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Molecular Signatures of MicroRNA in Obesity Associated Obstructive Sleep Apnea in Middle-Aged Adults

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ABSTRACT

Objective: *Obstructive sleep apnea (OSA) is a respiratory disorder with multiple clinical outcomes and is now being recognized as a serious health concern across the globe. However, the mechanisms of its pathophysiology are still elusive. Also, to date, evidence of miRNA regulation of sleep apnea in the Indian sub-population is unknown.* ***Methods:*** *In this study, we investigated the expression pattern of certain potential obesity-linked miRNAs in OSA subjects. Seventy adult subjects (20 obese OSA, 20 non-obese OSA and 30 healthy) were selected for this study. Total RNA was extracted and the expression of miR-21, miR-27, miR-29 and let-7 (normalized with internal control RNU48) was analyzed by SYBR Green-based qPCR.* ***Results:*** *We selected miR-21, miR-27, miR-29 and let-7 for their documented role in obesity. Relative miRNA expression profiles revealed differential expressions of all four above mentioned miRNAs in both obese and non-obese OSA subjects compared to healthy controls. Statistical analysis revealed a significant correlation between miRNA expression with obesity-associated parameters in OSA subjects.* ***Conclusion:*** *Our study demonstrates the involvement of four miRNAs (miR-21, miR-27, miR-29 and let-7) as potential molecular players of obesity-associated OSA. Identification of miRNA targets and in-depth analysis of their molecular mechanism in disease pathogenesis is further warranted.*

Keywords: Obstructive sleep apnea, microRNA, Obesity, Differential expression

Abbreviations: AHI: Apnea-Hypopnea Index; ALK.P: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: Body Mass Index; cDNA: Complementary DNA; CVD: Cardiovascular Disease; CRP: C-Reactive Protein; HDL: High Density Lipoprotein; miRNA: Micro-RNA; OSA: Obstructive Sleep Apnea; OSAS: Obstructive Sleep Apnea Syndrome; PBMC: Peripheral Blood Mononuclear Cells; PCR: Polymerase

INTRODUCTION

Obstructive sleep apnea (OSA) is a respiratory disorder that is distinguished by a repeated occurrence of upper airway obstruction and is usually linked with oxygen depletion and sleep disruption [1]. The severity of the disease is measured with the assistance of the apnea-hypopnea index (AHI). OSA is usually said to exist if the AHI is ≥ 5. AHI of 5-15 is categorized as mild OSA, 15-30 as moderate OSA and 30 and above as severe OSA [2]. OSA has now been recognized worldwide as a paramount health concern due to its consequences such as cardiovascular disorders (CVDs) and metabolic disorders, mood and behavioral changes [3]. OSA and OSAS (obstructive sleep apnea syndrome) are

diagnosed based on an overnight sleep study, known as polysomnography (PSG) [4]. The overall prevalence of OSA in middle-aged adults as per a recent study ranged from 6%-17% [5]. The most common risk factor associated with OSA is obesity that covers approximately 65-70% of the total patients screened with a high Body mass index (BMI) [6]. A recent piece of evidence suggested that more than 135 million Indians are obese. However, its prevalence varies due to a multitude of factors such as age, gender, geography, etc. [7]. It has also been proven that excessive presence of body fat is responsible for heightened metabolic and cardiovascular disorders in Asian Indians [8].

The severity in OSA is directly correlated to the measure of obesity which is further proportional to the risk of CVD [9,10]. Abundant studies have suggested an escalation in the inflammatory markers such as C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF-α), inflammatory cytokines and adhesion molecule as mentioned in our recently published review [11] in OSA indicative of an evident risk for cardiovascular diseases. However, these inflammatory markers are also dysregulated in most of the infections and diseases. Therefore a different dimension of biomarkers is warranted for better clinical utility.

MicroRNAs (miRNAs or miRs) are non-protein-coding RNAs, which mainly function through binding to the 3' untranslated region (3' UTR) of target mRNA, followed by mRNA degradation and/or translational inhibition [12]. miRNAs, which are now being recognized as potential diagnostic biomarkers, play a vital role in a variety of cellular events ranging from organogenesis to immunity [13,14]. Certain miRNAs have been identified that prove to be crucial biomarkers for risk assessment in obese patients [15]. Very recently, miR-664a-3p has been identified as a potential biomarker of atherosclerosis in OSA subjects [16]. In another study, the same group reported differentially expressed miRNAs in only six OSA subjects by next-generation sequencing (NGS). Although the expression of miRNAs was not validated further and no correlation analysis was performed with any of the clinical characteristics of OSA patients [17].

