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Research Article

The Influence of Obstructive Sleep Apnea-Induced Intermittent Hypoxia on Nonalcoholic Fatty Liver

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Abstract

Background: Obstructive Sleep Apnea Syndrome (OSAS) is a sleep disorder characterized by intermittent hypoxia with significant mortality and morbidity. Non-alcoholic fatty liver disease (NAFLD) varies widely from simple steatosis to fibrosis. Nocturnal hypoxemia caused by repetitive sleep apnea's and hypopneas can lead to NAFLD that is frequently concomitant with OSAS. We evaluated the association between nocturnal hypoxemia and NAFLD.

Methods: Patients complaining of snoring, daytime somnolence and who presented to sleep disorder clinic between January 2015 and June 2016 were evaluated. All patients performed a standard polysomnography. Biochemical parameters and abdomen ultrasonography were recorded. Alcohol abusers and patients with diabetes mellitus or chronic liver disease were excluded resulting in 112 enrolled patients. An Apnea Hypopnea Index above five indicated OSAS and NAFLD was graded by ultrasonography.

Results: Of the 112 patients, 78 had OSAS and 68 of these (87%) were NAFL positive. Of the 112 patients 86 had NAFLD. In the NAFL group the apnea-hypopnea index (AHI), oxygen desaturation index (ODI) and duration of saturation below 90% was significantly higher while minimum oxygen saturation was significantly lower ($p = 0.001$). The grade of hepatosteatosis correlated with AHI, ODI and duration of saturation below 90%, whereas minimum oxygen saturation inversely correlated with our findings ($p = 0.001$).

Conclusion: OSA parameters such as AHI, ODI, duration of saturation below 90%, contributed to NAFLD progress. Patients with severe nocturnal hypoxemia should be closely assessed for development of NAFLD and those with OSA and a suspicion of hepatosteatosis should have liver ultrasonography.

Keywords: *Obstructive Sleep Apnea; Non-Alcoholic Fatty-Liver Disease; Intermittent*

Introduction

Obstructive Sleep Apnea (OSA) is a common sleep disorder characterized by intermittent hypoxia, sleep partitions, increased respiratory effort, and increased sympathetic activity [1]. OSA has a prevalence of 2-4%, which can increase to 30% in obese subjects, and up to 60% in elderly obese subjects [2].

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease (30%) and has increased in frequency worldwide. The frequency also increases up to 60-70% in diabetic obese patients [3]. NAFLD has a wide spectrum from simple liver steatosis to chronic decompensated liver disease, cirrhosis or liver cancer [4].

OSA often accompanies systemic diseases such as hypertension [5], metabolic syndrome, dyslipidemia and insulin resistance [6]. These systemic disorders also contribute to the pathogenesis of NAFLD.

The association between OSA and NAFLD has been indicated in a few trials [7-9]. In our study we aimed to investigate the relationship of hypoxia with the severity and grade of hepatosteatosis (as assessed by biochemical tests and USG in OSA and control groups). What makes our study stand out from others is that diabetic patients were excluded and, as such, the effect of intermittent hypoxia on hepatosteatosis could be more clearly observed.

Methods

This cross-sectional study was approved by institutional review board of the Department of clinical investigations at Lutfi Kirdar Education and Research Hospital (Number: 89513317/1011/December 2014) and carried out in accordance with the principles of the Helsinki Declaration. All subjects gave written informed consent prior to participation in the study.

Study population

We recruited 172 adult patients (> 18 years old) who presented to the training hospital with witnessed apnea, daytime somnolence, and who performed a polysomnography between January 2015 and June 2016 as an inpatient.

Patient records were assessed and those with diabetes mellitus, chronic liver disease, unstable diseases of circulatory, respiratory and central nervous system were excluded. Patients who were history or current alcohol abusers, or who had a medication on regular hepatotoxic and hyperlipidaemia drugs were excluded (total of 60 patients were excluded) (Figure 1). Ultrasonography was performed on 112 patients. The patient's height and weight were measured. Body Mass Index (BMI) was calculated. A BMI ≥ 30 kg/m^2 was defined as obese.

