The role of systemic inflammation and dyslipidaemia in the pathogenesis of obstructive sleep apnoea

PhD thesis

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1. Introduction

Obstructive sleep apnoea (OSA) is frequently associated with abnormal lipid profiles, including elevated total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) levels and decreased high-density lipoprotein cholesterol (HDL-C) levels. Intermittent hypoxia (IH), sleep fragmentation, oxidative stress and consequential systemic inflammation have been considered as the main factors mediating OSA-associated metabolic dysfunction. Increased peripheral lipolysis, decreased lipoprotein clearance, and increased lipid production of the liver were postulated to lead dyslipidaemia in OSA. Other important mechanisms, such as genetic factors also have role in the OSA-associated metabolic dysfunction. There are several biomarkers which have anti-inflammatory and antioxidant properties and play role in lipid metabolism, suggesting their role in the OSA-associated metabolic dysfunction. High-molecular weight hyaluronic acid (HMW-HA) is an important glycosaminoglycan which is degraded by hyaluronidase-1 (HYAL-1) under hypoxic and inflammatory conditions. Soluble LDL-receptor related protein-1 (sLRP-1) has also anti-inflammatory effects and plays role in the clearance of apoE-containing lipoproteins. However, its ligand calreticulin (CALR) acts as a pro-inflammatory molecule. Atherogenic Index of Plasma (AIP) has been also considered as a biomarker of lipid alterations and atherosclerosis by showing dysregulation between anti- and pro-atherogenic lipoproteins. The hereby presented research was dedicated to investigate the role of previously not studied circulating biomarkers, genetic factors and AIP in the pathomechanism of OSA and its lipid alterations.

2. Objectives

1. To investigate the plasma levels of HMW-HA and HYAL-1 in patients with OSA.

2. To investigate the plasma levels of sLRP-1 and CALR in patients with OSA.

3. To investigate the heritability of the relationship between OSA and serum lipid levels.

4. To investigate the AIP values in patients with OSA in relation to the disease severity.

3. Methods

3.1. Study subjects

In total, 882 volunteers participated in the four studies.

Participants with suspected OSA (i.e., symptoms of snoring, witnessed apnoea, daytime sleepiness, obesity or cardiometabolic comorbidities) were recruited at the Department of Pulmonology, Semmelweis University, Budapest, Hungary and University of Medicine and Pharmacy Timisoara, Timisoara, Romania. OSA was diagnosed as apnoea-hypopnoea index (AHI) >5/h according to the International Classification of Sleep Disorders (Third Edition) criteria. None of the subjects was diagnosed with OSA before and they have not been treated with OSA therapies. The monozygotic and dizygotic twin participants were recruited from the Hungarian Twin Registry. Participants with, acute infection, autoimmune disorders, or uncontrolled chronic disease (for example acute heart failure, uncontrolled diabetes) were excluded.

3.2. Study design

The relationship between the hyaluronan metabolism and OSA was investigated in 40 healthy subjects and 68 patients with OSA.

To investigate the role of sLRP-1 and CALR in OSA, 30 healthy subjects and 46 volunteers with OSA were included.

Investigating the heritability of the relationship between OSA and serum lipid levels, we included 94 monozygotic and 44 dizygotic twin participants. Among them, 58 subjects were diagnosed with OSA.

The relationship between the AIP and OSA was studied in 99 control subjects and 461 patients with OSA.

AIP was calculated as log(TG (mmol/L)/HDL-C (mmol/L)).

All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. The local Ethics Committee approved the study (Semmelweis University, TUKEB 30/2014, RKEB 172/2018; University of Medicine and Pharmacy Victor Babes Timisoara 22/2014/24.07.2019). Each volunteer provided written informed consent.

3.3. Sleep studies

All participants underwent a full-night cardiorespiratory polygraphy (Somnoscreen RC device, Somnomedics GmbH, Germany) or polysomnography (Somnoscreen Plus Tele PSG, Somnomedics GmbH, Germany). The sleep studies were performed according to the American Academy of Sleep Medicine recommendations.

3.4. Biomarker measurements

In the morning following the full night sleep study fasting venous blood was collected for biomarker (HYAL-1 and HMW-HA; sLRP-1 and CALR) levels and lipid profile. Biomarker levels were measured using commercially available ELISA kits.