Considering the Indian subcontinent, there is very less evidence that highlights the biomarkers in OSA. For instance, Bhushan et al investigated the association of CRP [18] and TNF-α [19] levels with OSA in obese Indians. Recently, a study based on the South Indian population, elucidated the association between age and OSA severity whereby the authors stated that middle-aged overweight men are more likely to develop OSA and the severity increased with age [20,21]. No reports till now have surfaced that have demonstrated the role of miRNAs as promising biomarkers in the Indian OSA population.

The role of miRNAs has been speculated in obesity and associated disorders. However, their role in obesity-linked OSA pathophysiology has not been reported. Since obesity is one of the major risk factors for OSA, the study focusses on miRNA expression analysis with the direct correlation of obesity with OSA which has not been studied so far. Also, reports pertaining to miRNA profiling in the Indian sub-population of OSA are not known. Although lifestyle-related issues such as obesity in the metropolitan city sub-population is highly prevalent, therefore efforts are required to investigate and correlate miRNA expression with clinical characteristics of OSA patients. Here our study aims to establish the association between the miRNAs such as miR-21, miR-27, miR-29 obesity-linked and let-7 with OSA and differentiate the obese from the non-obese subjects based on the obtained miRNA signature. Interestingly, our study demonstrates a probable link between miRNA profiles and obesity-linked parameters in Indian patients with OSA.

METHODS

Selection of miRNAs

For this pilot study, we short-listed miR-21, miR-27, miR-29 and let-7 as they have validated roles in obesity. These miRNAs were also found to be dysregulated in a recent study by Li, et al., [17]. Here in this study, we extend to investigate the potential role of these miRNA targets in obesity-associated OSA.

Study Participants

For this prospective study, a total of 160 subjects were recruited in the Out-Patient Department at All India Institute of Medical Sciences, New Delhi who underwent screening procedures. Of these, subjects with cardiovascular and metabolic disorders such as diabetes (n=90) were excluded from the study. The remaining 70 subjects were divided into 3 groups based on their BMI-(i) obese subjects with polysomnography-proven OSA (n=20), (ii) non-obese with polysomnography-proven OSA (n=20) and (iii) control subjects who were exclusive of any disorders (n=30) as depicted in the patient flow diagram (Figure1). The subjects recruited were between 25-55 years of age of either gender. The clinical details such as blood lipid profile including serum triglycerides, total cholesterol and high-density

lipoprotein (HDL) were measured for all subjects. The study was approved by the institutional ethics committee at AIIMS, Delhi as well as Amity University, Noida and informed written consent was taken from all the subjects.

Peripheral Blood Mononuclear Cell (PBMC) Isolation

Five milliliters of peripheral blood was collected from all the subjects using a sterilized syringe in EDTA-coated sterile vacutainers by venipuncture. Blood was withdrawn after prior consent from the subjects ensuring total safety and PBMC isolation was performed. Isolated PBMC's were stored until RNA isolation.

RNA Isolation, cDNA Synthesis, and Real-Time Quantitative PCR

Total RNA was isolated from PBMCs using TRIzol Reagent (Thermo-Fisher). miRNA-specific stem-loop primers were designed, custom synthesized and reconstituted as per manufacturer's instructions. cDNA was synthesized with 700 ng of total RNA using stem-loop primers using the High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The stem-loop primer sequences for miR-21, miR-27, miR-29 and let-7 are as follows:

