]. Hepcidin's main function is performed by its interaction to the only known intracellular iron exporter, named ferroportin [6]. Increased hepcidin concentrations might be a risk of cardio-vascular disorders through iron mobilization in macrophages; iron turns them into atherogenic [7]. Different studies show the role of hepcidin in atherogenesis in dialysis patients [8].

Homocysteine is exclusively formed from methionine (Met) through the transmethylation pathway [9]. Elevated homocysteine is an independent, modifiable risk factor for atherosclerotic cardio-vascular diseases [10,11].

The aim of our study is to assess the connection between iron metabolism dysregulation in OSA and atherosclerotic changes of carotid arteries.

Materials and methods. Thirty-five OSA patients with brain-vascular atherosclerotic evidences were included in this study. The average age of the included OSA patients was 41.3 ± 5.8 years. The average age for the control group was similar – 42.1 ± 6.1 years. The study sets on the ethical principles of the Helsinki declaration and was approved by the review board and the local ethics committee of Medical University – Sofia, Bulgaria. The atherosclerotic changes were evaluated by intima-media thickness (IMT) of a. carotis and flow-mediated dilatation (FMD), representing main part of non-invasive measurements of atherosclerosis (NIMA). Their results were compared to an equal number of healthy volunteers without OSA and atherosclerotic changes in carotid arter-

ies. Complete blood count (CBC), erythrocyte indices (MCV, MCH, MCHC), serum iron and total iron-binding capacity (TIBC), serum transferrin, ferritin and hepcidin were quantified for iron metabolism evaluation. Homocysteine, hsCRP, vitamin B12, liver enzymes, Lactate dehydrogenase (LDH), fasting glucose and creatinine were measured in included OSA patients and the control group. For hematological, biochemical and immunological parameters analyzers by Siemens Healthineers Diagnostics were used. Hepcidin was quantified by previously validated ELISA method. During statistical analysis established results were presented as average value ± SD for parametric distribution. Pearson's correlation and paired t-test were used for the evaluation of significance and correlation between values. A level of $P < 0.05$ was considered statistically significant.

Results. Table 1 presents serum hepcidin concentration in the included groups – patients with OSA and atherosclerotic changes in carotid arteries and healthy volunteers.

Serum hepcidin levels are statistically significantly increased in OSA patients with atherosclerotic changes in carotid arteries compared to the control group; $P < 0.001$.

T a b l e 1

Benchmarking of serum hepcidin concentration in OSA patients with atherosclerotic changes in carotid arteries (ATH) and healthy controls expressed as average value (in µg/L) and standard deviation

	n	$\overline{x}$	SD
OSA with ATH	35	101.9	10.1
Healthy controls	35	18.5	2.5

T a b l e 2

Benchmarking of serum biochemical parameters, IMT and ABI in OSA patients with atherosclerotic changes in carotid arteries (ATH) and healthy controls expressed as average value and standard deviation

Group parameter	Controls	OSA with ATH	P
Iron (µmol/l)	17.7 ± 2.2	32.7 ± 8.5	$P < 0.001$
TIBC (µmol/l)	60.3 ± 8.1	64.1 ± 4.4	$P < 0.5$
TRSF (g/l)	2.1 ± 0.3	3.1 ± 0.4	$P < 0.01$
Ferritin (ng/ml)	117.4 ± 5.9	279.4 ± 25.7	$P < 0.001$
hsCRP (mg/l)	0.9 ± 0.3	14.7 ± 1.3	$P < 0.001$
Homocysteine (µmol/l)	1.1 ± 0.3	29.1 ± 3.6	$P < 0.001$
Vitamin B12 (pmol/l)	449.7 ± 21.4	71.7 ± 9.9	$P < 0.001$
IMT	0.34 ± 0.07	1.22 ± 0.19	$P < 0.001$
ABI	1.11 ± 0.06	1.71 ± 0.14	$P < 0.01$

TIBC – total iron-binding capacity; TRSF – transferrin; hsCRP – high sensitive C-reactive protein; IMT – intima-media thickness, ABI – ankle-brachial index

