Sleep and Breathing (2019) 23:171–178
https://doi.org/10.1007/s11325-018-1678-7

SLEEP BREATHING PHYSIOLOGY AND DISORDERS • ORIGINAL ARTICLE

Non-alcoholic fatty liver disease is an independent risk factor for inflammation in obstructive sleep apnea syndrome in obese Asian Indians

Surya Prakash Bhatt[1] · Randeep Guleria[1] · Naval K. Vikram[2] · A. K. Gupta[3]

Received: 26 August 2017 / Revised: 24 May 2018 / Accepted: 31 May 2018 / Published online: 22 July 2018
© Springer Nature Switzerland AG 2018

Abstract

Introduction Obstructive sleep apnea (OSA) has been estimated to affect 4–11% of the population and causes systemic inflammation which leads to metabolic syndrome (MS). Non-alcoholic fatty liver disease (NAFLD) is also associated with MS whether NAFLD is an additional risk factor for the systemic inflammation that occurs in OSA is unclear.

Objective In this study, we aimed to analyze the association of OSA and NAFLD with MS and systemic inflammation in Asian Indians.

Methods Total 240 (132 males and 108 females) overweight/obese subjects [body mass index (BMI > 23 kg/m^2)] were recruited; of these, 124 subjects had OSA with NAFLD, 47 had OSA without NAFLD, 44 did not have OSA but had NAFLD and 25 had neither OSA nor without NAFLD. Severity of NAFLD was based on abdomen ultrasound and of OSA on overnight polysomnography. Clinical examinations, anthropometry, body composition, metabolic parameters, and inflammatory biomarkers were recorded.

Results Serum levels of leptin, macrophage migration inhibitory factor (MIF), interleukin-6 (IL-6), high sensitive C-reactive protein (Hs-CRP), and tumor necrosis factor alpha (TNF-α) were significantly higher, and adiponectin levels were significantly lower in OSA with NAFLD subjects. Prevalence of MS was significantly increased in OSA and NAFLD subjects ($p = 0.001$). There was a strong association and correlation between leptin, IL-6, Hs-CRP, MIF, and TNF-α in OSA and NAFLD subjects. Multivariate logistic regression showed that OSA was positively associated with the NAFLD [odds ratio (OR), (95% confidence interval (CL) 3.12 (2.58–7.72), ($P = 0.002$)].

Conclusion NAFLD is an additional risk factor in OSA subject which contributes to systemic inflammation in Asian Indians.

Keywords Obstructive sleep apnea · Non-alcoholic fatty liver disease · Insulin resistance · Apnea hypopnea index · Metabolic syndrome

Introduction

Obstructive sleep apnea (OSA) is a common sleep breathing disorder characterized by repetitive upper airway collapse during sleep [1]. It is a common disorder, with prevalence estimated at 4% for men and 2% for women among adults in the Western countries, but the prevalence of OSA increases to as much as 30% to 50% in obese individuals [2, 3]. In the past few years, the association of OSA and non-alcoholic fatty liver disease (NAFLD) has raised considerable interest, and several studies have suggested the potential independent contribution of OSA and NAFLD in the pathogenesis of metabolic abnormalities, including type 2 diabetes mellitus (T2DM), metabolic syndrome (MS), and cardiovascular disease (CVD).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11325-018-1678-7) contains supplementary material, which is available to authorized users.

✉ Randeep Guleria
randeepg@hotmail.com

[1] Department of Pulmonary Medicine and Sleep Disorders, All India Institute of Medical Sciences, New Delhi 110029, India

[2] Department of Medicine, All India Institute of Medical Sciences, New Delhi, India

[3] Department of Radiology, All India Institute of Medical Sciences, New Delhi, India

It has been suggested that OSA may be associated with manifestation of metabolic dysfunction and NAFLD [4].

Obesity and OSA are independently associated with metabolic dysfunction and also with systemic inflammation [5], which has been implicated with poor cardiovascular outcomes [6]. The link between obesity and inflammation has been further illustrated by the increased levels of inflammatory markers including cytokines and acute phase proteins like high-sensitive C reactive protein (Hs-CRP) in obese subjects [7]. Further, animal study also has shown that OSA is a trigger for inflammation [8]. This might explain the associated increase in insulin resistance, dyslipidemia, and hypertension in these subjects.

Previous studies has been indicated that Asian Indians have higher predisposition to develop insulin resistance, MS, T2DM, OSA, NAFLD, and coronary heart disease than White Caucasians [9–11]. Misra and Khurana et al. [9] reported that Asian Indians have abnormal body composition consisting of higher body fat and abdominal adiposity that may partially explain the high prevalence of these clinical disorders [9]. Our previous study has shown high Hs-CRP levels in urban Asian Indian adolescents living in India [12]. Further, we showed NAFLD is closely associated with subclinical inflammation in North Indians NAFLD subjects [13]. Bhusan et al [14] reported that Hs-CRP levels were significantly higher in OSA subjects as compared to controls, and these levels were directly proportional to the severity of OSA.

There is paucity of data regarding sub-clinical inflammation in patients with OSA and NAFLD in Asian Indians. We hypothesized that sub-clinical inflammation closely correlates with OSA and NAFLD among Asian Indians and is significantly more than due to either disease process alone. This may increase the risk in this group to cardiovascular events. To test this hypothesis, we designed a study by analyzing clinical anthropometry, body composition, metabolic profiles, fasting insulin, adiponectin, leptin, macrophage migration inhibitory factor (MIF), interleukin-6 (IL-6), Hs-CRP, and tumor necrosis factor (TNF-α) levels in different groups of overweight/obese subjects with and without OSA and NAFLD.

