PLOS ONE

RESEARCH ARTICLE

Circulating levels of miR125a, miR126, and miR146a-5p in patients with obstructive sleep apnea and their relation with markers of endothelial dysfunction

Reza Fadaei[1], **Soudabeh Fallah**[2], **Mohammad-Taher Moradi**[1], **Masoumeh Rostampour**[1], **Habibolah Khazaie**[1]*

1 Sleep Disorders Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran,
2 Department of Clinical Biochemistry, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

* hakhazaie@gmail.com

Citation: Fadaei R, Fallah S, Moradi M-T, Rostampour M, Khazaie H (2023) Circulating levels of miR125a, miR126, and miR146a-5p in patients with obstructive sleep apnea and their relation with markers of endothelial dysfunction. PLoS ONE 18(11): e0287594. https://doi.org/10.1371/journal.pone.0287594

Editor: Slava Rom, Lewis Katz School of Medicine, Temple University, UNITED STATES

Received: February 27, 2023

Accepted: June 8, 2023

Published: November 2, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by a grant (Grant number: 990194) from Kermanshah University of Medical Sciences. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

obstructive sleep apnea (OSA) is a prevalent sleep disorder that is associated with increased risk factors for cardiovascular diseases (CVDs). Oxidative stress, insulin resistance, inflammation, and endothelial dysfunction are increased in OSA patients and microRNAs (miRs) are regulatory elements that influence these pathological mechanisms. miR125a, miR126, and miR146a-5p play a role in these pathological mechanisms and have not been evaluated in patients with OSA.

Method

This case-control study was performed on 90 OSA patients and 34 controls. Circulating levels of miR125a, miR126, and miR146a-5 were determined using real-time PCR, and serum levels of hsCRP, ICAM-1, and VCAM-1 were evaluated using ELISA kits.

Results

miR125a and miR146a were elevated in patients with OSA compared to controls while miR126 decreased significantly. All three miRs indicated a remarkable difference between the mild-OSA group compared to the severe-OSA group. Furthermore, patients with OSA showed elevated levels of hsCRP, ICAM-1, and VCAM-1. Multiple linear regression indicated an independent association of miR125a with ICAM-1 and hsCRP, miR126 associated with VCAM-1 and total cholesterol, and miR146a-5p represented an association with apnea-hypopnea index and ICAM-1. Furthermore, miR146a-5p illustrated a good diagnostic ability to differentiate between OSA and controls.

Competing interests: The authors have declared that no competing interests exist.

Conclusions

Circulating miR125a, miR126, and miR146a-5p fluctuations in patients with OSA and their relations with markers of endothelial dysfunction provide in vivo evidence and suggest a potential role for these miRs with endothelial dysfunction in patients with OSA.

Introduction

Obstructive sleep apnea (OSA) is one of the most prevalent sleep disorders which not only has an adverse impact on quality of life but also imposes several cardiovascular risk factors on the affected people [1]. Studies have reported a range of OSA prevalence from 9% to 38% and its prevalence increased with age, obesity, and being male [1, 2]. While the relationship of OSA with cardiovascular disease (CVD) is established the mechanism underlying this relationship is not fully understood. Several mechanisms have been proposed and investigated such as hypertension, endothelial dysfunction, insulin resistance, and dyslipidemia.

OSA is caused by recurrent partial or complete obstruction of the upper airway resulting in absence of inspiratory airflow [1]. This condition causes intermittent hypoxia which induces a situation like ischemia-reperfusion (I/R) results in increased ROS and oxidative stress [3]. Oxidative stress along with inflammation and sympathetic activation is considered the main driver of OSA consequences like insulin resistance, dyslipidemia, endothelial dysfunction, and atherosclerosis [1]. Exposing mice to intermittent hypoxia showed elevation of mitochondrial ROS that contributes to the development of type 2 diabetes mellitus [4]. In addition, intermittent hypoxia in rats leads to a decline in endothelial integrity and the number of endothelial cell progenitors [5]. Indeed, excessive ROS formation results in damage to biomolecules like DNA, proteins, and lipids in the body and in turn promotes an inflammatory cascade through transcription factor activation which causes upregulation of adhesion molecules and pro-inflammatory cytokines [6, 7]. Clinical studies have proven elevated inflammatory cytokines and adhesion molecules, which lead to endothelial dysfunction in OSA patients [8]. Moreover, the bioavailability of nitric oxide (NO) is reduced in intermittent hypoxia and in OSA patients which in turn promotes endothelial dysfunction [9].