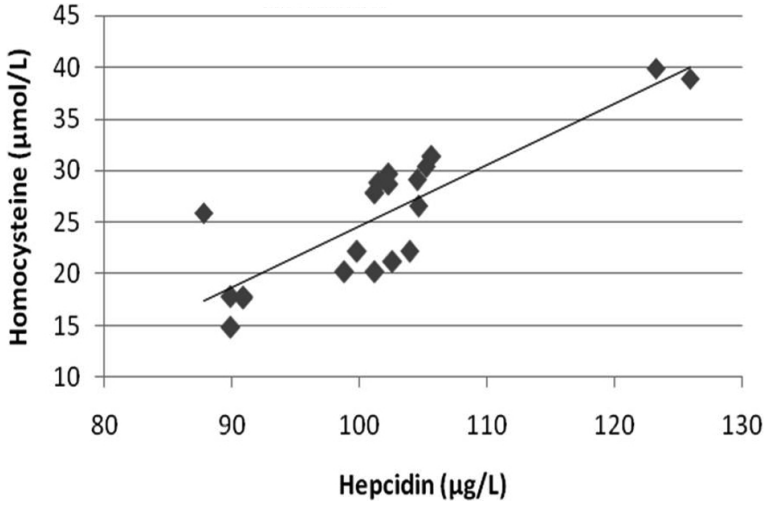

Fig. 1

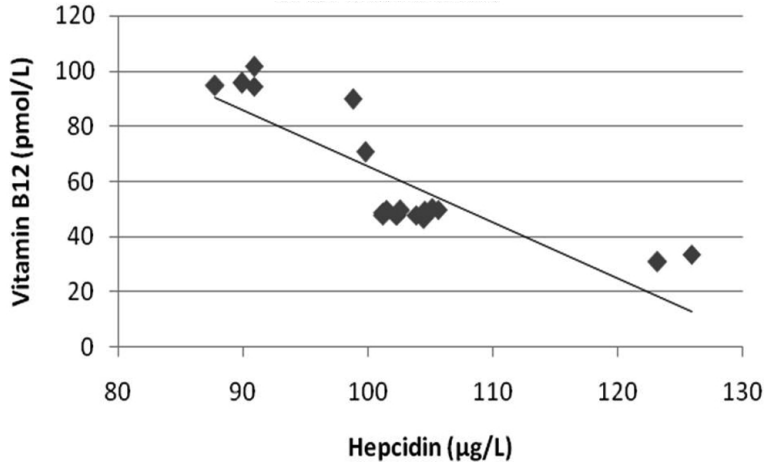

Fig. 2.

Table 2 presents evaluated biochemical parameters, IMT and ABI in OSA patients with atherosclerotic changes in carotid arteries and the control group.

There is a positive correlation between serum hepcidin levels and homocysteine in OSA patients with atherosclerotic changes in carotid arteries – level of correlation $r = 0.849$; significance $P < 0.001$ (Fig. 1).

A statistically significant negative relationship between serum hepcidin levels and vitamin B12 is observed in OSA patients with atherosclerotic changes in carotid arteries – level of correlation $r = -0.844$; significance $P < 0.001$ (Fig. 2).

A strongly positive correlation is found between serum hepcidin levels and IMT in OSA patients with atherosclerotic changes in carotid arteries – level of correlation $r = 0.904$; significance $P < 0.001$.

There is a positive correlation between serum hepcidin levels and ABI in OSA patients with atherosclerotic changes in carotid arteries as well ($r = 0.922$; $P < 0.001$).

Discussion. During desaturation episodes, the organism is a subject of chronic stress, which leads to decreased nitric oxide (NO) levels, increased interleukin-6 and tumour necrotic factor-α secretion. These changes lead to insulin resistance, arterial hypertension, metabolic syndrome, and others diseases occurrence; the risk of brain-vascular damages increases [2,3].

hcCRP is a relevant marker for inflammation in OSA patients and our study proved it once again [4]. Meta-analysis of 30 studies assessing the relationship between CRP and OSA confirmed that CRP was higher in OSA patients compared to the controls (pooled mean difference 1.77) [12]. In the study of Shamsuzzaman et al. [4], 22 otherwise healthy OSA patients and 20 subjects matched for age and body mass index (BMI) without OSA were included. CRP levels were significantly higher among the former (0.33 vs. 0.09 mg/dL, $P < 0.0003$), and they were independently associated with disease severity. We established increased concentrations of hsCRP in OSA patients with atherosclerotic changes in carotid arteries compared to control group ($P < 0.001$).