Methodology

Subjects

In this study, 240 overweight/obese (132 males and 108 females) subjects [body mass index (BMI > 23 kg/m^2)] were evaluated at All India Institute of Medical Sciences, New Delhi, India between May 2012 and December 2017. One hundred twenty-four subjects had OSA with NAFLD (group 1); 47 had OSA without NAFLD (group 2); 44 did not have OSA but had NAFLD (group 3), and 25 had neither OSA nor NAFLD (group 4) (Fig. 1). The institutional ethical clearance has been approved, and written informed consent was taken from each subject. NAFLD was diagnosed by abdominal ultrasonography [15] in subjects with alcohol intake of less than 20 g/day and OSA was diagnosed on the base of a overnight polysomnography as detailed below. Subjects with chronic obstructive pulmonary disease, advanced lung disease, mechanical upper airway obstruction, known T2DM, CVD, presence of other liver diseases (alcoholic liver disease, viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, biliary obstruction), severe organ damage, human immunodeficiency virus infection, pregnancy and lactation, or with any pro-inflammatory state were excluded from the study.

Clinical profile

Clinical details were recorded in a structured proforma. Blood pressure was measured over the right arm in sitting position according to the protocol defined by the eighth Report of Joint National Committee on Prevention, Detection and Treatment of High Blood Pressure using a standard mercury sphygmomanometer. Subjects were advised not have smoked or taken caffeine at least 30 min before the measurement and were rested for 5 min. In case of any abnormal reading (≥ 140/90 mmHg), another reading was obtained after an interval of 5 min.

Anthropometric measurements

For measurement of weight, subject was instructed to stand still on the platform. After removing heavy clothing, weight was measured to the nearest of 0.1 kg. Height was measured using stadiometer with head held in Frankfort plane to the nearest of 0.1 cm. Waist circumference (WC), hip circumference (HC), mid thigh circumference (MTC), and skinfold thickness (triceps, biceps, anterior axillary, suprailiac, subscapular, and lateral thoracic) were measured according to standard protocols [12]. All subjects were carefully examined physically.

Biochemical investigations

Fasting blood glucose (FBG), lipid profile and liver function test: The estimation of FBG was investigated after a 12-h overnight fasting with the help of commercially available kit (Randox lab ltd, United Kingdom). Estimation of FBG, total cholesterol (TC), serum triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were recorded as previously described [16].

Fasting insulin and homoeostasis modal assessment for insulin resistance (HOMA-IR): Fasting insulin levels were measured using commercially available radioimmunoassay kits (Immunotech, France) [17]. The intra and inter assay

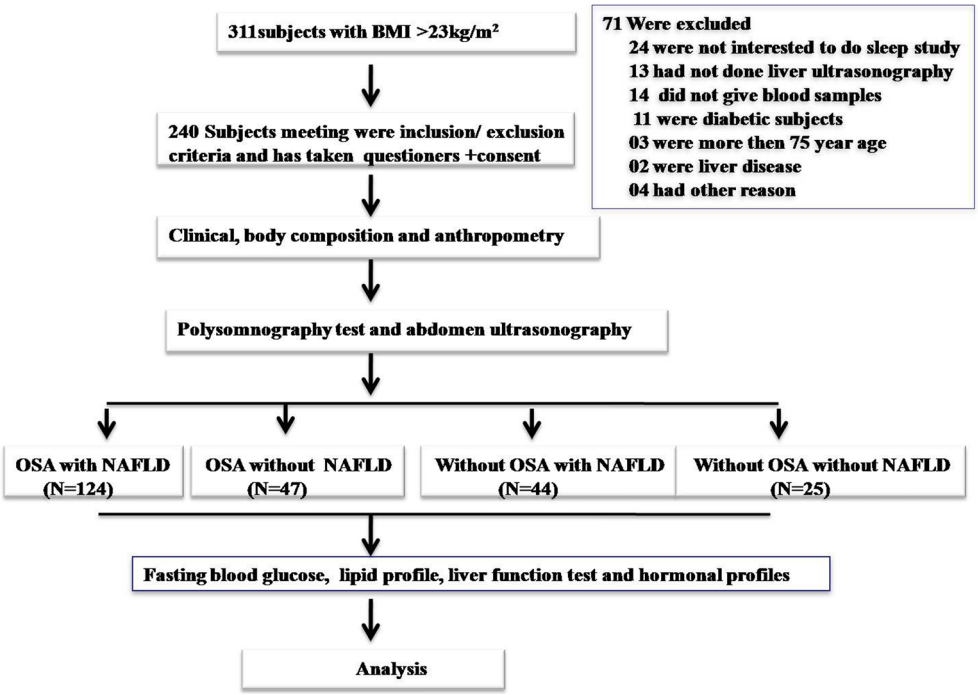

Fig. 1 Flow chart of patients included in the study

percentage coefficient and coefficient of variation was 2.03 and 1.9% for insulin and 3.2 and 4.2% for HOMA respectively.

Assessments of inflammatory biomarkers: Levels of adiponectin, Hs-CRP, and TNF-α were measured as previously described [12, 18, 19]. Levels of leptin, MIF, and IL-6 were recorded using enzyme-linked immunosorbent assay (Linco Research Inc., USA). The intra and inter assay percentage coefficient and coefficient of variation was 5 and 4%; 2.1 and 3.3%; 1.8 and 2.9%; 2.2 and 3.4%; 1.8 and 2.3%; 2.5 and 3.1% for adiponectin, hs CRP, TNF-α, leptin, MIF, and IL-6 respectively.