Several mechanisms in the body regulate endothelial functions at transcription, post-transcription, and post-translation levels. MicroRNAs (miRs) are small, single-stranded, non-coding RNAs of 18 to 25 nucleotides that play a substantial role in the physiological and pathological process of the cells at the post-transcriptional level [10]. miRs can bind to 3'UTR of the target genes to induce their degradation and inhibit translation [10]. miRs were considered as a marker for response to therapy in OSA patients and a cluster of CVD-associated miRs called the HIPARCO-Score, comprising miR-378a-3p, miR-100-5p, and miR-486-5p showed a good ability to predict desirable response to CPAP treatment [11]. While there are studies that tested the ability of miRs to predict response to treatment, most studies evaluated the diagnostic potential of miRs. One of the first studies showed that miR-574-5p was upregulated, while 199-3p, miR-107, and miR-485-5p were suppressed, in patients with OSA in comparison to controls [12]. Moreover, miR-181a, miR-133a, miR-340, miR-199b, miR-486-3p, and miR-345 were found to be lower in the plasma of male OSA patients compared to controls [13]. In addition to the diagnostic ability of miRs in sleep apnea, it has been shown that miRs can affect pathophysiological pathways and mechanisms related to OSA. Circulating exosomes containing miRs from subjects who were exposed to intermittent hypoxia considerably upregulated ICAM-1 and downregulated endothelial nitric oxide synthase (eNOS) [14]. There are

miRs that have close relationships with endothelial dysfunction, inflammation, and cardiovascular diseases which have not been evaluated in patients with OSA.

There is considerable literature that reported the relation of miR125a with underlying mechanisms of CVD [15], and lower levels of this miR have been reported in subjects suffering from insufficient sleep [16]. miR125a has been reported to have protective effects against I/R injuries in rats' myocardium [17], moreover, miR125a has a protective role in the inflammatory process through the PYD domains-containing protein 3 (NLRP3) [18]. miR126 plays a role in endothelial proliferation and in developmental angiogenesis [19, 20], and reports indicate that miR126 is released by endothelial cells [21]. miR126 was found to be downregulated in senescent endothelial cells and inhibition of miR126 resulted in a decrease in HIF-1α protein levels, that disrupt the wound healing process [22]. Additionally, miR126 showed an inverse relationship with the VCAM-1 and was found to be downregulated in senescent human aortic endothelial cells [23]. Furthermore, miR126 was decreased in OSA patients with hypertension compared to OSA patients with normal blood pressure [24]; chronic intermittent hypoxia in rats results in a decrease of miR126a-3p and an increase in HIF-1α in the rat [25]. miR146a-5p is a key regulator of several cancers, including prostate, breast, and gastric cancer [26–28], in addition, the levels of miR146a-5p have been found to be higher in animal and cell models of I/R [29]. Furthermore, miR146a-5p exacerbates injury induced by IH in H9c2 cells by reducing cell viability and by increasing apoptosis through the X-linked inhibitor of apoptosis protein (XIAP) [30]. Furthermore, miR146a-5p suppressed endothelial activation and pro-inflammatory signaling in endothelial cells [31].

While there are shreds of evidence for the relation between these three miRs and endothelial dysfunction, there is no study on the association of these miRs with OSA. Therefore, the present study sought to measure circulating levels of miR125a, miR126, and miR146a-5p in patients with OSA to determine if they were related to markers of endothelial dysfunction and inflammation.

Method

Study population and diagnosis of OSA

This case-control study was performed on 124 subjects (90 OSA and 34 control) who underwent polysomnography (PSG) in the sleep clinic of Farabi Hospital in Kermanshah, Iran, from March 2020 until March 2022. The diagnosis was based on the results of PSG [32, 33], subjects with an apnea-hypopnea index (AHI) $\geq$ 5 were categorized as OSA patients. Controls were subjects with AHI $<$ 5 and did not have any sleep disorders. Briefly, continuous PSG was performed overnight (7hrs) for all subjects via SOMNOscreen™ plus (SOMNOmedics GmbH, Randersacker, Germany). American Academy of Sleep Medicine (AASM 2012) guidelines were used to define hypopnea and apnea. Hypopnea is defined as reduced airflow by $\geq$30% along with reduced oxygen desaturation index by $\geq$3% or arousal and apnea are classified as a whole cessation of airflow for $\geq$10 seconds. The mean number of hypopneas plus apneas is considered AHI. The severity of the disease was defined according to the AHI value: 1) Mild: 5$\leq$AHI$<$15 (n = 30), 2) Moderate: 15$\leq$AHI$<$30 (n = 29), and 3) Severe: AHI$\geq$30 (n = 30). Subjects with evidence or history of cardiovascular diseases, autoimmune diseases, cancer, and diabetes (according to the criteria of American diabetes association) were excluded from the study.

Anthropometric and laboratory parameters

Fasting venous blood sample (5 mL) was obtained from participants, and serum was separated immediately by centrifugation and stored at—70˚ C. Systolic blood pressure (SBP) and

diastolic blood pressure (DBP) were measured in a was seated position using a standard sphygmomanometer. Body mass index (BMI) calculated using standard formula: weight (kg)/height (m^2). Levels of fasting blood glucose (FBG) and lipid profile, including triglyceride (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined using a spectrophotometric assay with commercially available kits (ParsAzmon, Tehran, Iran), and an auto-analyzer. Fasting insulin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Monobind, USA) according to the manufacturer's instructions.