3.5. Statistical analysis

We used commercially available statistical programs (JASP 0.11.1, Graph Pad Prism 5.0, MedCalc 19.5.3, SOLAR Eclipse version 8.1.1) for statistical analyses. The normality of the data was assessed with Shapiro-Wilk test or Kolmogorov-Smirnov test. We used non-parametric ANCOVA after adjustment on age, gender, BMI, smoking and lipid profile to evaluate the differences in biomarker levels between OSA and control groups as well as among the severity groups. ANCOVA was performed to evaluate

the relationship between lipids and OSA severity. Spearman test was used to compare the biomarker levels with clinical parameters and markers of sleep architecture. Multivariate logistic regression analysis was performed to investigate further potential relationship between biomarker levels and clinical variables as well as between lipid levels and AIP as well as OSA. The predictive value of AIP for OSA, cardiovascular disease, hypertension and diabetes was evaluated with the Receiver operating characteristic (ROC) analysis and compared to lipid levels with the DeLong test. Bivariate co-twin correlation (Pearson's test) was used to calculate a descriptive estimate of the genetic influence in MZ and DZ pairs. A bivariate Cholesky decomposition was calculated to derive the magnitude of covariation between the investigated phenotypes. A p-value <0.05 was considered significant.

4. Results

4.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

Plasma HYAL-1 concentrations were significantly higher (0.59/0.31–0.88/ ng/mL vs. 0.31/0.31–0.58/ ng/mL; p=0.005, *Figure 1a*) and HMW-HA levels were lower (31.63/18.11–59.25/ ng/mL vs. 46.83/25.41–89.95/ ng/mL; p=0.068, *Figure 1b*) in the OSA group compared to the controls after adjustment for age, gender, BMI and smoking. Plasma HMW-HA levels significantly and inversely correlated with AHI (ρ=−0.195, p=0.043). A significant correlation was found between plasma between HYAL-1 levels and AHI (ρ=0.30, p<0.01) and oxygen desaturation index (ODI; ρ=0.26, p<0.01). We observed a significant difference in HYAL-1 concentration (p=0.03), but not in HMW-HA levels (p=0.12) among the severity groups. HYAL-1 levels were directly related to glucose (ρ=0.32; p=0.002), CRP (ρ=0.30; p=0.005) and TG levels (ρ=0.24; p=0.014) and indirectly to HDL-C concentrations (ρ=−0.21; p=0.036).

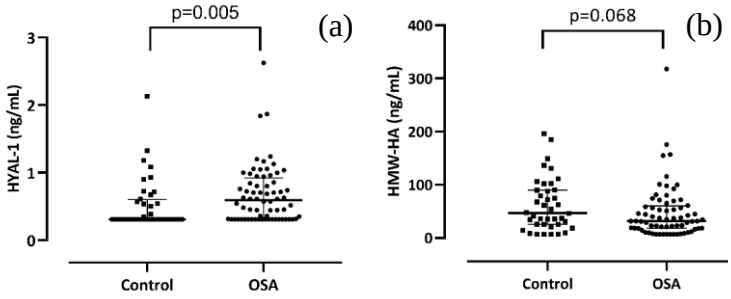

Figure 1. Circulating HYAL-1 (a) and HMW-HA (b) levels between OSA and control groups.

4.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

Plasma sLRP-1 concentrations were significantly lower (1.67 /0.90-2.11/ mg/L vs. 1.99 /1.53-3.51/ mg/L; p=0.04; *Figure 2a*) in the OSA group compared to the controls after adjustment for age, gender, BMI and lipid profile. CALR did not differ significantly between the two groups (0.23 /0.17-0.34/ ng/mL vs. 0.24 /0.20-0.36/ ng/mL p=0.76; *Figure 2b*). There was no difference between the severity groups, neither in sLRP-1 (p=0.15), nor in CALR levels (p=0.44). Plasma sLRP-1 levels significantly and inversely correlated with AHI (ρ=-0.29, p=0.01) and ODI (ρ=-0.23, p=0.04). CALR levels did not correlate with any sleep parameters (all p>0.05). A significant inverse correlation was found between plasma sLRP-1 levels and BMI (ρ=-0.35, p<0.01), LDL-C (ρ=-0.23, p=0.04), TG (ρ=-0.27, p=0.02) and AIP (ρ=-0.27, p=0.02).