Ultrasound imaging

All subjects were assessed by a liver ultrasound using 3.5-MHz curvilinear probe (Siemens-G 60 S 2004, Germany). The definition of NAFLD was based on a comparative assessment of image brightness relative to the kidneys, in line with previously reported diagnostic criteria [15]. Severity of NAFLD was classified according to the brightness compared to the kidneys, blurring of gall bladder wall, of hepatic veins, and of portal vein. The radiologist performing the ultrasound was unaware of the clinical history, anthropometry profiles, biochemical investigations, and sleep study results.

Polysomnography

All subjects were called for overnight polysomnography (PSG, (Medi palm; Braebon Medical Corp., Canada) at 9:00 P.M. and were attached to Alice 3 adult computerized PSG system using the various leads and devices through standard gold cup electrodes. All subjects were classified according to apnea–hypopnea index (AHI). The various parameters monitored were electrooculogram, electroencephalogram, electromyogram, electrocardiogram and airflow (with an oro-nasal thermistor), chest and abdominal efforts, and arterial oxyhemoglobin saturation by pulse oximeter. Continuous positive airway pressure (C-PAP) titration was also done. At least 6 h of PSG was considered as a complete study. The recordings were analyzed with 60-s epoch, and sleep stages were scored according to the standard criteria [20]. Obstructive apneas and hypopneas are typically distinguished from central events by the detection of respiratory efforts during the event.

Statistical analysis

The data was confirmed for approximate normality. Categorical data was analyzed by Chi-squared test, with Fisher correction when appropriate, and expressed as absolute number (%). Continuous variables were expressed as the mean ± standard deviation to summarize the variables. All continuous values were performed using the *Z* score method. The influence of the groups (1 vs 2, 1 vs 3, 1 vs 4, 2 vs 3, and 2 vs 4) was estimated by the analysis of covariance test with multiple comparisons. Pearson's correlation coefficient and significance of "*r*" was used to compare between the inflammatory markers and clinical parameters. A multivariate logistic regression analysis of covariance was used to assess differences in leptin, adiponectin, MIF, IL-6, Hs-CRP, and TNF-α are among the groups, with adjustment for WC, fasting

insulin, and TG to avoid possible confounder effects. All analyses were conducted using SPSS (Chicago, USA). Values of $p < 0.05$ was considered statistically significant.

Results

Demographic, clinical, body composition and anthropometric profiles

Demographic, clinical, body composition and anthropometric profiles and detailed multi variable comparison (group 1 vs 2, 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4, and 3 vs 4) are presented in Tables 1 and 2. The prevalence of MS in groups 1, 2, 3, and 4 was 80, 75, 62, and 48% respectively. AHI levels were significantly increased in group 1 as compared to groups 2, 3, and 4 ($p = 0.001$). Alcohol intake was significantly higher in group 1 but there was no significant difference between tobacco intakes was seen in all groups. Percentages of regular tobacco and alcohol users were 16.0 and 2.5% respectively. It was observed that the mean values of blood pressure (systolic and diastolic), BMI, fat mass (kg), fat-free mass, % body fat, WC, HC, MTC, neck, subscapular, suprailiac, lateral thoracic, and thigh were significantly higher in OSA with NAFLD group as compared to the other groups.

Biochemical profiles and inflammatory markers

The biochemical and inflammatory investigations of the four groups and multi variable comparison are given in Tables 3 and 4. Mean values of FBG ($p = 0.004$), serum TG ($p = 0.01$), TC ($p = 0.02$), HDL ($p = 0.005$), LDL ($p = 0.002$), AST ($p = 0.01$), ALT ($p = 0.03$), ALP ($p = 0.05$), fasting insulin ($p = 0.001$), and HOMA-IR ($p = 0.001$) were significantly increased in OSA with NAFLD group as compared to other groups. Values of IL-6 ($p = 0.05$), leptin ($p = 0.006$), MIF ($p = 0.001$), Hs-CRP ($p = 0.01$), and TNF-α ($p = 0.001$) were significantly higher, and serum adiponectin levels ($p = 0.04$) were lower in OSA with NAFLD group (Fig. 2).

Multivariate logistic regression

Multivariate logistic regression showed that OSA was positively associated with the NAFLD [Odds ratio (OR), (95% confidence interval (CL) 3.12 (2.58–7.72), ($p = 0.002$)].

Correlation analysis of inflammatory biomarkers with other variables

Hs-CRP (supplementary Table 1): In OSA and NAFLD group, Hs-CRP levels correlated with AHI ($r = 0.497$, $p = 0.001$), BMI ($r = 0.6059$, $p = 0.002$), fat mass ($r = 0.5949$, $p = 0.05$), % body fat ($r = 0.5831$, $p = 0.04$), MIF ($r = 0.5956$, $p = 0.04$), IL-6 ($r = 0.6071$, $p = 0.005$), and TNF-α ($r = 0.6971$, $p = 0.004$) levels. A strong negative correlation was found between Hs-CRP and serum adiponectin ($r = -0.5654$, $p = 0.001$).