Serum levels of adhesion molecules

ELISA kits (Quantikine, R&D Systems; USA) were used to measure serum levels of ICAM-1 and VCAM-1. Intra-assay and inter-assay coefficients of variation (CV) for ICAM-1 and VCAM-1 were <6.5% and <7% respectively. Moreover, the minimum detectable range for ICAM-1 and VCAM-1 were 0.096 ng/mL and 0.6 ng/mL, respectively.

Determining miRs circulating levels

The miRNA was extracted from serum samples using the QIAzol reagent (Qiagen, USA) according to the manufacturer's protocol. The concentration and purity of RNA were tested by a NanoDrop (Thermo Fisher Scientific, USA). The miR complementary DNA (cDNA) was synthesized using TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, USA). The levels of circulating miRs were measured using TaqMan Advanced miRNA Assays (Thermo Scientific, USA) based on the manufacturer's protocol. miR-361-5p was used as the internal control and specific primers and probes were applied for each of the miRNAs. Relative quantification of miRs was determined by the $2^{-\Delta Ct}$ method [34].

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 25 was used for statistical analysis. The chi-square test was used to compare categorical data between the groups and results are represented by frequency and percentage. The mean between two groups was compared using either Student's t-test or Mann-Whitney U test, depending on the normality test results. One-Way ANOVA or Kruskal Wallis test were used to compare continuous variables between more than two groups according to normality results. Analysis of Covariance (ANCOVA) was performed to adjust for the possible impact of covariates on miRNA levels. Data that were not normally distributed were transformed logarithmically before including in correlation tests or regression analyses. The Pearson correlation test was used to test the correlation between miRs and other variables. The correlation for PLMS was tested using the Spearman test because there was a zero in PLMS. To identify the independent association between miRs and continuous variables, all variables that were found to be correlated were included in a linear regression analysis. Binary logistic regression was used to test the relation of miRs with the risk of OSA. The receiver operating characteristic (ROC) curve was plotted to test the diagnostic ability of circulating miRs. The sample size was calculated for a case-control study comparing the levels of three circulating miRs (miR146a, 126, and 125) between the OSA group and the control group separately. The calculation was performed with a power of 80% and a significance level (alpha) of 0.05, and the highest sample size required was determined. A p-value of less than 0.05 was considered statistical significance.

Results

The basic characteristic of the study population

Table 1 represents the detailed characteristic of the studied population. Age and BMI indicated no considerable difference between the groups. While patients with OSA had a bigger neck circumference (p = 0.019), waist circumference didn't reach the significant threshold (p = 0.078). SBP and DBP were remarkably higher in the OSA group compared with controls ($p<0.01$ for both). As expected, both AHI and RDI were considerably higher in OSA patients compared to controls ($p<0.001$ for both). Moreover, total sleep time and sleep efficiency indicated no considerable difference between the groups while periodic limb movements in patients with OSA was higher compared to controls. While average SpO2 indicated no remarkable difference between the groups (p = 0.121), minimal SpO2 declined in patients compared to controls ($p<0.001$). FBG illustrated no considerable difference between the groups but, insulin and HOMA-IR showed a considerable elevation in the OSA group compared to the control group. TC and LDL-C were not significantly different between the groups. HDL-C was reduced in the OSA group compared to controls (p = 0.037) while TG was elevated in the patients compared to controls (p = 0.043).

Circulating hsCRP and soluble adhesion molecules

Serum levels of hsCRP, as an inflammatory marker, were elevated in patients with OSA (5.86 ± 2.2) compared with controls (2.5 ± 0.92 mg/L, $p<0.001$). According to the comparison

Table 1. The basic characteristic of the studied population.

Variables	Control (n = 34)	OSA (n = 90)	P Value
Age (year)	44.56 ± 9.34	45.28 ± 12.85	0.733
Sex (male /female)	18 (52.1%)/16(47.9%)	63 (0%)7/27(30%)	0.06
BMI (kg/m^2)	26.53 ± 3.25	26.93 ± 2.61	0.477
Neck circumference (cm)	37 (33, 39)	39 (37, 41)	0.019
Waist circumference (cm)	93.38 ± 9.37	96.86 ± 9.86	0.078
SBP (mmHg)	110 (110, 120)	120 (115, 125)	0.003
DBP (mmHg)	70 (70, 80)	80 (70, 80)	0.004
AHI (event/h)	2.2 (1.25, 2.9)	18.9 (9.25, 37)	<0.001
RDI (event/h)	4.82 ± 3.92	28.42 ± 20.53	<0.001
AverageSpO2 (%)	92.15 ± 4.57	89.79 ± 8.34	0.121
MinimalSpO2 (%)	89.26 ± 5.65	84.22 ± 9.33	<0.001
TST (h)	6.5 (6.29, 7.27)	6.54 (5.53, 7.33)	0.831
SE (%)	86.6 (81.1, 93.3)	86.45 (74.6, 93.7)	0.827
PLMS	0.9 (0, 3.2)	3.35 (0.7, 8.5)	0.001
Insulin (μU/ml)	3.2 (2.5, 4.4)	4.5 (2.45, 7.16)	0.009
HOMA-IR	0.73 (0.57, 1.09)	1.08 (0.57, 1.59)	0.010
FBS (mg/dL)	94.63 ± 11.428	95.84 ± 10.68	0.583
TC (mg/dL)	164.31 ± 34.37	167.03 ± 45.96	0.755
LDL-C (mg/dL)	94.82 ± 23.72	99.44 ± 31.69	0.383
TG (mg/dL)	121 (94, 157)	147 (109.5, 183)	0.043
HDL-C (mg/dL)	44.1 (41.5, 51.75)	42 (37, 48)	0.037

