

Scientific Letter

Circulating Exosomal MicroRNAs and Subclinical Atherosclerosis in Obstructive Sleep Apnea


To the Director,

Obstructive sleep apnea (OSA) is characterized by periodic collapse of the upper airway during sleep leading to intermittent hypoxia (IH) and arousals from sleep.¹ These events are associated with acute cardiometabolic changes and increased risk of cardiovascular disease and mortality.² Early development of subclinical atherosclerosis (SA) has been associated with OSA.³ SA is defined as the presence of at least one atheroma plaque in the absence of any symptoms, being a risk factor by itself for developing cardiovascular events.⁴ The mechanism by which OSA promotes early onset of SA is unknown.

Exosomes participate in cell-to-cell communication through their cargo, which includes microRNA (miRNA), proteins, or lipids.⁵ Exosomes from children with OSA exhibited an altered miRNA cargo which promotes endothelial dysfunction.⁶ Changes in the exosomal miRNA profile have also been reported in healthy young adults exposed to IH⁷ and we showed that exosome from adults with OSA enhanced endothelial dysfunction *in vitro*.⁸ Here, we compare eleven miRNAs (previously linked to SA, [Table S1](#)) in exosomes from patients with OSA and SA (OSA-SA), without SA and healthy control subjects. Participants were selected from the “Epigenetic Modifications in Obstructive Sleep Apnea Study” (EPIOSA) (NCT014575421), an ongoing long-term longitudinal cohort study at the Hospital Universitario Miguel Servet (Zaragoza, Spain).⁹ We followed current guidelines and regulations, the recommendations of the Declaration of Human Rights and the Conference of Helsinki. The experimental protocols were approved by the Instituto Investigación Sanitaria de Aragón Institutional Review Board (IRB #15/2013). All participants gave written informed consent. Home sleep tests were conducted, and patients diagnosed with OSA received management based on current Spanish national guidelines. The carotid intima-media thickness (CIMT) measurement was performed using a Philips IU22 ultrasound system (Philips Healthcare, USA) with linear high-frequency two-dimensional probes using the Bioimage Study protocol for carotid arteries. SA was defined as the presence of ≥ 1 atheromatous plaque, identified as a focal vessel wall thickness of ≥ 1.5 mm in any examined carotid artery. Fasting blood samples were taken and we followed our described methodology for exosome isolation and miRNA analysis.¹⁰ Assessment of exosome integrity was performed by transmission electron microscopy, nanoparticle tracking analysis and dot blot ([Fig. S1](#)). Relative miRNA expression was determined using the $\Delta\Delta Ct$ method.

At the exploratory study, we included 50 OSA patients (apnea-hypopnea index – AHI ≥ 10 events/hour sleep; e/h) and 16

age- and sex-matched healthy subjects (AHI ≤ 10 e/h) ([Table S2](#)). Three exosomal miRNAs were overexpressed in patients with OSA-SA ([Fig. 1](#)): miR-21-5p (relative expression, RE = 2.91), miR-145-5p (RE = 2.12) and miR-320a-3p (RE = 4.06). We evaluated the predictive potential of SA. The univariate ROC curve analysis displayed significant values for miR-21-5p ($p = 0.0284$), miR-145-5p ($p = 0.0072$) and miR-320a-3p ($p = 0.0001$) but only miR-320a-3p ([Fig. S2](#)) showed potential to discriminate SA in OSA with a value of area under the curve (AUC) of 0.8125 (0.6834–0.9416). miR-320a-3p has been related to atherogenic progression in response to electrophilic stress response induced by oxidized phospholipids,¹¹ which is consistent with its increase in OSA-SA. In heart failure studies, miR-320a-3p was directly linked to an increase in all-cause mortality within 90 days of the event.¹² This finding could be related to the direct consequences of OSA on the cardiovascular system.

For validation, 50 OSA patients, 38 OSA-SA and 24 matched controls were selected. All subjects were also re-evaluated after 1 year, when OSA was divided into CPAP treated ($n = 28$) and those who refused CPAP treatment ($n = 22$). A similar partition was performed in the OSA-SA group, yielding 25 patients with and 13 without CPAP treatment. Blood pressures, ApoB, hsCRP and triglycerides were elevated in both OSA groups. At the 1-year follow-up period OSA patients irrespective of SA status also presented an increase in their CIMT values. Of note, OSA-SA NT patients showed a significant increase in ApoB, total cholesterol and LDL ([Table S3](#)). MiR-320a-3p recapitulated previous findings (RE = 2.66 among OSA-SA, AUC of 0.798) ([Fig. 1](#)). We also fitted miR-320a-3p to the multivariate logistic regression model including AHI and APO B (ROC analysis: AUC = 0.885; $p < 0.0001$).