UGUCGGGUAGCUUAUCAGACUGAUGUUGACUGUUGAAUCUCAUGGCAACACCAGUCGAUGGGCU-GUCUGACA (miR-21), CUGAGGAGCAGGGCUUAGCUGCUUGUG AGCAGGGUCCACACCAAGUCGU-GUUCACAGUGGCUAAGUUCCGCCCCCCAG (miR-27), AUGACUGAUUUCUUUUGGUGUUCAGAGU-CAAUAUAAUUUUCUAGCA CCAUCUGAAAUCGGUUAU (miR-29) and UGGGAUGAGGUAGUAGGUU-GUAUAGU UUUAGGGUCACACCCACCACUGGGAGAUAACUAUACAAUCUACUGUCUUU-CCUA (let-7)

miRNA expression analysis was performed using Power-Up SYBR Green master-mix (Applied Biosystems, Austin, TX) based quantitative PCR. cDNA samples from a total of 70 subjects were run in triplicates and normalized with RNU48 (21) and non-template controls. RNU6 was not considered as an endogenous control due to inconsistency in the Ct values, unlike RNU48 which was consistent and showed minimal variation. ΔCt was calculated using the formula: ΔCt=Ct (test gene)-Ct (reference gene). The result of the experiment was represented as relative fold expression ($2^{-\Delta\Delta Ct}$) of miRNA in diseased subjects relative to healthy subjects.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics Software (Version 22.0). Student's t-test was employed to generate p-values. Spearman's correlation test was employed to test a correlation between two variables. Multivariate analysis was performed using the Logistic Regression Method. $p<0.05$ was considered as statistically significant.

RESULTS

Selection of miRNAs

Obesity specific miRNAs such as miR-21, miR-27, miR-29 and let-7 were selected for further investigation in our study.

Study Participants

A total of 70 subjects (40 OSA and 30 healthy) were selected for this pilot study (Figure 1) of which 20 were obese (BMI=35.77 ± 3.93), 20 were non-obese (BMI=27.11 ± 1.91) and 30 were healthy volunteers (BMI=24.35 ± 1.41). The clinical characteristics and anthropometric measurements of the participants is presented in Table 1. The participants in each group included 11 males and 9 females (obese), 18 males and 2 females (non-obese) and 15 males and 15 females (control) respectively. The levels of serum triglycerides in the OSA subjects was higher than the non-obese OSA and the control population ($p=0.013$). The total cholesterol levels were also higher in the obese subjects with OSA than the healthy controls ($p<0.0001$). However, there was no difference in the total cholesterol levels in the obese and non-obese subjects with OSA.