IL-6 (supplementary Table 2): In group 1, IL-6 levels correlated with AHI ($r = 0.613$, $p = 0.003$), % body fat ($r = 0.6138$, $p = 0.004$), fasting insulin ($r = 0.6135$, $p = 0.005$), leptin ($r = 0.5939$, $p = 0.03$), Hs-CRP ($r = 0.6071$, $p = 0.005$), and TNF-α ($r = 0.5338$, $p = 0.004$) levels. In without OSA and with NAFLD group, IL-6 levels had a positive correlation

Table 1 Demographic, clinical, and body composition investigations

Variables		Group 1 ($n = 124$)	Group 2 ($n = 47$)	Group 3 ($n = 44$)	Group 4 ($n = 25$)	Overall p value
Age (yrs)		44.8 ± 9.1 ‡‡	44.2 ± 9.1	39.5 ± 10.5 #, ‡	41 ± 8.5	0.08
Sex	Males	64 (51.6)	25 (53.2)	26 (59)	17 (68)	0.05
	Females	60 (48.4)	22 (46.8)	18 (41)	08 (32)	
Apnea hypopnea index (events per hour of sleep)		22.9 ± 9.4 *,†	13.5 ± 6.4 ‡,@	4.3 ± 2.4	2.3 ± 1.1	0.001
SYBP (mmHg)		131.4 ± 11.5 ¶	130.2 ± 15.6	126 ± 20.6 #, @	120 ± 16.9	0.001
DYBP (mmHg)		84.4 ± 14.4 ¶	83.5 ± 13.6	82.2 ± 15.4 @	80.1 ± 13.6	0.003
Pulse rate		79.70 ± 7.8	79.71 ± 5.9	76.6 ± 5.1	76.83 ± 4.6	0.11
BMI (kg/m^2)		33.3 ± 7.9 ¶	32.5 ± 6.9	31.0 ± 8.3 #,@	28.5 ± 8.6	0.003
Fat mass (kg)		40.45 ± 17.4 *	35.6 ± 14.2	31.1 ± 14	30.5 ± 9.5	0.02
Fat-free mass (kg)		54.1 ± 12.1 *	52.4 ± 11.7	48.03 ± 12.1 @	45.7 ± 9.7	0.002
Total body water (kg)		40.6 ± 8.6	38.2 ± 9.6	35.2 ± 8.7	33.2 ± 8.3	0.5
Body fat (%)		40.2 ± 13.6 †	38.2 ± 11.6	37.1 ± 12.8 #,@	34.6 ± 11.6	0.002

Results are shown as mean ± SD. OSA with NAFLD (group 1), OSA without NAFLD (group 2), without OSA with NAFLD (group 3), and without OSA and without NAFLD (group 4). p value ≤ 0.05 is statistically significant. * group 1 vs 2, 1 vs 3, and 1 vs 4 ($p \leq 0.05$); # group 3 vs 4 ($p \leq 0.05$); † group 1 vs 3 and 1 vs 4 ($p \leq 0.05$); ¶ group 1 vs 4 ($p \leq 0.05$); ‡‡ group 1 vs 3 ($p \leq 0.05$); ‡ group 2 vs 3 ($p \leq 0.05$); @ group 2 vs 4 ($p \leq 0.05$). *SYBP* systolic blood pressure, *DYBP* diastolic blood pressure, *BMI* body mass index

Table 2 Anthropometry profile

Variables	Group 1 ($n = 124$)	Group 2 ($n = 47$)	Group 3 ($n = 44$)	Group 4 ($n = 25$)	Overall p value
WC (cm)	106.9 ± 13.3†	104.2 ± 14.6	102 ± 13.5$^{@}$	100 ± 15.6	0.001
HC (cm)	109.5 ± 13.2*	106.5 ± 23.5	103 ± 16.9 $^{\#,@}$	101.7 ± 15.9	0.003
MTC (cm)	55.8 ± 7.4¶	54.1 ± 8.6	53.2 ± 8.9	52 ± 7.9	0.005
Mid arm	32.16 ± 7.6¶	30 ± 6.5	29.7 ± 6.4	24.6 ± 5.6	0.6
Neck (mm)	38.74 ± 5.6¶	38.3 ± 3.6	36.2 ± 4.04	32.1 ± 3.1	0.0004
Biceps (mm)	16.8 ± 7.07	15.4 ± 6.7	17.36 ± 5.4	15.2 ± 5.5	0.9
Triceps (mm)	25.0 ± 10.5	24.3 ± 9.3	24.3 ± 7.7	22.2 ± 9.3	0.44
Subscapular (mm)	30 ± 8.1	29.2 ± 9.8	27 ± 5.6	26 ± 6.5	0.2
Anti-axillary (mm)	17 ± 6.0	14.6 ± 5.3	13.27 ± 5.2	13.7 ± 5.1	0.5
Suprailiac (mm)	31.8 ± 9.8*	29.2 ± 10.5	28.1 ± 9.5	27 ± 8.9	0.02
Lateral thoracic (mm)	33.7 ± 11.1¶	31.9 ± 12.5	30.1 ± 11.9	28.9 ± 9.8	0.02
Thigh (mm)	30.1 ± 11.3†	26 ± 9.9	25.4 ± 6.6	24.7 ± 8.1	0.05

Results are shown as mean ± SD. OSA with NAFLD (group 1), OSA without NAFLD (group 2), without OSA with NAFLD (group 3), and without OSA and without NAFLD (group 4). p value ≤ 0.05 is statistically significant. * group 1 vs 2, 1 vs 3 and 1 vs 4 ($p \le 0.05$); $^{\#}$ group 3 vs 4 ($p \le 0.05$); † group 1 vs 3 and 1 vs 4 ($p \le 0.05$); ¶ group 1 vs 4 ($p \le 0.05$); ‡‡ group 1 vs 3 ($p \le 0.05$); ‡ group 2 vs 3 ($p \le 0.05$); $^{@}$ group 2 vs 4 ($p \le 0.05$). *WC* waist circumference, *HC* hip circumference, *MTC* mid thigh circumference

with serum TG ($r = 4752$, $p = 0.05$), TC ($r = 0.4753$, $p = 0.05$), and LDL ($r = 0.5333$, $p = 0.01$).