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AHI, apnea-hypopnea index; RDI, respiratory disturbance index; averageSpO2, average saturation of peripheral oxygen; MinimalSpO2, minimal saturation of peripheral oxygen; TST, total sleep time; SE, Sleep efficiency; PLMS, Periodic limb movements in sleep; HOMA-IR, homeostatic model assessment for insulin resistance; FBS, fasting blood sugar; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol

https://doi.org/10.1371/journal.pone.0287594.t001

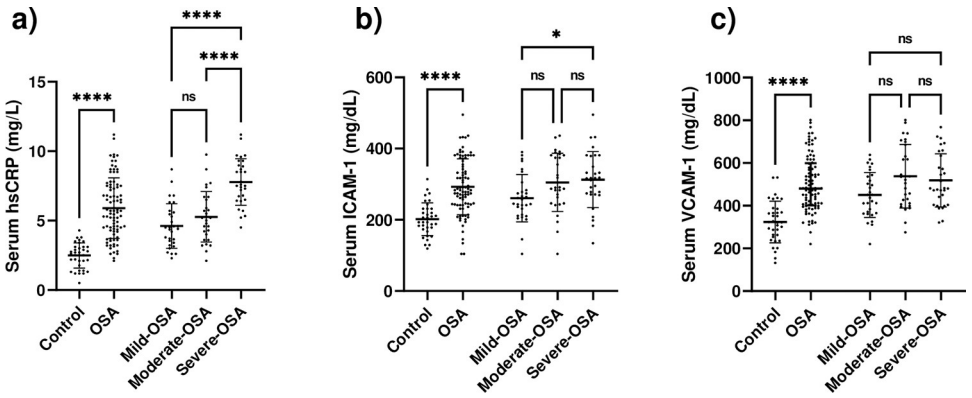

Fig 1. Serum levels of hsCRP, ICAM-1, and VCAM-1. a) Serum levels of hsCRP were elevated in OSA patients compared to controls, and the severe-OSA group represents a higher concentration of hsCRP compared with mild-OSA and moderate-OSA groups. b) Serum concentration of ICAM-1 was higher in the OSA group compared to controls, and the severe-OSA group indicated higher ICAM-1 compared to the mild-OSA group. c) Patients with OSA were found to have a higher concentration of VCAM-1 compared to controls. ns, not significant; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

https://doi.org/10.1371/journal.pone.0287594.g001

between OSA categories, severe OSA (7.78 ± 1.68 mg/L) had higher hsCRP levels than mild (4.62 ± 1.61 mg/L) or moderate OSA (5.27 ± 1.82 mg/L) ($p<0.001$ for both), but mild and moderate OSA did not differ significantly from each other (Fig 1A). ICAM-1 concentration was higher in OSA patients (200.23 ± 45.99 mg/dL) compared to controls (292.27 ± 78.12 mg/dL, $p<0.001$), furthermore, severe-OSA group indicated a higher concentration of ICAM-1 compared to mild-OSA (260.8 ± 66.30 mg/dL) ($p = 0.048$), and there was no considerable difference between moderate-OSA (304.9 ± 81.7 mg/dL) with mild-OSA and severe-OSA (312.9 ± 78.83 mg/dL) (Fig 1B). Similarly, VCAM-1 (480 (399, 599) vs. 322 (258.5, 387)) increased in patients with OSA compared to controls ($p<0.001$ for both), but there was no remarkable difference between mild-OSA (444 (364.5, 538)), moderate-OSA (521 (408.5, 692)) and severe-OSA (480.5 (404, 639.3)) groups (Fig 1C).