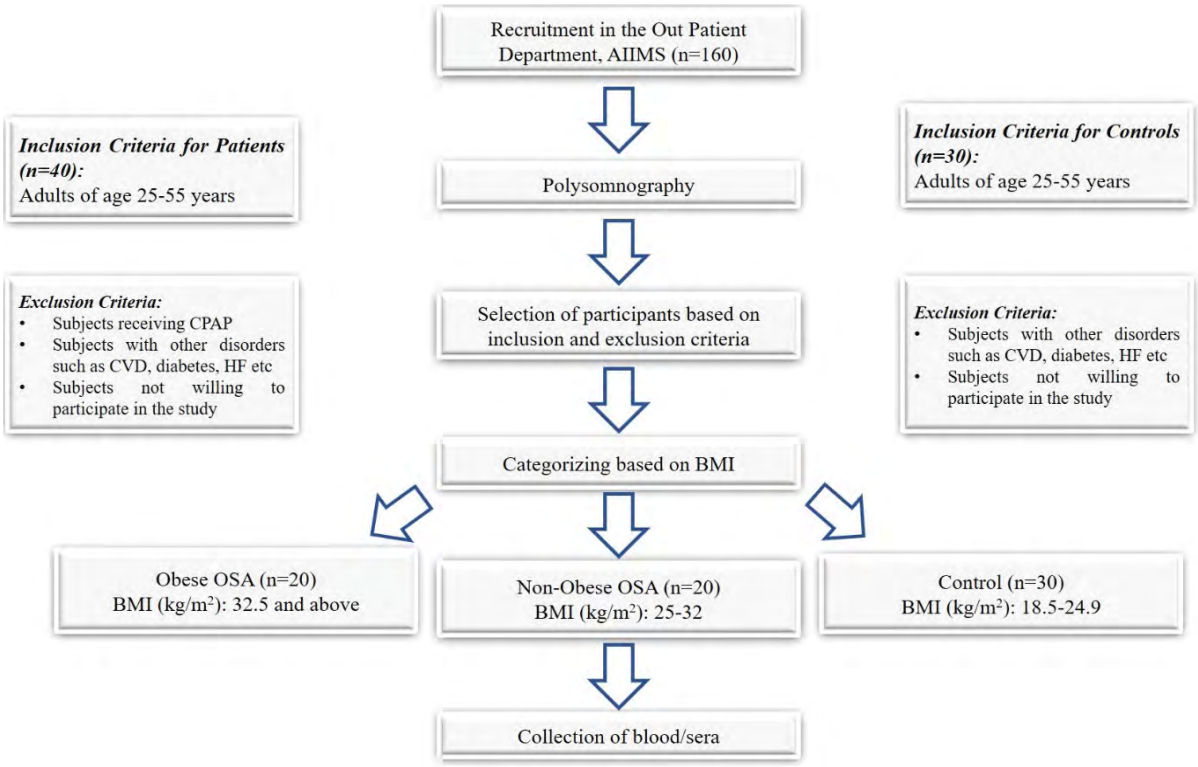

Figure 1 Patient Flow Diagram. Patients were screened and enrolled for this prospective study based on the inclusion and exclusion criteria. Those with disorders such as cardiovascular, diabetes, heart failure, etc were ruled out from the study. A total of 70 subjects were included for the study among which 20 were obese OSA, 20 were non-obese OSA and 30 were healthy controls. CVD: Cardiovascular Diseases; HF: Heart Failure; CPAP: Continuous Positive Airway Pressure; BMI: Body Mass Index

Table 1 Clinical characteristics and anthropometric measurements of the study participants

Parameter	Obese OSA	Non-obese OSA	Healthy Controls	Total Mean ± SD	p-value
Gender (M/F)	11, 9	18, 2	15/15	44/26	-
Age (years)	48.75 ± 11.11	47.5 ± 11.15	30.00 ± 6.98	42.08 ± 13.04	<0.0001
Height (cms)	160.25 ± 9.98	166.3 ± 9.81	167.42 ± 9.13	164.66 ± 9.99	0.058
Weight (kgs)	92.81 ± 11.88	75.14 ± 9.28	71.41 ± 9.50	79.79 ± 13.82	<0.0001
BMI (kg/m^2)	35.77 ± 3.93	27.11 ± 1.91	24.35 ± 1.41	29.07 ± 5.55	<0.0001
ALK P (mg/dL)	141.95 ± 76.73	142.6 ± 61.29	70.75 ± 23.88	118.23 ± 66.66	0.004
AST (mg/dL)	49.32 ± 29.62	25.01 ± 9.37	39.57 ± 15.51	37.56 ± 21.68	<0.0001
ALT (mg/dL)	49.47 ± 22.29	43.00 ± 22.62	25.84 ± 16.12	39.26 ± 22.52	0.002
TG (mg/dL)	130.25 ± 35.11	115.05 ± 26.08	104.76 ± 13.19	116.69 ± 27.99	0.013
TC (mg/dL)	169.65 ± 34.51	174.35 ± 19.96	136.17 ± 28.92	159.06 ± 32.37	<0.0001
HDL (mg/dL)	39.71 ± 9.54	39.73 ± 8.91	39.39 ± 12.31	39.61 ± 10.18	0.993

N: number of subjects in each group; BMI: Body Mass Index; AHI: Apnea-Hypopnea Index; ALK.P: Alkaline Phosphatase; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; TG: Triglycerides; TC: Total Cholesterol; HDL: High-Density Lipoprotein

Differential Expression of miRNAs in OSA than Healthy Controls

To investigate the miRNA profile in OSA, a panel of miRs (miR-21, miR-29, miR-27, and let-7) were selected and their expression was checked using quantitative PCR. We observed differential expression of all these miRNAs in OSA subjects compared to healthy controls as depicted in Figure 2. Real-time analysis revealed a marginal increase in the expression level of miR-21 (OSA: ΔCt=4.25 ± 0.28; Controls: ΔCt=4.48 ± 0.48; p<0.0001), and significant decrease in the expression levels of miR-27 (OSA: ΔCt=5.36 ± 0.39; Controls: ΔCt=4.41 ± 0.28; p<0.0001) , miR-29 (OSA: ΔCt=5.45 ± 0.47; Controls: ΔCt=3.11 ± 0.21; p<0.0001) and let-7 (OSA:ΔCt=5.76 ± 0.61; Controls: ΔCt=4.41 ± 0.28; p<0.0001) in OSA subjects compared to the healthy controls. Multivariate analysis (Table 2) in different age

groups suggested significant associations between miRNA expression and various clinical parameters. Age category of 25-35 revealed significant associations of miR-27 (p=0.003) and let-7 (p=0.013). In the age group of 35-45, we observed significant associations between miR-21 (p<0.0001) and miR-29 (p=0.005). Interestingly, within the age group of 45-55 significant associations were observed for all four miRNAs (p<0.05).