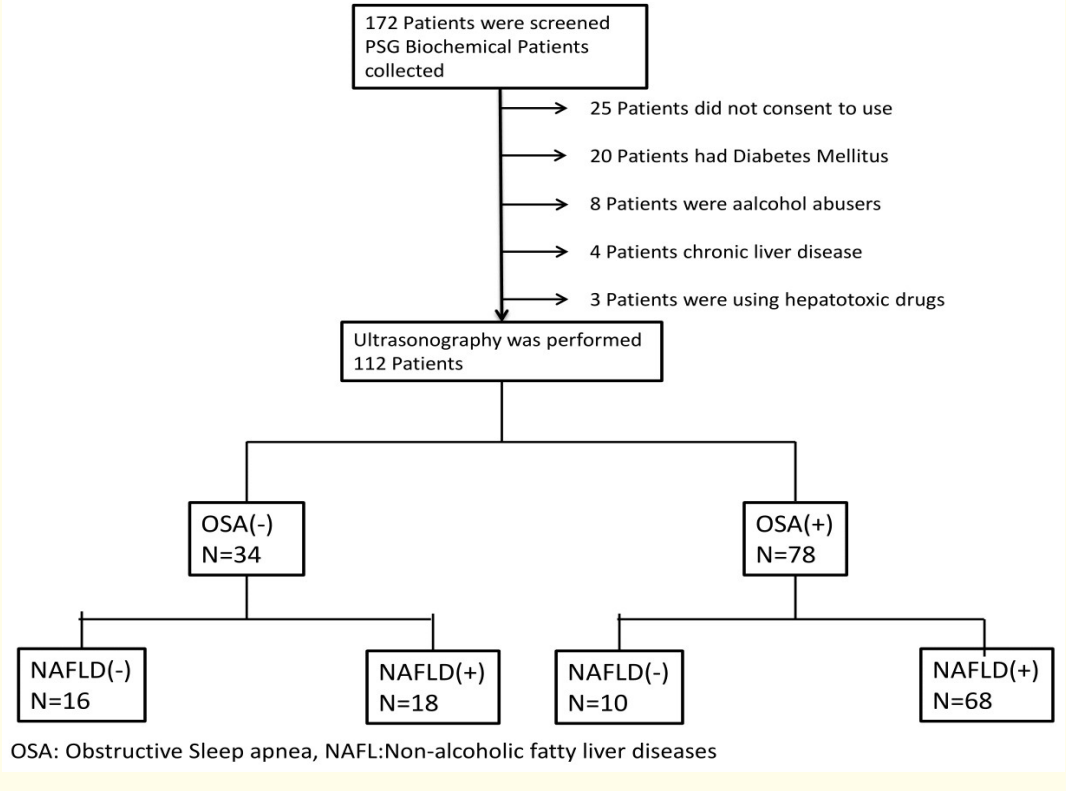

Figure 1: *Flow Chart.*

Biochemical tests including glucose, liver function tests, total cholesterol, low density lipoprotein, high density lipoprotein, and HbA1c were analysed. Blood samples were drawn into heparinized tubes, and centrifuged at 3000 rpm for 10 minutes to separate the plasma. The samples were analysed within 2h. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, total serum cholesterol and triglycerides, low-density lipoprotein, high-density lipoprotein (HDL), and hemoglobin A1C level were measured using standard techniques. Diabetes have been defined by biochemical identification as summarised above.

Polysomnography

The standard polysomnography included electrooculography, electroencephalography, bilateral leg and submental and electrocardiography recordings. We measured airflow with an oronasal thermistor, and a nasal pressure transducer. Respiratory effort was measured via respiratory inductance plethysmography and arterial oxyhaemoglobin saturation via a finger pulse oximeter (Commet grass; Astro med, Inc., West Warwick, RI, USA).

The same sleep specialist scored sleep stages using the 2012 American Academy of Sleep Medicine (AASM) scoring system [10].

Apnea's were defined as a 90% decrease in air flow from basal and for a minimum of 10 seconds. Apnea's were classified as central, mixed, or obstructive according to thoracic-abdominal movements. Hypopneas were defined as a 30% decrease in air flow from basal for a minimum of 10 seconds, and were related to a > 3% decrease in oxygen saturation (SaO_2). The apnea-hypopnea index (AHI) was calculated as the average number of apnea and hypopnea per hour during a sleep period. An AHI > 5 was diagnosed as OSA. The SaO_2 during sleep was analysed automatically, and after the elimination of probable artefacts, the average SaO_2 and minimum SaO_2 were calculated. OSA patients were categorized according to the AASM 1999 criteria as mild (AHI 5 - 14.9/h), moderate (AHI 15 - 29.9/h), and severe (AHI ≥ 30/h) [11].