Circulating MicroRNAs

The levels of circulating miR125a were higher in OSA patients than in controls ($p<0.001$). Moreover, patients with mild OSA had higher levels of miR125a than those in the moderate and severe OSA groups (Fig 2A). Conversely, the levels of miR126 were lower in patients with OSA than in controls ($p<0.001$), and miR126 was also decreased in the severe OSA group compared to the mild OSA group (Fig 2B). In addition, miR146a-5p levels were higher in OSA patients than in controls ($p<0.001$), but miR146a-5p was found to be lower in the mild OSA group compared to the moderate and severe OSA groups (Fig 2C). All the results remained consistent even after adjusting for age, sex, and BMI.

Association of miRs with other variables

Correlation analysis was performed in each group separately and the results are shown in Table 2. In the patients with OSA, miR125a indicated a positive association with miR146a-5p, AHI, RDI, FBS, hsCRP, ICAM-1, and VCAM-1, and an inverse correlation with minimal SpO2. Furthermore, miR126 indicated an inverse correlation with AHI, insulin, HOMA-IR, TC, ICAM-1, and VCAM-1. miR146a-5p represented a positive correlation with AHI, RDI, neck circumference, FBS, insulin, HOMA-IR, ICAM-1, and VCAM-1, and an inverse correlation with minimal SpO2 and HDL-C. Multiple stepwise linear regressions were performed to

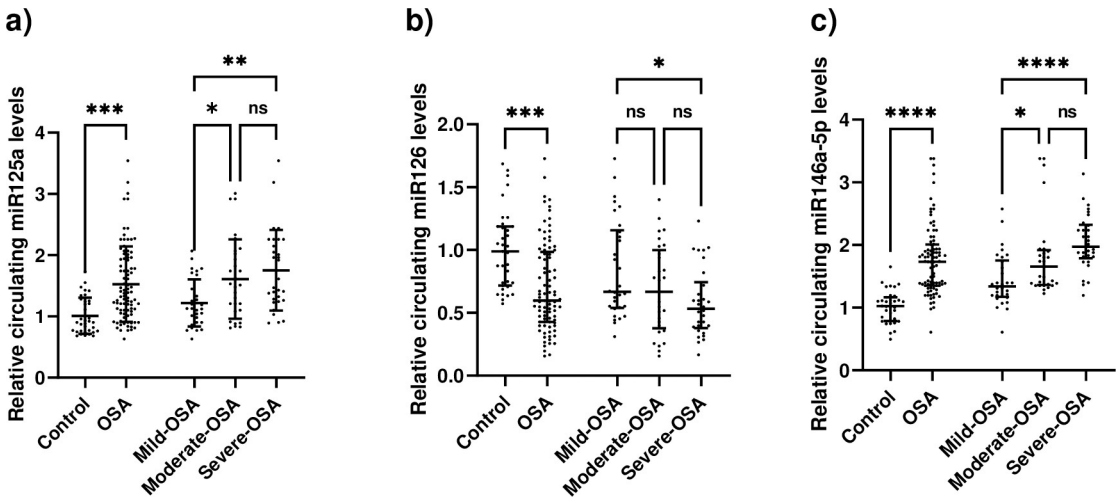

Fig 2. Circulating levels of miRs. a) miR125a levels increased in patients with OSA compared to controls, and in the severe-OSA group compared to the mild-OSA group. b) miR126 concentration was lower in OSA compared to controls and in the severe-OSA group compared to the mild-OSA group. c) circulating mi146a were found to be higher in OSA patients in comparison to controls, and mild-OSA had a lower concentration of miR146a-5p compared with moderate-OSA and severe-OSA groups. ns, not significant; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ and **** $p<0.0001$.

https://doi.org/10.1371/journal.pone.0287594.g002

find independent associations of miRs with correlated variables. miR125a was found to independently associated ICAM-1 [B (95% CI) = 0.002 (0.001, 0.004), p = 0.003] with hsCRP [B (95% CI) = 0.099 (0.047, 0.151), p<0.001] and miR126 showed an independent association with VCAM-1 [B (95% CI) = -0.651 (-1.050, 0.252), p = 0.002] and TC [B (95% CI) = -0.001 (-0.002, 0.000), p = 0.027]. Furthermore, miR146a-5p demonstrated an independent association with the AHI [B (95% CI) = 0.141 (0.067, 0.216), p<0.001] and ICAM-1 [B (95% CI) = 0.001 (0.000, .001), p = 0.007]. The relation of miRs with sex was tested and there was no considerable difference between men and women in terms of miR125a and miR126, however, miR146a-5p indicated a higher level in men compared to women.

Association of miRs with OSA

The association of circulating miRs with the risk of OSA was tested using binary logistic regression. The results showed that levels of miR are associated with the risk of OSA and the relation remained significant after adjusting for age, sex, and BMI (Table 3).

The diagnostic ability of the miRs was assessed using ROC analysis and the results showed that miR125a had a relatively good ability to distinguish between OSA and control [area under the curve (AUC) and 95% CI: 0.787 (0.702, 0.873), p<0.001] (Fig 3A). Similarly, miR126 represented a relatively good potential diagnosis of OSA [AUC and 95% CI: 0.745 (0.66, 0.83), p<0.001] (Fig 3B). Moreover, miR146a-5p had a high potential for OSA diagnosis with an AUC and 95% CI: 0.933 (0.890, 0.976), p<0.001 (Fig 3C).