Leptin (supplementary Table 3): In OSA and NAFLD group, leptin levels correlated with AHI ($r = 0.497$, $p = 0.001$), BMI ($r = 0.6159$, $p = 0.003$), % body fat ($r = 0.6089$, $p = 0.002$), fasting insulin ($r = 0.5968$, $p = 0.005$), IL-6 ($r = 0.5938$, $p = 0.005$), and MIF ($r = 0.6038$, $p = 0.001$).

MIF (supplementary Table 4): In OSA and NAFLD group, MIF levels correlated with AHI ($r = 0.643$, $p = 0.001$), BMI ($r = 0.6013$, $p = 0.03$), % body fat ($r = 0.6156$, $p = 0.007$), TC ($r = 0.6123$, $p = 0.01$), IL-6 ($r = 0.6036$, $p = 0.003$), leptin ($r = 0.6036$, $p = 0.003$), and Hs-CRP ($r = 0.5956$, $p = 0.005$).

TNF-α (supplementary Table 5): TNF-α levels correlated with WC ($r = 6139$, $p = 0.004$), AST ($r = 0.6158$, $p = 0.003$), IL-6 ($r = 0.5339$, $p = 0.005$), and Hs-CRP ($r = 0.6971$, $p = 0.003$) in OSA and NAFLD group. In without OSA and with NAFLD group, TNF-α levels were significantly correlating with serum TG ($r = 0.6012$, $p = 0.005$). In without OSA and with NAFLD group, TNF-α levels were significantly correlating with BMI ($r = 0.6076$, $p = 0.005$).

Table 3 Biochemical profiles of the subjects

Variables	Group 1 ($n = 124$)	Group 2 ($n = 47$)	Group 3 ($n = 44$)	Group 4 (n = 25)	Total p value
FBG (mg/dl)	103 ± 25.2 †	104.1 ± 38.4$^{@}$	98.14 ± 21.2	90.3 ± 24.4	0.004
TG (mg/dl)	189 ± 40.6 *	177 ± 46.9 ‡‡	158.1 ± 55.2#	151 ± 58.9	0.01
T.C (mg/dl)	185 ± 38.3*	180 ± 44.6 ‡‡	178 ± 43.6#	171 ± 39.8	0.02
HDL (mg/dl)	42.4 ± 8.3¶	43.8 ± 11.7	44.6 ± 9.1 #	52.3 ± 10.2	0.005
LDL (mg/dl)	112.6 ± 40.2¶	109 ± 36.5‡‡	109 ± 35.6	98 ± 30.8	0.002
VLDL (mg/dl)	33.5 ± 11.2	32 ± 12.3	31 ± 11.3	30.0 ± 9.6	0.4
AST (IU/L)	44.5 ± 15.9 †	41.4 ± 22.1‡	39.6 ± 19.6#	31.6 ± 15.9	0.01
ALT (IU/L)	60.9 ± 10.3*	54.2 ± 12.9‡‡	52.3 ± 11.9	50.9 ± 10.9	0.03
ALP (IU/L)	240.6 ± 74.3†	242 ± 76.5	235 ± 72.9	235 ± 69.8	0.05
Insulin (μU/ml)	12 ± 4.3*	11.1 ± 4.8$^{@}$	9.3 ± 3.6	9.3 ± 3.8	0.001
HOMA-IR	2.9 ± 0.92*	2.5 ± 0.98‡‡	1.9 ± 0.86	1.6 ± 0.76	0.001

Results are shown as mean ± SD. OSA with NAFLD (group 1), OSA without NAFLD (group 2), without OSA with NAFLD (group 3), and without OSA and without NAFLD (group 4). p value ≤ 0.05 is statistically significant. *group 1 vs 2, 1vs 3 and 1 vs 4 ($p \le 0.05$); #group 3 vs 4 ($p \le 0.05$); † group 1 vs 3 and 1 vs 4 ($p \le 0.05$); ¶ group 1 vs 4 ($p \le 0.05$); ‡ group 2 vs 3, 2 vs 4, and 3 vs 4 ($p \le 0.05$); $^{@}$ group 2 vs 3 and 2 vs 4 ($p \le 0.05$). ‡‡ group 2 vs 4 ($p \le 0.05$). *FBG* fasting blood glucose, *TG* serum triglyceride, *TC* total cholesterol, *HDL* high density lipoprotein, *LDL* low-density lipoprotein, *VLDL* very low density lipoprotein, *ALP* Alkaline phosphate, *ALT* alanine transaminase, *AST* aspartate transaminase, *HOMA-IR* homoeostasis modal assessment for insulin resistance