Several studies have reported that elevated plasma levels of total homocysteine are related to an increased risk of cardiovascular disease [13–15]. In our study increased level of serum homocysteine was established in OSA patients with atherosclerotic changes in carotid arteries compared to control group; 1.1 µmol/l vs. 29.1 µmol/l; $P < 0.001$.

Vitamin B12 deficiency may increase the risk of carotid atherosclerosis by elevating total homocysteine [16,17]. Serum vitamin B12 levels were decreased in OSA patients with atherosclerotic changes in carotid arteries compared to the controls (449.7 ± 21.4 pmol/l vs. 71.7 ± 9.9 pmol/l; $P < 0.001$).

In our study NIMA parameters – IMT and ABI were also increased in OSA patients in comparison to healthy volunteers; $P < 0.001$. Similar results were obtained by Szabóová et al. [18]. They found that IMT (max) was increased in subjects with mild to moderate OSA alone (AHI = 20.4 ± 8.7/h) vs. healthy controls (0.83 ± 0.14 mm vs. 0.63 ± 0.08 mm, $P < 0.01$). Other authors reported similar results [19].

Evaluation of hepcidin is still a novelty in our routine practice although some studies show the role of hepcidin in atherogenesis in OSA patients [20]. Our findings contribute to the clarification of hepcidin's role in atherosclerotic changes in obstructive sleep apnoea patients, and might be helpful in right therapeutic choice of accompanying metabolic distortions. We established increased serum hepcidin levels in OSA patients with atherosclerotic changes in carotid arteries ($P < 0.001$). The connection between hepcidin concentration and hepcidin/ferritin ratio with presence of atherosclerotic plaques and ABI, confirms hepcidin role in atherosclerotic process. In our study we found a significant positive correlation between

serum hepcidin levels and ABI and IMT in OSA patients with atherosclerotic changes in carotid arteries ($P < 0.01$ and $P < 0.001$).

Conclusions. A connection between iron homeostasis and atherosclerotic changes in OSA was established during this study. Increased hepcidin and homocysteine concentrations might be connected to an elevated risk of cardio- and brain-vascular diseases occurrence in OSA patients. The use of these markers in the routine practice may be very useful for early atherosclerosis assessment in OSA.

REFERENCES

[1] Grigg-Damberger M. M. (2021) The AASM scoring manual four years later, J. Clin. Sleep Med., **8**, 323–332, https://doi.org/10.5664/jcsm.1928.

[2] Lavie L. (2003) Obstructive sleep apnoea syndrome – an oxidative stress disorder, Sleep Med. Rev., **7**, 35—51, https://doi.org/10.1053/smrv.2002.0261.

[3] Lévy P., M. Kohler, W. T. McNicholas, F. Barbé, R. D. McEvoy et al. (2015) Obstructive sleep apnoea syndrome, Nat. Rev. Dis. Primers, **25**(1), 15015, https://doi.org/10.1038/nrdp.2015.15.

[4] Shamsuzzaman A. S. M., M. Winnicki, P. Lanfranchi, R. Wolk, T. Kara et al. (2002) Elevated C-reactive protein in patients with obstructive sleep apnea, Circulation, **105**(21), 2462–2464, https://doi.org/10.1161/01.CIR.0000018948.95175.03.

[5] Knutson M. D., M. Oukka, L. M. Koss, F. Aydemir, M. Wessling-Resnick (2005) Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin, Proc. Natl. Acad. Sci. USA, **102**, 1324—1328, https://doi.org/10.1073/pnas.0409409102.

[6] Nemeth E., T. Ganz (2006) Regulation of iron metabolism by hepcidin, Annu. Rev. Nutr., **26**, 323—342, https://doi.org/10.1146/annurev.nutr.26.061505.111303.