Discussion

The present study established a substantial difference in circulating levels of three miRs which are involved in vascular inflammation and endothelial cell dysfunction [35–37]. miR125a indicated a higher concentration in OSA patients and showed a positive association with disease severity. This is the first study of miR125a in patients with OSA while there are studies that have reported perturbation in circulating levels of miR125a in diseases conditions such as cancer and cardiovascular diseases [15, 38]. It has been established that the lack of this miR can

Table 2. Pearson correlation of miR125a, miR126, and miR146a-5p with other variables.

Variables	Groups					
	Control			OSA		
	miR125a	miR126#	miR146a-5p#	miR125a	miR126#	miR146a-5p#
miR125a	1	-0.135	-0.183	1	-0.106	0.325**
miR126#	0.089	1	-0.059	-0.111	1	0.007
miR146a-5p#	-0.107	-0.059	1	0.333**	0.007	1
Age	-0.006	-0.122	0.297	0.091	0.055	0.152
BMI	0.078	-0.311	0.123	0.099	-0.133	0.154
AHI#	-0.263	-0.069	0.219	0.356**	-0.259*	0.428**
RDI	0.061	-0.242	0.164	0.352**	-0.176	0.435**
AverageSpO2	-0.044	0.004	-0.249	-0.026	0.100	-0.019
MinimalSpO2	-0.305	0.152	-0.091	-0.228*	0.000	-0.234*
TST#	0.257	-0.107	0.093	0.019	0.029	-0.063
SE#	0.260	-0.098	0.056	0.011	0.023	-0.060
PLMS$	0.116	-0.073	0.297	-0.147	-0.028	-0.041
Neck circumference	-0.057	-0.249	0.207	0.024	-0.110	0.216*
Waist circumference	0.110	-0.250	0.264	-0.028	-0.159	0.164
SBP#	0.167	-0.266	0.219	0.151	-0.045	0.038
DBP#	0.181	-0.336	0.142	-0.038	-0.129	-0.152
FBS	0.210	-0.118	0.185	0.220*	-0.076	0.219*
Insulin#	0.206	0.202	0.125	0.134	-0.267*	0.242*
HOMAIR#	0.238	0.168	0.158	0.162	-0.267*	0.265*
TC	-0.207	0.037	0.242	0.089	-0.291**	0.145
TG#	-0.063	0.172	0.194	0.035	-0.150	0.119
LDL	0.007	0.271	0.261	0.118	-0.120	0.198
HDL#	-0.226	0.080	-0.213	-0.164	-0.105	-0.213*
HsCRP	0.313	-0.132	0.288	0.384**	-0.073	0.189
ICAM-1	0.044	-0.064	-0.173	0.322**	-0.317**	0.357**
VCAM-1#	0.001	-0.100	-0.196	0.244*	-0.368**	0.266*

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; AHI, apnea-hypopnea index; RDI, respiratory disturbance index; averageSpO2, average saturation of peripheral oxygen; MinimalSpO2, minimal saturation of peripheral oxygen; TST, total sleep time; SE, Sleep efficiency; PLMS, Periodic limb movements in sleep; HOMA-IR, homeostatic model assessment for insulin resistance; FBS, fasting blood sugar; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol

* $p<0.05$

** $p<0.01$

Logarithmically transformed

$ Spearman correlation test

https://doi.org/10.1371/journal.pone.0287594.t002

lead to defects in the development of the cardiovascular system. In contrast with the present study, a decline in levels of miR125a was detected in systemic lupus erythematosus and juvenile-onset lupus patients [39]. Additionally, Hijmans et al, indicated a lower concentration of this miR125a in a subject suffering from insufficient sleep. It is worth to noting that there is no consensus on the circulating level of miR125a in different diseases, so it seems likely that miR125a has a complex regulation mechanism and disease setting can have a huge impact on circulating levels of miR125a. Interestingly, there was a significant positive correlation between miR125a and two important OSA-related parameters (i.e. AHI and RDI), with an inverse correlation with minimal SpO2. Repetitive hypoxia/reoxygenation is an important aspect of OSA

Table 3. Odds ratio (OR) of the OSA presence according to circulating levels of miRs.