Table 4 Inflammatory marker investigations

Variables	Group 1 ($n = 124$)	Group 2 ($n = 47$)	Group 3 ($n = 44$)	Group 4 ($n = 25$)	p value
Leptin (ng/ml)	20.2 ± 9.2*	16.8 ± 9.5††	13.5 ± 7.5$^{\#}$	9.8 ± 3.6	0.006
Adiponectin (μg/ml)	10.1 ± 4.6¶	12.1 ± 6.1	13.3 ± 5.6	14.1 ± 6.0	0.04
MIF (ng/ml)	5.9 ± 2.5*	4.7 ± 1.9††	3.6 ± 1.3$^{\#}$	2.8 ± 2.0	0.001
IL-6 (pg/ml)	20.1 ± 5.6†	17.5 ± 6.5††	16.5 ± 4.6	15.4 ± 3.5	0.05
Hs-CRP (mg/L)	4.2 ± 2.2*	3.6 ± 1.5††	3.1 ± 1.3$^{\#}$	1.4 ± 0.7	0.0001
Log TNFα (pg/ml)	3.86 ± 0.18*	3.6 ± 0.14‡	3.2 ± 0.06	2.86 ± 0.2	0.001

Results are shown as mean ± SD. OSA with NAFLD (group 1), OSA without NAFLD (group 2), without OSA with NAFLD (group 3), and without OSA and without NAFLD (group 4). p value ≤ 0.05 is statistically significant. * group 1 vs 2, 1 vs 3, 1 vs 4 ($p \le 0.05$); $^{\#}$ group 3 vs 4 ($p \le 0.05$); † group 1 vs 3 and 1 vs 4 ($p \le 0.05$); ¶ group 1 vs 4 ($p \le 0.05$); ‡ group 2 vs 3 and 2 vs 4; †† group 2 vs 4 ($p \le 0.05$). *MIF* macrophage migration inhibitory factor, *IL-6* interleukin 6, *Hs-CRP* high-sensitivity C-reactive protein, *TNF α* tumor necrosis factor alpha

Adiponectin (supplementary Table 6): In OSA and NAFLD group, a strong negative correlation was found between adiponectin, AHI ($r = -0.523$, $p = 0.001$), BMI ($r = -0.6103$, $p = 0.004$), and Hs-CRP ($r = -0.5654$, $p = 0.001$).

Discussion

In this study, we observed that blood pressure, body composition, metabolic profiles, and inflammatory biomarkers showed significant abnormal values in OSA and NAFLD group as compared to the other groups. Furthermore, inflammatory markers showed a significant correlation in the OSA and NAFLD group. Most importantly, this study suggests that OSA and NAFLD operate as an independent contributors to the increased systemic inflammation that occurs in overweight/obese subjects. Such an independent relationship of OSA and NAFLD is being reported for the first time.

OSA is a risk factor for obesity, hypertension, T2DM, and CVD; its relationship with NAFLD is poorly researched, and it is also debated whether OSA promotes liver injury independently of coexisting comorbidities, including obesity, insulin resistance, and MS. In obese subjects, a study has been indicated that chronic intermittent hypoxia (CIH) increased liver injury and inflammation, in which relationship between OSA to NAFLD is controversial [21]. Another study has been reported that OSA is one of the elements promoting the evolution of NAFLD from steatosis to NASH [22]. Although, some small studies have found an independent relationship between OSA and liver histology [4, 23]. This study also showed independent relationship between OSA and NAFLD.

Obesity and OSA have been independently associated with metabolic dysfunction and systemic inflammation [5]. Further, Hs-CRP is one of the markers of systemic inflammation which has been found to be an important predictor of the severity of atherosclerosis and cardiovascular outcomes [24].

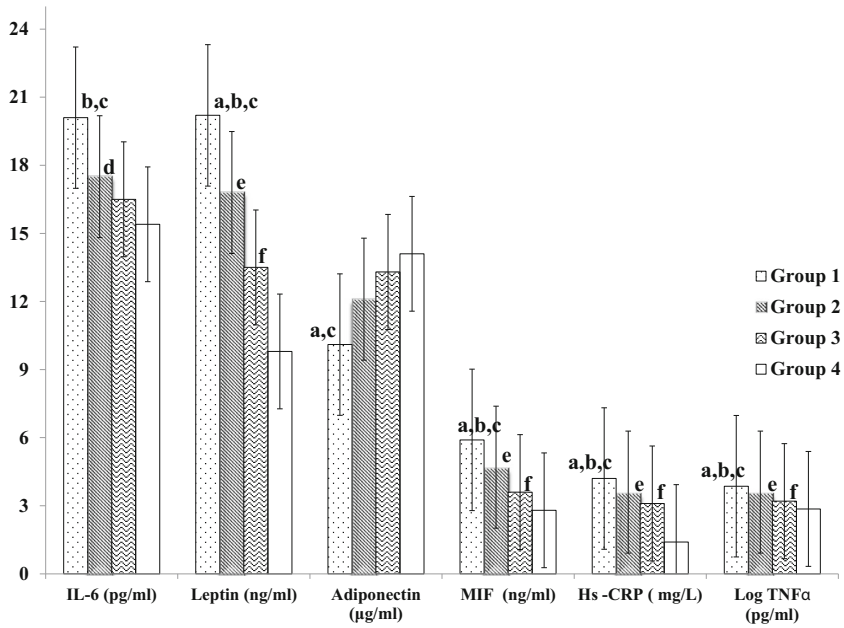

Fig. 2 Association of inflammatory markers with OSA and NAFLD (group 1), OSA without NAFLD (group 2), without OSA with NAFLD (group 3), and without OSA and without NAFLD (group 4). (a) Group 1 vs group 2. (b) Group 1 vs group 3. (c) Group 1 vs group 4. (d) Group 2 vs group 3. (e) Group 2 vs group 4. (f) Group 3 vs group 4 ($p < 0.05$)

Similarly, we showed obesity leads to high systemic inflammation in this study.