Ultrasonography

USG examinations were done by a radiologist who was blind to the study. Abdominal ultrasonography was verified with a 3.5 MHz curve linear probe (PVT-375BT, Toshiba Japan). Both subcostal and intercostal sections were measured.

The presence or absence, and severity of fatty infiltration were graded on a scale from 0 to 3, indicating absent, mild, moderate, and severe hepatosteatosis, respectively, as reported by Mihmanli., *et al* [12]. These correspond to increasing degrees of hepatic echogenicity with a weak image of the intrahepatic vessels and diaphragm. Subjects with normal liver echogenicity were graded 0 (non-NAFL), while those with a minimal diffuse increase in hepatic echogenicity, and a normal image of the diaphragm and walls of the intrahepatic vessels were graded 1 (mild hepatosteatosis). Subjects with a moderate diffuse increase in hepatic echogenicity, and with a slightly impaired image of the hepatic veins and diaphragm were graded 2 (moderate hepatosteatosis). Those with a marked increase in echogenicity and with weak penetration of the right liver lobe, and very weak or no image of the hepatic veins and diaphragm were graded 3 (severe hepatosteatosis).

Statistical Analysis

The data obtained from patient records were analysed with a Statistical Package for Social Sciences (SPSS) with the Windows 21.0 programme. Qualitative data was used as well as descriptive statistical methods, such as frequency, percent, mean, and standard deviation. The Mann Whitney U Test was used to compare two groups for quantitative variables. The mean (± SD medians and ranges, and 95% confidence intervals were determined for all continuous variables. The statistical significance of the groups for AHI and ODI duration of saturation below90% was compared with the Kruskal Wallis test. A p value less than 0.05 was considered to indicate statistical significance.

Results

Of the 172 participants, 112 were eligible for the study. 32 (28.6%) of the participants were women and 80 (71.4%) of them were man. The mean age was 48.75 ± 10,39 years.

Patients were split into two groups; OSA and non-OSA. Of the 112 participants, 78 were OSA while 34 of them were non-OSA. NAFL was present in 87% of the patients diagnosed with OSA, significantly different to the non-OSA patients (p = 0.005). There was a total of 86 NAFL patients, 68 of whom were in the OSA group, and 18 in the non-OSA group. The demographic and biochemical parameters of the OSA and non-OSA patients are shown in table 1. There was no statistically difference among age, BMI, and biochemical parameters between the OSA and non-OSA groups. The NAFLD group was divided into three subgroups according to USG findings: grade 1 hepatosteatosis (nine patients, 11%), grade 2 (59 patients, 68%), and grade 3 (18 patients, 21%). There were 14, 18, and 46 mild, moderate, and severe OSA cases, respectively. OSA parameters such as AHI, minimum oxygen saturation, and mean oxygen saturation were related with NAFLD severity. The AHI values and standard deviations of the NAFLD patients compared to the grade I AHI parameters were as follows: Mild NAFLD: AHI: 18 ± 16.4, Average NAFLD AHI: 49.9 ± 25.7, AHI in severe NAFLD cases: 64.6 ± 28.5.

	No OSA N:34	**Mild OSA N:14**	**Moderate OSA N:18**	**Severe OSA N:46**	**P**
Age, year	46.6 ± 11	55.7 ± 10	50.2 ± 7	47.6 ± 11	0.23
BMI, kg/m^2	29.7 ± 2	31.7 ± 6	30.9 ± 3	33.5 ± 8	0.22
ALT, U/L	27.9 ± 15	24 ± 5	29.4 ± 18	27.5 ± 14	0.9
AST, U/L	27.5 ± 8	23.3 ± 3	24 ± 9	27 ± 14	0.75
LDL, mg/dL	141 ± 30	136 ± 37	229.2 ± 294	134 ± 30	0.21
TG, mg/dL	194.3 ± 125	136 ± 43	161.6 ± 109	204.6 ± 159	0.61
AHI	2.8 ± 1	11 ± 3	21.2 ± 3	54.4 ± 20	0.001
Mean SAT, %	96 ± 0.6	86.2 ± 20.1	93 ± 1.2	87.5 ± 6.6	0.006
HbA1C	5.7 ± 0.1	6 ± 0.1	5.5 ± 0.1	5.7 ± 1	0.87
Urica, mg/dL	5.7 ± 0.3	5.2 ± 0.6	5.7 ± 0.8	6 ± 1.7	0.61
GGT, U/L	28.9 ± 7	34 ± 15	35.5 ± 11	35.2 ± 21	0.6
Glucose, mg/dL	94.6 ± 7	99.3 ± 9	101 ± 6	99.5 ± 9	0.17