[7] Kuragano T., K. Itoh, Y. Shimonaka, A. Kida, M. Furuta et al. (2011) Hepcidin as well as TNF-α are significant predictors of arterial stiffness in patients on maintenance hemodialysis, Nephrol. Dial. Transplant., **26**, 2663–2667, https://doi.org/10.1093/ndt/gfq760.

[8] Saeed O., F. Otsuka, R. Polavarapu, V. Karmali, D. Weiss et al. (2012) Pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis, Arterioscler. Thromb. Vasc. Biol., **32**, 299–307, https://doi.org/10.1161/ATVBAHA.111.240101.

[9] Finkelstein J. D. (1990) Methionine metabolism in mammals, J. Nutr. Biochem., **1**, 228–237, https://doi.org/10.1016/0955-2863(90)90070-2.

[10] Nygård O., J. E. Nordrehaug, H. Refsum, P. M. Ueland, M. Farstad et al. (1997) Plasma homocysteine levels and mortality in patients with coronary artery disease, N. Engl. J. Med., **337**, 230–236, https://doi.org/10.1056/NEJM199707243370403.

[11] Baggott J. E., T. Tamura (2015) Homocysteine, iron and cardiovascular disease: a hypothesis, Nutrients, **7**(2), 1108–1118, https://doi.org/10.3390/nu7021108.

[12] Nadeem R., J. Molnar, E. M. Madbouly, M. Nida, S. Aggarwal et al. (2013) Serum inflammatory markers in obstructive sleep apnea: a meta-analysis, J. Clin. Sleep Med., **9**(10), 1003–1012, https://doi.org/10.5664/jcsm.3070.
[13] Özkan Y., E. Özkan, B. Imşek (2002) Plasma total homocysteine and cysteine levels as cardiovascular risk factors in coronary heart disease, Int. J. Cardiol., **82**(3), 269–277, https://doi.org/10.1016/S0167-5273(02)00010-4.
[14] Van den Brandhof W. E., K. Haks, E. G. Schouten, P. Verhoef (2001) The relation between plasma cysteine, plasma homocysteine and coronary atherosclerosis, Atherosclerosis, **157**(2), 403—409, https://doi.org/10.1016/s0021-9150(00)00724-3.
[15] Rehman T., M. A. Shabbir, M. Inam-Ur-Raheem, M. F. Manzoor, N. Ahmad et al. (2020) Cysteine and homocysteine as biomarker of various diseases, Food Sci. Nutr., **8**(9), 1–12, https://doi.org/10.1002/fsn3.1818.
[16] Robertson J., F. Iemolo, S. P. Stabler, R. H. Allen, J. D. Spence (2005) Vitamin B12, homocysteine and carotid plaque in the era of folic acid fortification of enriched cereal grain products, CMAJ, **172**, 1569—1573, https://doi.org/10.1503/cmaj.045055.
[17] Ji X., X. Y. Leng, Y. Dong, Y. H. Ma, W. Xu et al. (2019) Modifiable risk factors for carotid atherosclerosis: a meta-analysis and systematic review, Ann. Transl. Med., **7**(22), 632, https://doi.org/10.21037/atm.2019.10.115.
[18] Szabóová E., Z. Tomori, V. Doničˇ, J. Petrovičová, P. Szabó (2007) Sleep apnoea inducing hypoxemia is associated with early signs of carotid atherosclerosis in males, Resp. Physiol. Neurobiol., **155**(2), 121–127, https://doi.org/10.1016/j.resp.2006.05.004.
[19] Zhou M., B. Guo, Y. Wang, D. Yan, C. Lin et al. (2017) The association between obstructive sleep apnea and carotid intima-media thickness: A systematic review and meta-analysis, Angiology, **68**(7), 575–583, https://doi.org/10.1177/0003319716665985.
[20] Archontogeorgis K., E. Nena, N. Papanas, P. Steiropoulos (2014) Biomarkers to improve diagnosis and monitoring of obstructive sleep apnea syndrome: current status and future perspectives, Pulm. Med., 930535, https://doi.org/10.1155/2014/930535.

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