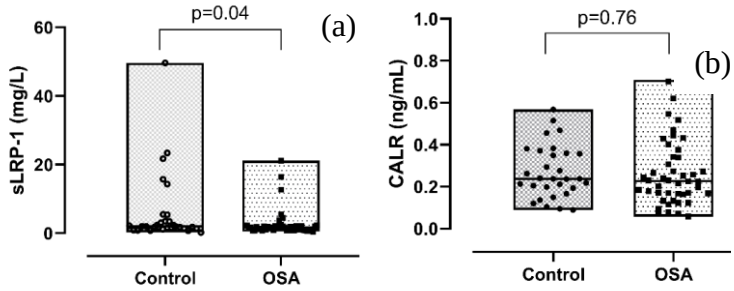

Figure 2. Circulating sLRP-1 (a) and CALR (b) levels between OSA and control groups.

4.3. Heritable factors in the pathomechanism of dyslipidaemia in OSA

There was a significant correlation between AHI, ODI and the percentage of total sleep time spent with saturation below 90% (TST90%), as well as blood pressure, serum

TG, lipoprotein(a) and glucose levels (all $p<0.05$). The bivariate analysis revealed a common genetic background for the correlations between serum TG and ODI ($r=0.63$, $p=0.03$), as well as TST90% ($r=0.58$, $p=0.03$) after adjustment for age, gender and BMI and after exclusion of participants taking antidiabetic and lipid lowering drugs. None of the other correlations were significantly genetically or environmentally determined.

4.4. Atherogenic Index of Plasma in OSA

Higher AIP, lower HDL-C and surprisingly lower TC levels were associated with OSA after adjusting for age, gender and BMI (all $p<0.05$). AIP values significantly correlated with Epworth Sleepiness Scale ($r=0.19$), AHI ($r=0.40$), ODI ($r=0.43$), TST90% ($r=0.36$) and minimal oxygen saturation ($r=-0.28$, all $p<0.05$). AIP was not a better predictor for self-reported cardiovascular disease or diabetes than HDL-C. According to the ROC analysis, HDL-C, TG and AIP were found to be significant predictors (all $p<0.05$). HDL-C was associated with significantly higher area under curve (AUC) than AIP ($p=0.03$) and TG ($p<0.01$). AIP was associated significantly higher AUC than TG ($p<0.01$; *Figure 3*).

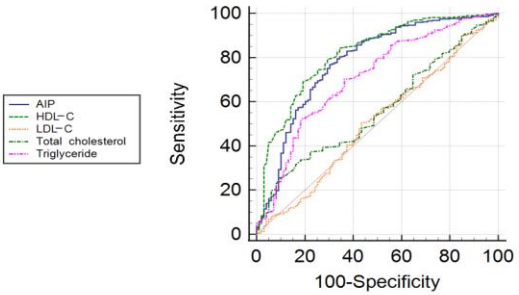

Figure 3. Receiver operating characteristic curve for specific lipid fractions to detect OSA.

5. Conclusions

5.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

- Plasma HYAL-1 levels were higher and plasma HMW-HA levels were lower in the OSA group compared to the healthy subjects.
- There was a direct relationship between HYAL-1 levels and disease severity and markers of nocturnal hypoxaemia.
- There was an inverse relationship between HMW-HA levels and the disease severity.

5.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

- Plasma sLRP-1 levels were lower in the OSA group compared to controls.
- There was an inverse relationship between sLRP-1 levels and lipid parameters, as well as markers of nocturnal hypoxaemia.
- Plasma CALR did not differ between the two groups.

5.3. Heritable factors in the pathomechanism of dyslipidaemia in OSA

- The relationship of OSA and serum TG levels was heritable, suggesting a common genetic background.

5.4. Atherogenic Index of Plasma in OSA

- AIP were higher in the OSA group compared to the controls and related to the disease severity.
- AIP did not have a stronger predictive utility than the other lipid parameters in assessing dyslipidaemia in OSA.