	Model	B	S.E.	Wald	OR	95% C.I.for OR		p
						Lower	Upper	
miR125a	Crude	2.874	0.713	16.245	17.705	4.377	71.616	<0.001
	Adjusted*	2.872	0.721	15.875	2.872	4.302	72.537	<0.001
miR126#	Crude	-2.227	0.606	13.486	0.108	0.033	0.354	<0.001
	Adjusted*	-2.275	0.624	13.293	0.103	0.030	0.349	<0.001
miR146a-5p#	Crude	7.324	1.556	22.166	1516	71.9	31990	<0.001
	Adjusted*	8.769	1.949	20.249	6430	141.092	293042	<0.001

Logarithmically transformed

*An adjustment was performed for age, sex, and BMI.

https://doi.org/10.1371/journal.pone.0287594.t003

pathogenesis, creating a condition similar to ischemia/reperfusion (I/R). There is evidence suggesting a role for miR125a in I/R [40]. Furthermore, miR125a has been shown to protect rat myocardium against I/R injuries through urocortin [40] and increased levels of miR125a may represent a response to combat such injuries [17]. On the other hand, miR125a modulates inflammation through the inhibition of tet methylcytosine dioxygenase 2 (TET2). This function of miR125a results in mitochondrial dysfunction, elevation of oxidative stress, activation of nuclear factor-κB, and increased generation of pro-inflammatory cytokines [41]. The results of the present study showed a positive relation between miR125a and hsCRP as a marker of inflammation. On the other hand, overexpression of miR125a resulted in a decline in ICAM-1 and VCAM-1 expression in human brain microvessel endothelial cells [42], and our result showed that miR125a positively correlated with markers of endothelial dysfunction (i.e. ICAM-1 and VCAM-1). This controversy may be due to the fact that increased levels of miR125a in vivo may not be as high as the levels achieved through *in vitro* overexpression, which could impact the observed effects of miR125a. There is inconsistency in how miR125a is related to inflammation, I/R, and markers on vascular function [43, 44]. While there is evidence for the protective role of miR125a against I/R injuries, and its ability to improve vascular function by reducing ICAM-1 and VCAM-1, it also has a negative impact on inflammation and oxidative stress [17, 43, 44]. Moreover, miR-125a inhibits Hyaluronan Synthase 1 [45], and clinical reports have shown that levels of hyaluronic acid reduced in patients with OSA,

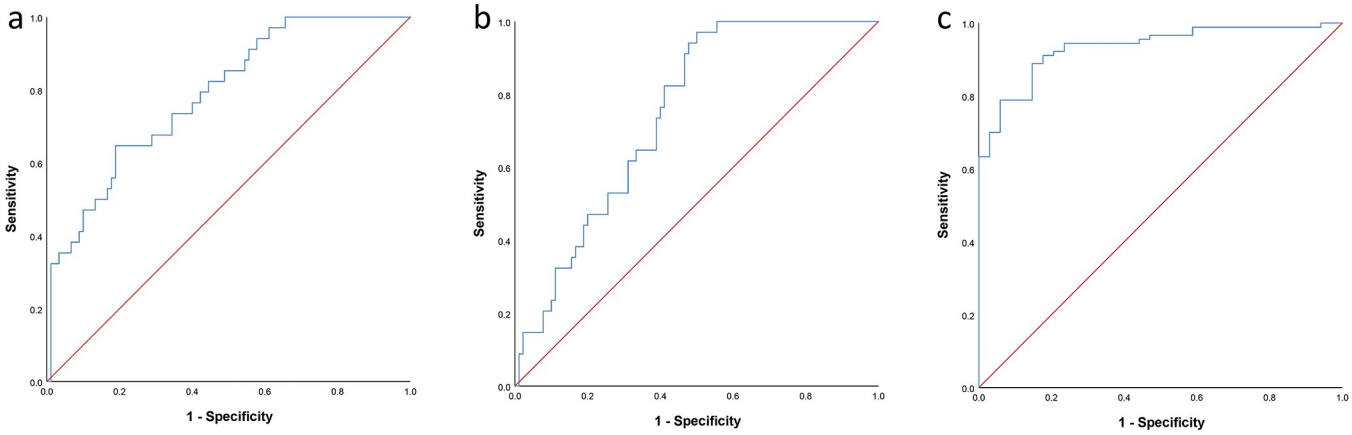

Fig 3. ROC curve for the diagnostic ability of a) miR125a, b) miR126, and c) miR146a-5p.

https://doi.org/10.1371/journal.pone.0287594.g003

which is associated with inflammation and endothelial dysfunction in these patients [46]. Regarding these findings, our results suggest a compensatory increase in miR125a in response to I/R and endothelial dysfunction, but there is no evidence of the effectiveness of elevated miR125a to reduce levels of ICAM-1 and VCAM-1, and instead of miR-125a being a modulator of inflammation, it might be that inflammation upregulates miR-125a. These results suggest that the role of miR-125a in OSA patients and pathological conditions such as endothelial dysfunction is complex and requires further investigation.