Table 1: *Demographic and Biochemical Parameters in OSA and non-OSA patients.*

All variables are given Mean ± SD BMI: Body- mass index ; ALT: Alanine aminotransferase; AST: Aspartate Aminotransferase; LDL: Low Density Lipoprotein Cholesterol; TG: Triglycerides; AHI: Apnea-Hypopnea Index; Mean Sat: Mean Saturation; HbA1C: Hemoglobin A1C; Urica: Uric acid; GGT: Gamma-Glutamyl Transferase

The severity of NAFLD in patients were found to be highly statistically correlated with AHI (p = 0.001).

The minimal oxygen saturation level during sleeping according to the grade of NAFLD patients as; Mild NAFLD: 83.3 ± 9.7, moderate NAFLD: 72.3 ± 12.9 and severe NAFLD: 64.6 ± 13.5. there was also a statistically significant correlation between groups. The mean oxygen saturation level during sleeping according to the grade of NAFLD patients were also showed statistically significant correlation as mild NAFLD: 91.4 ± 10.7; moderate NAFLD: 87.4 ± 8 and severe NAFLD: 84 ± 2 (p = 0.001).

On the other hand, as showed in table 2, the time interval during the oxygen saturation level below 90% with NAFLD grade has also present a statistically significant correlation (mild NAFLD: 11.4 ± 17.8; moderate NAFLD: 52.6 ± 29.7 and severe NAFLD: 90 ± 10.3) (p = 0.001).

		N	Mean	Sd.	p
AHI	n-NAFLD	26	13,25	18,39	0,000*
	NAFLD Mild	9	18,16	16,43	
	NAFLD Moderate	59	49,93	25,70	
	NAFLD Severe	18	64,67	28,54	
Min. O_2 sat.	n-NAFLD	26	84,38	18,10	0,001*
	NAFLD Mild	9	83,30	9,77	
	NAFLD Moderate	59	72,31	12,99	
	NAFLD Severe	18	64,67	13,58	
Mean O_2 sat.	n-NAFLD	26	95,00	1,91	0,000*
	NAFL Mild	9	91,44	10,73	
	NAFLD Moderate	59	87,49	8,18	
	NAFLD Severe	18	84,00	2,00	
Dur. O_2 sat. < 90%	n-NAFLD	26	1,23	3,03	0,000*
	NAFLD Mild	9	11,40	17,81	
	NAFLD Moderate	59	52,60	29,71	
	NAFLD Severe	18	90,00	10,39	

Table 2: *Comparison of OSA parameters in NAFLD and non-NAFLD groups.*

AHI: Apnea-Hypopnea Index; Min O_2 sat: Minimum Oxygen Saturation; Mean O_2 saturation: Mean Oxygen Saturation; Dur. 0_2 sat. < 90%: Elapsing Time O_2 Saturation < 90%; NAFLD: Non-Alcoholic Fatty-Liver Disease; n-NAFLD: Non-Nonalcoholic Fatty-Liver Disease

Discussion

NAFLD was present in most of the patients diagnosed OSA and was significantly higher compared with non-OSA patients. Parameters such as AHI, ODI, and duration of saturation below 90% correlated with the grade of NAFLD, and there was negative correlation between NAFLD grade and the minimum and mean oxygen saturation. NAFLD grade also increased as OSA severity increased. This could be explained by the fact that nocturnal hypoxic levels have an important role in the pathogenesis of NAFLD.