6. Publications of the candidate

6.1. Publications related to the PhD thesis

1. Meszaros M, A. Kis, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2020) *The role of hyaluronic acid and hyaluronidase-1 in obstructive sleep apnoea.* Sci Rep, **10**: 19484.
   Impact factor: 4.380
   Quartile: D1

2. Meszaros M, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2021) *The role of soluble low-density lipoprotein receptor-related protein-1 in obstructive sleep apnoea.* J Clin Med, **10**: 1494.
   Impact factor: 4.964
   Quartile: Q1

3. Meszaros M*, A.D. Tarnoki*, D.L. Tarnoki*, D.T. Kovacs, B. Forgo, J. Lee, J. Sung, J. Vestbo, V. Müller, L. Kunos, and A. Bikov. (2020) *Obstructive sleep apnea and hypertriglyceridaemia share common genetic background: Results of a twin study.* J Sleep Res, **29**: e12979.
   ** Shared first authorship.*
   Impact factor: 3.981
   Quartile: Q1

4. Bikov, A., M. Meszaros, L. Kunos, A.G. Negru, S.M. Frent, and S. Mihaicuta. (2021) *Atherogenic index of plasma in obstructive sleep apnoea.* J Clin Med, **10**: 417.
   Impact factor: 4.964
   Quartile: Q1

Σ Impact factor: 18.289

6.2. Other publications

1. Meszaros, M., P. Horvath, A. Kis, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2021) *Circulating levels of clusterin and complement factor H in patients with obstructive sleep apnea.* Biomark Med, **15**: 323-330.
2. Meszaros, M., T.D. Latshang, S.S. Aeschbacher, F. Huber, D. Flueck, M. Lichtblau, S. Ulrich, E.D. Hasler, P.M. Scheiwiller, L. Reinhard, S. Ulrich, K.E. Bloch, M. Furian, and E.I. Schwarz. (2021) *Effect of nocturnal oxygen on blood pressure response to altitude exposure in COPD - Data from a randomized placebo-controlled cross-over trial.* Int J Chron Obstruct Pulmon Dis, **16**: 3503-3512.
3. Meszaros, M., A.G. Mathioudakis, M. Xanthoudaki, V. Sircu, E. Nena, J. Vestbo, A. Corlateanu, P. Steiropoulos, and A. Bikov. (2021) *The association between beta-blocker therapy and daytime sleepiness in obstructive sleep apnoea.* Sleep Biol Rhythms, **19**: 399-408.
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5. Bikov, A., S.M. Frent, M. Meszaros, L. Kunos, A.G. Mathioudakis, A.G. Negru, L. Gaita, and S. Mihaicuta. (2021) *Triglyceride-glucose index in non-diabetic, non-obese patients with obstructive sleep apnoea.* J Clin Med, **10**: 1932.

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7. Bikov, A., M. Mészáros, and L. Kunos. (2020) *[Characteristics of Hungarian patients with obstructive sleep apnoea].* Orvosi Hetilap, **161**: 2117-2123.
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9. Csoma, B., E. Bárczi, M. Mészáros, L. Büdi, P. Horváth, T. Erdélyi, K. Vincze, Z. Süttő, A. Biró, B. Bombai, M. Vámos, D. Fekete, and V. Müller. (2020) *Tapasztalatok a járvány kezdetén észlelt első COVID-19 betegek ellátásával kapcsolatban a Semmelweis Egyetem Pulmonológiai Klinikán.* Med Thor, **73**: 192-195.
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11. Horváth, P., Z. Lázár, G. Gálffy, R. Puskás, L. Kunos, G. Losonczy, M. Mészáros, Á.D. Tárnoki, D.L. Tárnoki, and A. Bikov. (2020) *Circulating P-selectin glycoprotein ligand 1 and P-selectin levels in obstructive sleep apnea patients.* Lung, **198**: 173-179.
12. Bikov A, Frent S Bikov, A., S. Frent, R. Pleava, L. Kunos, S. Bokhari, M. Meszaros, and S. Mihaicuta. (2020) *The burden of associated comorbidities in patients with obstructive sleep apnea-regional differences in two Central-Eastern European sleep centers.* J Clin Med, **9**: 3583.

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15. Scarlata, S., P. Finamore, M. Meszaros, S. Dragonieri, and A. Bikov. (2020) *The role of electronic noses in phenotyping patients with chronic obstructive pulmonary disease.* Biosensors, **10**: 171.
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18. Bikov, A., Z. Lazar, P. Horvath, D.L. Tarnoki, A.D. Tarnoki, L. Fesus, M. Horvath, M. Meszaros, G. Losonczy, and L. Kunos. (2019) *Association between serum lipid profile and obstructive respiratory events during REM and non-REM sleep.* Lung, **197**: 443-450.
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