miR126 represented a lower concentration in OSA patients compared to controls. This study presents the first report of miR126 in OSA patients. Our results showed a correlation between miR126 with AHI, which is the main indicator of OSA and its severity. Hypoxia was found to be a factor that downregulates miR126 in RF/6A cell line [47]. Previous studies have reported a lower concentration of miR126 in patients with type 2 diabetes mellitus and prediabetes [48]. Consistent with these findings, our results showed an inverse correlation between reduced miR126 and HOMA-IR and insulin levels in OSA patients. Moreover, miR126 is inversely correlated with markers of endothelial function in OSA patients (e. i. ICAM-1 and VCAM-1). Several lines of evidence have shown the beneficial impact of miR126 on endothelial function [49] which may explain the association between miR126 and ICAM-1 and VCAM-1 in the present study. A study found that miR126 downregulation leads to an increase in VCAM-1 expression in endothelial cells [49]. Another study reported that miR126 was lower in patients with intracerebral hemorrhage and that using miR126 mimics to downregulate VCAM-1 in the rat model of intracerebral hemorrhage [50]. In addition, miR126 increases endothelial cell viability and promotes activation of endothelial nitric oxide synthase (eNOS) by suppressing phosphoinositide 3-kinase (PI3K)/AKT/eNOS [51]. Collectively, miR126 reduction in patients with OSA and its relation with vascular adhesion molecules provide *in vivo* evidence for the association of this miR with the pathological aspects of OSA.

miR146a-5p was found to be present at higher levels in patients with OSA which demonstrated good diagnostic ability. Studies have reported that miR146a can mediate several endothelial pathophysiological mechanisms. miR146a was found to be upregulated under intermittent hypoxia and mediates its effects in H9c2 cells [30]. Similarly, miR146a is upregulated during I/R in rat myocardium [29]. In the current study, miR146a-5p represented a positive correlation with insulin resistance and levels of insulin and FBS. Transfection of adipocytes with a miR146a inhibitor resulted in reduced insulin sensitivity [52] and another study has shown that patients with diabetes mellitus have lower levels of miR146a in their peripheral blood mononuclear cells compared to controls [53]. The relation of miR146a with insulin and glucose metabolism indicators in the present study, suggests that it might be a response to insulin resistance that is not effective in reducing insulin sensitivity. Furthermore, we found a positive correlation between miR146a-5p with ICAM-1 and VCAM-1, and previous study has shown a relation of this miR with inflammation [54]. Treatment of endothelial cells with LPS and pro-inflammatory cytokines upregulate miR146a and ICAM-1 and VCAM-1 and the present study showed a positive relation between miR146a and these factors [31, 55]. While studies have shown that miR146a can downregulate ICAM-1 and VCAM-1 [31, 55] and it suppresses inflammation by targeting TNF receptor-associated factor 6 (TRAF6) [56], our results showed a positive correlation between miR146a and adhesion molecules. This finding suggests that miR146a-5p levels *in vivo* might not be as high as levels of upregulated miR146a-5p *in vitro* to suppress ICAM-1 and VCAM-1 and other mechanisms might be more effective in regulating ICAM-1 and VCAM-1 expression, and elevation of miR146a-5p might result from the inflammatory milieu in OSA patients or from a compensatory response. The exact mechanism underlying this finding is unclear and further studies are needed to investigate it.

In conclusion, the present study is the first report on the change of circulating miR125a, miR126, and miR146a-5p in patients with OSA, and provides *in vivo* evidence for the relationship of these miRs with pathological consequences of OSA like endothelial dysfunction, and could be potential therapeutic targets for vascular dysfunction and inflammation in OSA patients, although more studies are required in this regard.

The present study was conducted on subjects with PSG-confirmed OSA and without diabetes and matched according to age, sex, and BMI. While there are some limitations, the sample size and cross-sectional design limited us to conclude a causal relation, and direct measurement of vascular functions was not taken.

Supporting information

S1 Data. Raw data.
(XLS)

Acknowledgments

The authors wish to thank professor Leonard Kritharides, and Dr. Maaike Kockx from Atherosclerosis and Vascular Biology Laboratory, ANZAC Medical Research Institute for helpful feedback and comments in preparation of the manuscript.

Author Contributions

Conceptualization: Reza Fadaei, Habibolah Khazaie.

Data curation: Soudabeh Fallah, Mohammad-Taher Moradi.

Formal analysis: Reza Fadaei.

Funding acquisition: Reza Fadaei.

Investigation: Reza Fadaei, Mohammad-Taher Moradi, Masoumeh Rostampour, Habibolah Khazaie.

Methodology: Reza Fadaei, Soudabeh Fallah, Mohammad-Taher Moradi, Masoumeh Rostampour, Habibolah Khazaie.

Project administration: Habibolah Khazaie.

Resources: Reza Fadaei.

Supervision: Reza Fadaei, Habibolah Khazaie.

Validation: Reza Fadaei.

Writing – original draft: Reza Fadaei, Soudabeh Fallah, Mohammad-Taher Moradi, Masoumeh Rostampour, Habibolah Khazaie.

Writing – review & editing: Reza Fadaei, Soudabeh Fallah, Mohammad-Taher Moradi, Masoumeh Rostampour, Habibolah Khazaie.

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