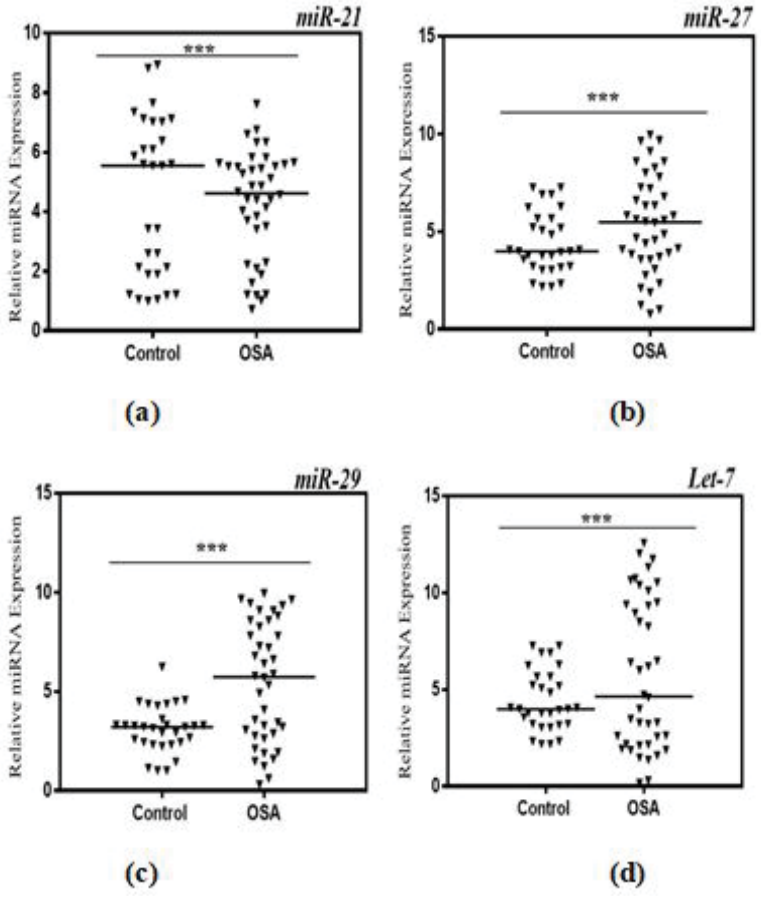

Figure 2 Relative expression levels of miRNAs in OSA and Control groups. The plots have been generated using ΔCt values. The horizontal lines indicate the median. Of the four miRNAs only miR-21 was found to be upregulated in OSA (n=40) compared to the healthy controls (n=30). The data is representative of 5 consecutive experiments. ***p<0.0001

Table 2 Association of miRNA expression with anthropometric and clinical parameters in different age groups

Age Group	miRNA	AGE	BMI	TG	TC	HDL	ALK.P	AST	ALT	Multivariate
25-35	miR-21	0.999	0.874	0.028	0.172	0.17	0.154	0.471	0.114	0.093
	miR-27	0.101	0.744	0.359	0.201	0.211	0.464	0.268	0.177	0.003
	miR-29	0.357	0.458	0.047	0.226	0.094	0.171	0.117	0.068	0.093
	let-7	0.274	0.198	0.528	0.963	0.343	0.111	0.406	0.867	0.013
35-45	miR-21	0.202	0.771	0.351	0.101	0.021	0.073	0.196	0.023	<0.0001
	miR-27	0.392	0.934	0.539	0.293	0.436	0.884	0.201	0.37	0.439
	miR-29	0.838	0.38	0.044	0.172	0.251	0.1	0.038	0.383	0.005
	let-7	0.568	0.281	0.435	0.658	0.533	0.846	0.292	0.869	-
45-55	miR-21	0.632	0.627	0.283	0.094	0.177	0.002	0.154	0.61	0.002
	miR-27	0.966	0.735	0.161	0.107	0.032	0.002	0.038	0.103	0.002
	miR-29	0.948	0.451	0.141	0.067	0.149	<0.001	<0.001	<0.001	0.026
	let-7	0.606	0.745	0.032	0.011	0.01	0.072	<0.001	<0.001	0.049