Chronic inflammation is indicated by an increased expression of inflammatory cytokines and increased infiltration of macrophages into the adipose tissue. The relationship between obesity and inflammation has been further illustrated by the increased levels of several inflammatory markers including cytokines and proteins like Hs-CRP in obese individuals [25]. Xu et al. [26] showed that diet-induced obesity is associated with infiltration of macrophages into white adipose tissue. Similarly, we observed that MIF and IL-6 levels were significantly associated and correlated with OSA and NAFLD.

During nocturnal hypoxia, adipocytes and monocytes secreted IL-6 through the NF-κB pathway. Previous reports have indicated that IL-6 was an important stimulus of CRP production in the liver [27] and plays an important role in inflammatory processes in OSA. Further, free fatty acid flux to the liver induced by IH [28] and upregulate IκB kinase β resulting in phosphorylation and degradation of IκB by activation of NF-κB and synthesis of TNF-α and IL-6 [29–31]. An interesting study has shown that the induction of NF-κB by CIH leads to upregulation of inflammatory biomarkers [32]. Further, TNF-α and IL-6 levels are not only increased in OSA; they are also significantly associated with insulin resistance, MS, and obesity [33]. A study done by our group has been identified that the TNF-α − 308 polymorphism was associated with OSA. In addition, serum TNF α levels were significantly higher in OSA subjects [19]. In this study, we found that the increase in inflammatory markers in the OSA and NAFLD group was independent of the degree of obesity. Furthermore, all six biomarkers altered by OSA and NAFLD in our subjects have been ascribed to play a pathophysiological role in cardiovascular dysfunction, thereby suggesting that OSA and NAFLD in obese subjects might predispose them to a more severe cardiovascular phenotype and to earlier development of cardiovascular morbidities.

Adiponectin is an important biomarker and produced by adipocytes. It has been indicated that the severity of NAFLD was correlated to decreased adiponectin levels [34], and in adipose tissue, the expression of adiponectin was decreased by hypoxia [35]; the consequence could be the increased expression of inflammatory cytokines. Our study also indicated that serum adiponectin levels were decreased and negatively correlated in OSA and NAFLD group.

It has been argued that other methods such as magnetic resonance spectroscopy and liver biopsy are better methods for defining NAFLD and could be considered as "gold standard" to be used in the present study. In a recent meta-analysis regarding ultrasonography for diagnosis of NAFLD, the overall sensitivity and specificity of abdominal ultrasound for the detection of moderate to severe NALFD was good when compared to histology [36]. Also sensitivity and specificity of abdominal ultrasound was similar to other imaging techniques [37]. Finally, abdomen ultrasound for liver fat is simple to perform, non-invasive, cost-effective, easier to do in a clinical setting, and can also be used in large epidemiological studies.

Conclusion

Systemic inflammation is more pronounced in overweight/obese Asian Indians subjects with OSA and NAFLD. Subjects with OSA and NAFLD are at a higher risk for MS. Further studies are needed to investigate the role of these biomarkers on endothelial dysfunction.

Acknowledgements The authors acknowledge the contribution of Mr. Kirti Pratap who performed many of the biochemical investigations. Finally, the cooperation of the subjects who took part in the study is greatly appreciated.

Financial disclosure Funding for this study was provided through the Ministry of Science and Technology, Department of Science and Technology (Ref: SR/SO/HS-0146/2010), Government of India. The sponsors had no role in the design or conduct of this research.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S (1993) The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 328(17):1230–1235
2. Punjabi NM, Sorkin JD, Katzel LI et al (2002) Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. Am J Respir Crit Care Med 165(5):677–682
3. Tufik S, Santos-Silva R, Taddei JA, Bittencourt LRA (2010) Obstructive sleep apnea syndrome in the Sao Paulo Epidemiologic Sleep Study. Sleep Med 11(5):441–446
4. Polotsky VY, Patil SP, Savransky V, Laffan A, Fonti S, Frame LA, Steele KE, Schweizter MA, Clark JM, Torbenson MS, Schwartz AR (2009) Obstructive sleep apnea, insulin resistance, and steatohepatitis in severe obesity. Am J Respir Crit Care Med 179: 228–234
5. Vgontzas AN, Papanicolaou DA, Bixler EO, Hopper K, Lotsikas A, Lin HM, Kales A, Chrousos GP (2000) Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. J Clin Endocrinol Metab 85:1151–1158