NAFL pathogenesis has been described as a two-step process. The first step occurs with increased activity of the hormone sensitive lipase. The regulation of lipolysis is thus affected and consequently the uptake of free fatty acids by the liver increases leading to triglyceride synthesis and accumulation, insulin resistance and the adipocyte increase associated with obesity [13,14]. The second step occurs because of oxidative stress (caused by intermittent desaturations as a result of the development of OSA) and lipid peroxidation and pro-inflammatory cytokine release that can lead to abnormal mitochondrial activity. In addition, some microbial disturbances and gene polymorphisms can contribute to the development of liver inflammation and hepatic steatosis. Oxygen plays a major role in the vital functions of the liver and other organs. Hypoxia aggravates steatohepatosis [15]. A study that used a modified Berlin sleep apnea questionnaire on patients with NAFLD found that 46% had sleep related respiratory disorders [16].

Similar to a study by Turkay., *et al*. [7] we found no significant difference in aminotransferase levels between NAFLD and non-NAFLD patients. In another study where polysomnography (PSG) was performed on patients who had liver biopsies during obesity surgery, alanine aminotransferase was found to be higher in patients who were obese and who had OSA [17]. Our results may differ from this study as the BMIs in our study (average 32.6) were lower than that of their study population (average 55.1). In addition, we excluded patients with diabetes mellitus. Also, most of our subjects (70%) had grade 2 hepatosteatosis on USG and the decreased incidence of severe steatosis may be the cause of normal ranges of aminotransferases. Another difference between our study and Kalwitz., *et al*. consists in definition of OSA as Kalwitz stated OSA AHI > 15/h [17].

Proinflammatory cytokines, such as tumour necrosis factor alpha and interleukin 1 beta, have been found to be higher in patients with sleep apnea than in the normal population [18]. Hypoxia can modulate hypoxia-inducible factors (HIF) through targeted gene expression. HIF are known to be composed of alpha and beta subunits; the alpha subunits are produced by normoxic cells and degraded by hydroxylation by 3-prolyl hydroxylases. Active HIF levels have been demonstrated to increase due to chronic intermittent hypoxia as occurring in OSAS patients [19]. A recent animal model study showed that chronic intermittent hypoxia induced hyperlipidaemia and hepatic lipid peroxidation in non-obese subjects [20]. In a study conducted in our country, the BMI index was found to be high in OSA patients and it was considered as an effective factor in association with intermittent hypoxia in the formation of hepatosteatosis [9]. Basic factors leading to NAFL are obesity, diabetes, alcohol usage and dyslipidaemia. In our study among these factors, no difference was seen within OSA and Non OSA groups. However, we did find a positive correlation between the severity of hypoxic parameters, such as ODI, minimum oxygen saturation, and the elapsed time of saturation below 90% and the grade of hepatosteatosis. This situation can be explained by oxidative stress which plays an important role in pathogenesis. In the study that used both, cellular and animal models HIF's activated several cellular pathways resulting in hepatic triglyceride accumulation. Necroinflammation and fibrosis occur in the presence of hypoxia [21].

Limitations

Limitations of our study include the fact that although a liver biopsy is the gold standard for the diagnosis of NAFLD we performed abdominal USG; ethical reasons and patient disapproval being the major factors. Although liver biopsy is the gold standard for evaluating prognosis in hepatosteatosis, the procedure itself has serious complications and many patients refuse to have one. An abdominal USG is a non-invasive alternative method. While Mishra., *et al*. found a correlation between USG findings and histological evidence [22], Razavizade., *et al*. 2012 rather consider ultrasonography as a non-invasive gold standard [23]. A further limitation of our study was that it was a single-centre study, thus limiting the generalizability of the results.

A strength of this trial was that it comprised a large population. NAFLD was frequent in patients diagnosed with OSA. There was an apparent correlation with the duration and frequency of intermittent nocturnal hypoxemia and NAFLD grade.

Our results suggest that chronic intermittent hypoxia contributes in the development of hepatosteatosis. Patients with OSA should be closely followed up and monitored for the evolution of hepatosteatosis. Ultrasonography is highly recommended. In addition, these patients should be encouraged to use a PAP device to reduce progression NAFLD development.

Conclusion

OSA parameters such as AHI, ODI, duration of saturation below 90%, contributed to NAFLD progress. Patients with severe nocturnal hypoxemia should be closely assessed for development of NAFLD and those with OSA and a suspicion of hepatosteatosis should have liver ultrasonography.

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Conflict of Interest

The authors declare that they have conflict of interests.

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