After 1 year of CPAP treatment, exosomal miR-320a-3p remained elevated in all groups of patients with OSA ([Fig. 2](#)). This was surprising considering that this miRNA has been linked to atherogenic progression through regulation of serum response factor¹³ and response to electrophilic stress.¹¹ To reconcile these a priori contradictory findings, re-evaluation of CIMT at the 1-year follow-up allowed us to assess the predictive value of this miRNA as a marker of the progression of atherosclerosis. We found that higher levels of miR-320a-3p were related to a higher increase in CIMT from baseline ($p = 0.0003$), and therefore a persistent higher cardiovascular risk ([Fig. 2](#)). Consequently, CPAP treatment that effectively corrects intermittent hypoxia, seems not enough to reverse the pathophysiological processes previously activated by the presence of OSA. The temporal changes in miR-320a-3p support the validity of this assumption. Notably, this hypothesis has been partially corroborated by animal studies. In a mouse model of OSA, discontinuing intermittent hypoxia did not eliminate the pro-inflammatory activity of vascular wall macrophages. These cells exhibited epigenetic modifications in gene pathways associated with atherogenesis.¹⁴ Similarly, in a murine model, “adherent

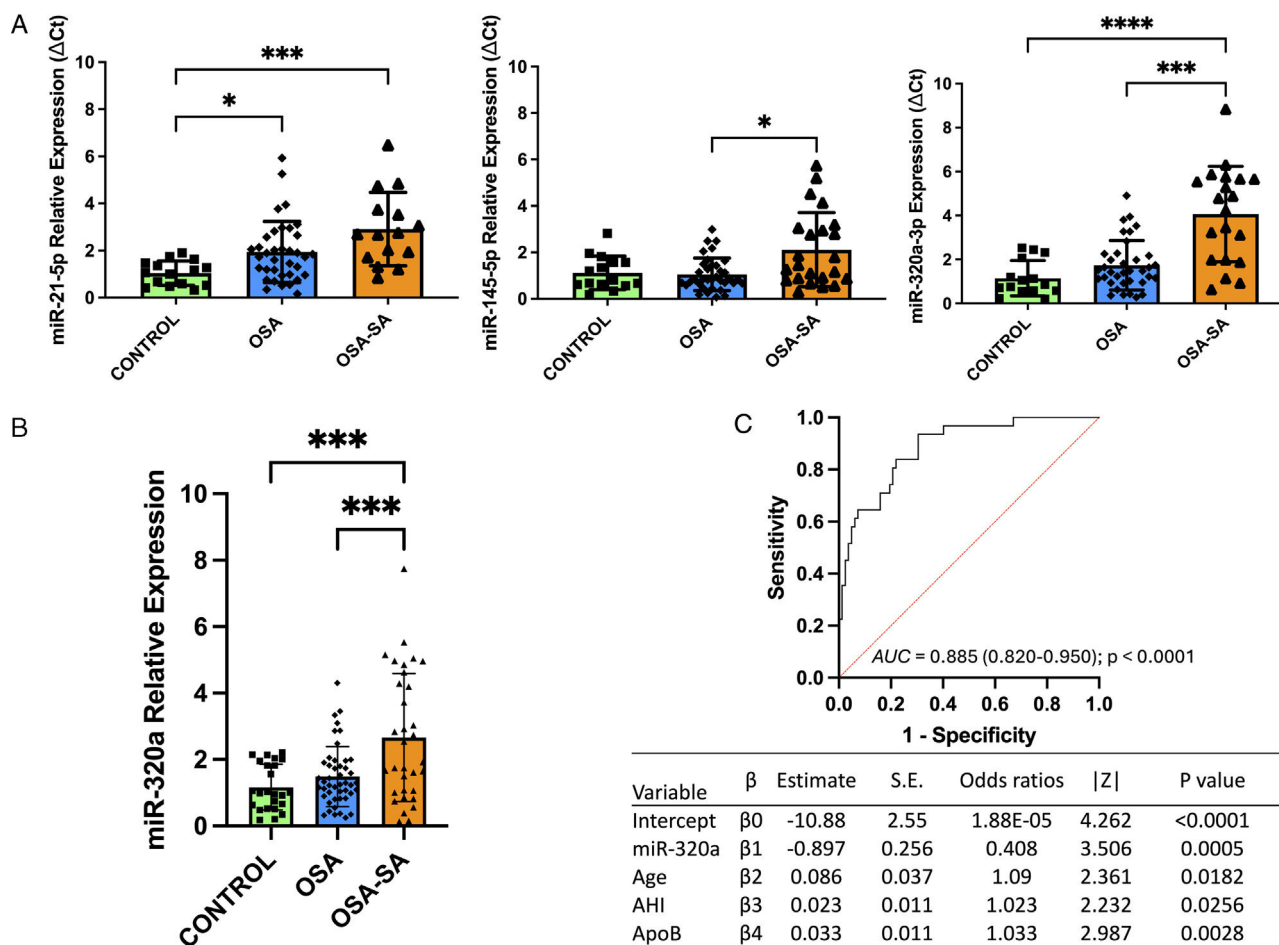


Fig. 1. (A) Relative expression of exosomal miRNAs exhibiting differential expression patterns in the derivation arm of the study. (B) Validation of exosomal miR-320a-3p which showed increased relative expression in OSA-SA. (C) Adjusted multivariable logistic regression model included miR-320a, age, AHI and ApoB for prediction of SA with a promising AUC value in the ROC curve analysis. OSA: obstructive sleep apnea; OSA-SA: overlap OSA and subclinical atherosclerosis; AHI: apnea hypoapnea index; ApoB: apolipoprotein B; ROC: receiver operating characteristic; AUC: area under the curve. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

treatment” for OSA failed to reverse many OSA-induced end-organ morbidities, likely involving senescence-related pathways.¹⁵