ALK.P: Alkaline Phosphatase; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; BMI: Body Mass Index; HDL: High-Density Lipoprotein; TG: Triglycerides; TC: Total Cholesterol

Differential Expression of miRNAs in Obese and Non-obese Subjects with OSA

OSA being well documented to be associated with obesity, here we investigated whether these miRNAs are also differentially expressed between obese and non-obese OSA subjects (Figure 3). Interestingly, we observed a significant increase in the relative expression of miR-21 in non-obese patients (ΔCt=3.80 ± 0.40) than the obese OSA (ΔCt=4.71 ± 0.39) and healthy subjects (ΔCt=4.48 ± 0.48) (p=0.0001). miR-27 expression was also significantly higher in non-obese (ΔCt=4.50 ± 0.37) as compared to obese OSA subjects (ΔCt=6.25 ± 0.65) but marginally lower than healthy subjects (ΔCt=4.41 ± 0.28) (p<0.0001). A similar trend was observed for let-7 in non-obese subjects (ΔCt=2.55 ± 0.35) compared to obese (ΔCt=8.77 ± 0.58) and controls (ΔCt=4.41 ± 0.28) (p<0.0001). However, miR-29 expression was found to be significantly higher in the obese OSA subjects (ΔCt=2.93 ± 0.37) compared to the non-obese (ΔCt=7.98 ± 0.35) and marginally higher than controls (ΔCt=3.11 ± 0.21) (p<0.0001).

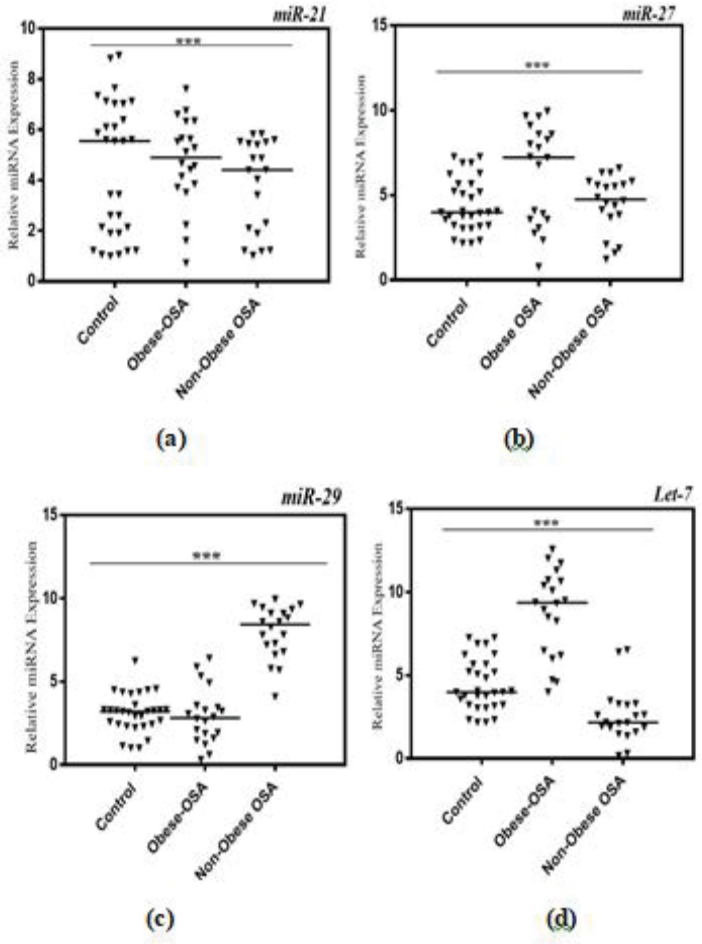

Figure 3 Association of miRNA expression with obesity. Quantitative PCR data showing relative miRNA expression. The plots have been generated using ΔCt values. The upregulation of miR-21 was observed in non-obese subjects with OSA compared to the obese subjects and healthy controls. miR-27 expression was found to be lesser in obese subjects than non-obese and healthy. miR-29 was downregulated in non-obese subjects whereas let-7 was downregulated in obese subjects compared to the other groups. The data is representative of 5 consecutive experiments. *** p<0.0001