6. Manco M, Fernandez-Real JM, Equitani F, Vendrell J, Valera Mora ME, Nanni G, Tondolo V, Calvani M, Ricart W, Castagneto M, Mingrone G (2007) Effect of massive weight loss on inflammatory adipocytokines and the innate immune system in morbidly obese women. J Clin Endocrinol Metab 92:483–490
7. Matsuzawa Y (2006) Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 3:35–42
8. Iiyori N, Alonso LC, Li J, Sanders MH, Garcia-Ocana A, O'Doherty RM, Polotsky VY, O'Donnell CP (2007) Intermittent hypoxia causes insulin resistance in lean mice independent of autonomic activity. Am J Respir Crit Care Med 175(8):851–857
9. Misra A, Khurana L (2008) Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 93:S9–S30
10. Misra A, Khurana L (2009) The metabolic syndrome in south Asians: epidemiology, determinants, and prevention. Metab Syndr Relat Disord 7:497–514
11. Bhatt SP, Guleria R (2014) Obstructive sleep apnea and non alcoholic fatty liver disease: the growing link and management issues. World Journal of Respirology 4(2):1–7
12. Vikram NK, Misra A, Dwivedi M, Sharma R, Pandey RM, Luthra K, Chatterjee A, Dhingra V, Jailkhani BL, Talwar KK, Guleria R (2003) Correlations of C-reactive protein levels with anthropometric profile, percentage of body fat and lipids in healthy adolescents and young adults in urban North India. Atherosclerosis 168:305–313
13. Nigam P, Bhatt SP, Misra A et al (2013) Non-alcoholic fatty liver disease is closely associated with sub-clinical inflammation: a case-control study on Asian Indians in North India. PLoS One 8(1)
14. Bhushan B, Guleria R, Misra A, Pandey RM, Luthra K, Vikram NK (2009) Obstructive sleep apnoea correlates with C-reactive protein in obese Asian Indians. Nutr Metab Cardiovasc Dis 19(3):184–189
15. Tam KM, Wu JS (1986) Ultrasonographic diagnosis of fatty liver. Taiwan Yi Xue Hui Za Zhi 85:45–53
16. Bhatt SP, Nigam P, Misra A, Guleria R (2013) Independent associations of low 25 hydroxy vitamin D and high parathyroid hormonal levels with non alcoholic fatty liver disease in Asian Indians residing in North India. Athroscorosis 230(1):157–163
17. Bajaj P, Nigam A, Luthra RM et al (2009) A case-control study on insulin resistance, metabolic co-variates & prediction score in non-alcoholic fatty liver disease. Indian J Med Res 129:285–292
18. Vikram NK, Misra A, Pandey RM, Dwivedi M, Luthra K (2004) Adiponectin, insulin resistance, and C-reactive protein in postpubertal Asian Indian adolescents. Metabolism 53:1336–1341
19. Bhushan B, Guleria R, Misra A, Luthra K, Vikram NK (2009) TNF-alpha gene polymorphism and TNF-alpha levels in obese Asian Indians with obstructive sleep apnea. Respir Med 103:386–392
20. BaHammam AS, Obeidat A, Barataman K et al (2014) A comparison between the AASM 2012 and 2007 definitions for detecting hypopnea. Sleep Breath 18(4):767–773
21. Musso G, Olivetti C, Cassader M, Gambino R (2012) Obstructive sleep apnea-hypopnea syndrome and nonalcoholic fatty liver disease: emerging evidence and mechanisms. Semin Liver Dis 32:49–64
22. Piguet A-C, Stroka D, Zimmermann A, Dufour JF (2010) Hypoxia aggravates non-alcoholic steatohepatitis in mice lacking hepatocellular PTEN. Clin Sci 118(6):401–410
23. Musso G, Cassader M, Olivetti C, Rosina F, Carbone G, Gambino R (2013) Association of obstructive sleep apnoea with the presence and severity of NAFLD. A systematic review and meta-analysis. Obes Rev 14:417–431
24. Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe C, Pfeffer MA, Braunwald E (2005) Pravastatin or atorvastatin evaluation and infection therapy-thrombolysis in myocardial infarction 22 (PROVE IT-TIMI 22) investigators. N Engl J Med 352(1):20–28
25. Trayhurn P, Wood IS (2005) Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans 33(5):1078–1081
26. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Investig 112(12):1821–1830
27. Heinrich PC, Castell JV, Andus T (1990) Interleukin-6 and the acute phase response. Biochem J 265(3):621–636
28. Drager LF, Li J, Reinke C, Bevans- Fonti S, Jun et al (2011) Intermittent hypoxia exacerbates metabolic effects of diet-induced obesity. Obesity (Silver Spring) 19:2167–2174
29. Kim JK, Kim YJ, Fillmore JJ, Chen Y, Moore I, Lee J, Yuan M, Li ZW, Karin M, Perret P, Shoelson SE, Shulman GI (2001) Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 108:437–446
30. Sinha S, Perdomo G, Brown NF, O'Doherty RM (2004) Fatty acid-induced insulin resistance in L6 myotubes is prevented by inhibition of activation and nuclear localiza- tion of nuclear factor kappa B. J Biol Chem 279:41294–41301
31. Boden G, She P, Mozzoli M, Che- ung P, Gumireddy K, Reddy P et al (2005) Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor- kappaB pathway in rat liver. Diabetes 54:3458–3465
32. Imeri L, Opp MR (2009) How (and why) the immune system makes us sleep. Nat Rev Neurosci 10(3):199–210
33. Tasali E, Ip MSM (2008) Obstructive sleep apnea and metabolic syndrome: alterations in glucose metabolism and inflammation. Proc Am Thorac Soc 5(2):207–217
34. Savvidou S, Hytiroglou P, Orfanou-Koumerkeridou H, Panderis A, Frantzoulis P, Goulis J (2009) Low serum adiponectin levels are predictive of advanced hepatic fibrosis in patients with NAFLD. J Clin Gastroenterol 43(8):765–772
35. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, Shimomura I (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 56(4):901–911
36. Mathiesen UL, Franzén LE, Aselius H et al (2002) Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis 34:516–522
37. Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM (2011) Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 54(3):1082–1090