Target prediction and enrichment analyses of miR-320a-3p suggested an interesting role of this miRNA in the context of OSA-SA (Tables S4–7). Alterations in cell adhesion have been linked to cell senescence and lately to the atherogenic process.¹⁶ Additionally, OSA has been shown to accelerate biological aging.¹⁷ T-cell metabolic disruptions have also been reported in OSA, which are associated with an increased incidence of various types of malignant tumors.¹⁸ Pathway enrichment analysis revealed the involvement of the Wnt signaling pathway, previously linked to plasma exosomal miRNAs from OSA patients with reverse dipping blood pressure.¹⁹ Furthermore, dysregulation of the cadherin pathway, particularly VE-cadherin, has been observed in OSA patients. This dysregulation may increase endothelial permeability, promote endothelial-to-mesenchymal transition, and ultimately contribute to atheroma plaque formation.²⁰

This study has several limitations. First, this work evaluated a population with no comorbidities other than OSA avoiding confounding factors such as hypertension so, our findings cannot be applied to the general population. Second, we evaluated a restricted subset of exosomal miRNAs related to atherosclerosis. This selection could lead to underestimation of the role of exosomes. Future analyses will assess a broader and more comprehensive profile of miRNAs. Finally, our study describes alterations in the exosomal miRNA profile and does not implicitly ascribe causality; however, further functional assays coupled to treatment and experimental

models should contribute to further understanding on the roles of those miRNAs in OSA.

In summary, exosomal miRNAs are altered in the progression of atherogenic processes associated with OSA. Notably, the increased abundance of exosomal miR-320a-3p in OSA patients with SA was confirmed in two independent studies, thereby conferring validity to the potential use of this miRNA as a biomarker of SA in OSA. Finally, CPAP treatment did not reverse the altered levels of miR-320a-3p which suggests that CPAP may not be sufficient to reverse the atherosclerosis related pathways initiated and propagated by OSA.

CRedit Authorship Contribution Statement

Conceptualization: D.S.-R., I.M.-B., and J.M.M.; methodology: D.S.-R., I.M.-B., J.R.-S., A.K., and J.M.M.; investigation: D.S.-R., I.M.-B., J.R.-S., M.M.-O., and J.M.M.; patient recruitment: J.R.-S., M.M.-O., and J.M.M.; data curation: D.S.-R., J.R.-S. and J.M.M.; writing – original draft preparation: D.S.-R.; writing – review and editing: D.S.-R., I.M.-B., J.R.-S., M.M.-O., A.K., M.S.-T., D.G. and J.M.M.; project coordination: D.S.-R.; funding acquisition: D.S.-R. and J.M.M. All authors have read and agreed to the submitted version of the manuscript.