Association of miRNA Expression Levels with Anthropometric and Clinical Characteristics

We further correlated miRNA expression with various clinicopathological parameters such as levels of TG, ALT, AST, HDL, BMI, and AHI (Table 3). A significant positive correlation was observed for all miRNAs and AHI as shown in Table 3, indicating a direct link with OSA. Similarly, miR-21 expression was also positively correlated with AST suggesting a possible role of miR-21 in liver function (r=0.43, p=0.016). A significant negative correlation was observed for miR-27 expression with triglyceride levels (r=-0.37, p=0.041), BMI (r=-0.48, p=0.006) and AST (r=-

0.37, p=0.041) while miR-29 showed positive correlation with BMI (r=0.39, p=0.028) only. However, let-7 did not show any significant correlation with any parameter.

Table 3 Correlation between miRNA expression profiles and clinical and anthropometric characteristics using Spearman Correlation Test

Parameter	miR-21	miR-27	miR-29	Let-7
AHI	r=0.39	r=0.08	r=-0.15	r=-0.30
	p=0.03	p=0.044	p=0.02	p=0.01
BMI	r=0.19	r=-0.48	r=0.39	r=-0.24
	p=0.29	p=0.006	p=0.028	p=0.19
TG	r=0.06	r=-0.37	r=-0.08	r=-0.04
	p=0.72	p=0.041	p=0.66	p=0.80
AST	r=0.43	r=-0.35	r=0.02	r=-0.12
	p=0.016	p=0.041	p=0.89	p=0.50
ALT	r=-0.19	r=-0.10	r=-0.25	r=0.08
	p=0.31	p=0.57	p=0.170	p=0.64
HDL	r=0.10	r=0.13	r=0.06	r=-0.13
	p=0.59	p=0.47	p=0.71	p=0.47

AHI: Apnea-Hypopnea Index; ALK.P: Alkaline Phosphatase; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; BMI: Body Mass Index; HDL: High-Density Lipoprotein; TG: Triglycerides, TC: Total Cholesterol

DISCUSSION

OSA is a major public health concern associated with numerous societal consequences like mood swings, impaired work performances and has multiple implications that lead to various metabolic and cardiovascular risks such as coronary artery disease (CAD) [22], ischemic stroke [23], atherosclerosis [24], hypertension [25], diabetes [26]. One of the major risk factors for OSA is obesity. The prevalence of obesity in India is due to various reasons such as age, lifestyle, geographical differences, etc. More than 100 million Indians suffer from obesity and obesity-related metabolic and cardiovascular disorders.

Abundant studies have suggested an escalation in the inflammatory profile in OSA subjects indicative of an evident risk for cardiovascular diseases. These inflammatory markers like CRP, TNF-α, interleukins (IL-6, IL-8), etc. are also dysregulated in most of the infections and diseases. Moreover, the prevalence of metabolic diseases is increasing worldwide including India. Therefore, identification of OSA-specific biomarkers is warranted for better clinical relevance and understanding of the pathophysiology of OSA.

Studies have demonstrated the role of miR-21 during early stages of embryonic development [27]. Similarly miR-27, miR-29, and let-7 are reported to play a vital role in embryonic stem cell differentiation and cell proliferation [28], respectively. Many studies have attempted to establish miRNAs as potential biomarker candidates for the diagnosis, prognosis, and treatment of many diseases such as obesity, cardiovascular and metabolic disorders. However, the critical linkage between such disorders through experimental analysis is limited. The role of epigenetics in various metabolic regulation which gets dysregulated in OSA and OSA linked diseases is emerging as mentioned in our recent review. In this study, we investigated a miRNA signature in OSA and elucidated their association with obesity by differentiating the obese from non-obese subjects based on the obtained miRNA expression profiles. We have selected four miRNAs (miR-21, miR-27, miR-29 and let-7) for their dysregulation in obesity. Our study demonstrated that these miRNAs are differentially expressed in OSA as compared to healthy subjects.