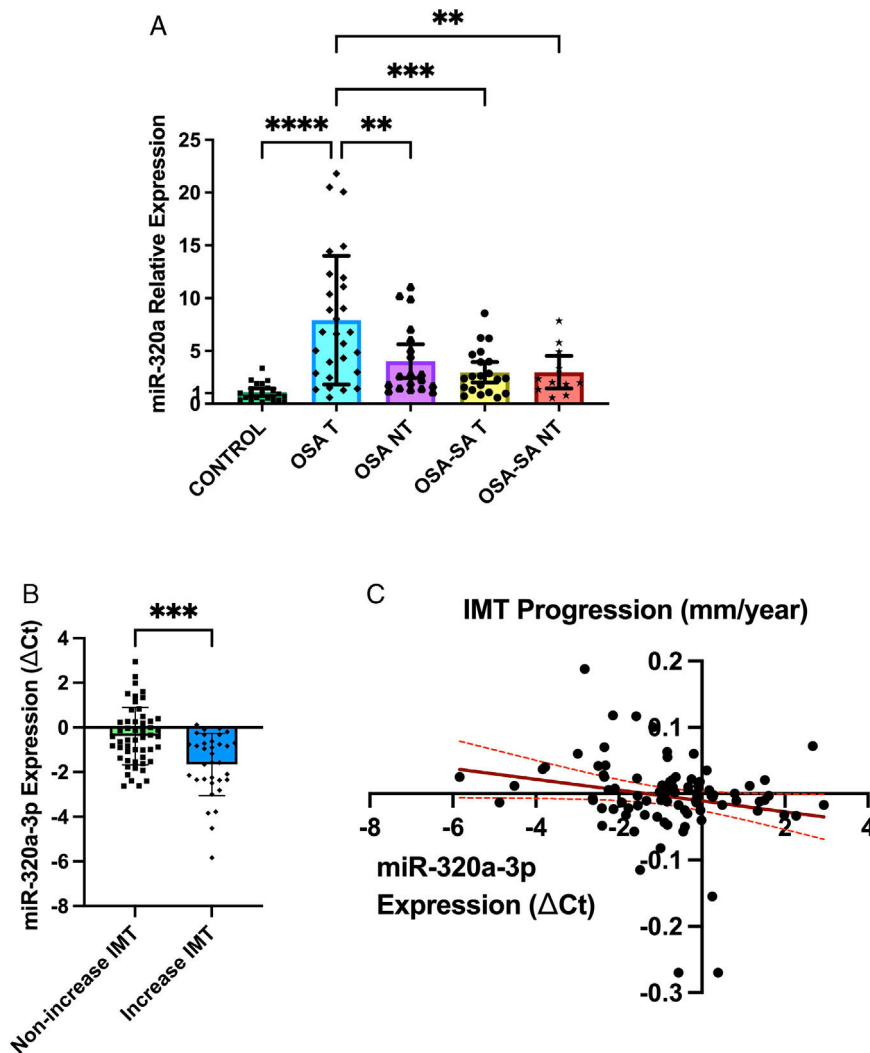


Fig. 2. Progression of carotid intima-media thickness (CIMT) and exosomal miR-320a-3p at 1-year follow-up. (A) Relative expression of exosomal miR-320a-3p at 1-year follow-up. (B) Delta Ct of exosomal miR-320a-3p in OSA at baseline classified according to their CIMT progression in the follow-up. (C) Lineal regression between miR-320a-3p and IMT progression. OSA: obstructive sleep apnea; OSA-SA: OSA and subclinical atherosclerosis; T: treated with CPAP; NT: not treated with CPAP. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

None of the content was produced with the help of an artificial intelligence software or tool.

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Conflicts of Interest

The authors declare not to have any conflicts of interest that may be considered to influence directly or indirectly the content of the manuscript.

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Appendix A. Supplementary Data

Supplementary data associated with this article can be found in the online version available at <https://doi.org/10.1016/j.arbres.2024.12.005>.

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