Obesity is a well-known risk factor of OSA and is associated with poor clinical outcomes such as CVD and diabetes [29,30]. Therefore, identification of OSA-specific biomarkers such as miRNAs that can correlate with obesity is essential to understand the pathophysiology of the disease. In our study design, we included obesity and the OSA patient cohort population as our initial step to understanding the role of molecular signatures here as miRNA. We have found that the level of miR-21 was inversely correlated with obesity which is also supported by a Spanish study by Murri, et al., [31]. However, as per recent evidence, miR-21 has also been seen to be up-regulated in obesity pointing towards its dual role [32]. We also found an increase in the expression of miR-29 in obese subjects with OSA as compared to the non-obese OSA subjects. There is limited literature on the involvement of miR-29 in obesity and OSA. Further, de-

creased expressions of miR-27 and let-7 were found in the obese subjects than non-obese subjects with OSA. miR-27 and let-7 are key players in the regulation of adipogenesis. Overexpression of these miRNAs are known to lower the levels of Peroxisome proliferator-activated receptor-gamma (PPARG) and CCAAT/enhancer-binding protein alpha (C/EBPa) two major adipogenesis regulators that are largely responsible for adipogenic differentiation [33,34]. The functions of miRNAs as stimulators or inhibitors of adipogenesis have been well elucidated in a previous study [35].

We also tried to establish a correlation between the expression of miRNAs with various obesity-linked parameters. We found positive correlations of miR-21, miR-27, miR-29 and let-7 expression with AHI and miR-29 expression with BMI whereas negative correlations of miR-27 expression with BMI, TG and AST (Table 3). This points towards a close relationship between these miRNAs with OSA and obesity, respectively. TG and AST are known to play crucial roles in obesity-associated lipid abnormalities [36,37]. We also observed a significant positive correlation between AHI and AST ($r=0.421$, $p=0.020$) suggesting that OSA influences lipid metabolism as corroborated by a recent study [36].

As mentioned in our recent review [11], risk factors such as age, obesity, craniofacial anatomy, etc. deeply influence the onset, pathophysiology, and severity of OSA. Older people experience the easy collapse of the airway because the efficiency of the dilator muscles in the upper airway reduces with age [38].

Our multivariate analysis of miRNA expression in different age groups of OSA subjects indicates significant associations of miRNA expression in middle age group OSA subjects. The maximum significant associations were observed in the age group of 45-55 indicating that age and OSA might be related at a molecular level. Therefore this study elucidates that miRNAs have a potential role and may serve as a probable molecular player for OSA.

miRNA regulated target genes are crucial as they can play a critical role in various metabolic pathways. For instance, miR-21 regulates the level of transforming growth factor, beta receptor II through TGF-β/Smad signaling pathway [39], adiponectin and activator protein-1 expression to promote adipogenesis during mesenchymal stem cell differentiation [40]. Similarly, the upregulation of miR-29 is attributed to a substantial decrease in IRS-1 expression levels resulting in impaired insulin signaling [41]. A study by Kang et al demonstrated that miR-27 suppresses adipogenesis by targeting prohibitin and impairs the mitochondrial signaling in adipose-derived stem cells [42]. Likewise, evidence states that let-7 regulates body weight and size in obesity by targeting high-mobility group AT-hook 2, cyclin-dependent kinase-6, DOT-1 like histone H3 methyltransferase and Lin-28 gene [43]. All these targets are associated with obesity and hence may have a crucial role in OSA. Our preliminary *in-silico* study also points towards certain potential targets for these differentially expressed miRNAs in obesity-linked OSA.

Recently, Li, et al., [17] reported 104 differentially expressed miRNAs in OSA. However, as mentioned earlier, miRNA profiling was done only in 3 OSA subjects and 3 controls and no further validation was carried out. Moreover, miRNA profiling for OSA in the Indian population is still elusive. Hence, our study is of clinical relevance since we not only report the differential expression of miRNAs in obese and non-obese patients of OSA and controls but also correlated miRNA expression with various clinical characteristics such as AHI, BMI and lipid profiles.

CONCLUSION

In summary, our study throws light on a specific miRNA signature (miR-21, miR-27, miR-29, and let-7) with significant correlations with various clinical and anthropometric parameters (AHI, TG, BMI, and AST) in Indian OSA subjects. We conclude that these miRNAs, when correlated with such parameters, can be implicated as potential players in OSA. Further analysis involving the identification of molecular targets of these miRNAs is warranted to understand the molecular mechanisms underlying the pathophysiology of OSA. These miRNAs will be further studied in OSA associated cardiovascular and metabolic disorders.

DECLARATIONS

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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