Association Between the Severity of Nocturnal Hypoxia in Obstructive Sleep Apnea and Non-Alcoholic Fatty Liver Damage

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Abstract

Background: Obstructive sleep apnea (OSA) is a major disease that can cause significant mortality and morbidity. Chronic intermittent hypoxia is a potential causal factor in the progression from fatty liver to nonalcoholic steatohepatitis.

Objectives: This study evaluated the association between the degree of liver steatosis and severity of nocturnal hypoxia.

Patients and Methods: In this study, between December 2011 and December 2013, patients with ultrasound-diagnosed NAFLD evaluated by standart polysomnography were subsequentally recorded. Patients with alcohol use, viral hepatitis and other chronic liver diseases were excluded. We analyzed polysomnographic parameters, steatosis level and severity of obstructive sleep apnea (OSA) in consideration of body mass index (BMI), biochemical tests and ultrasonographic liver data of 137 subjects. Patients with sleep apnea and AHI scores of < 5, 5 - 14, 15 - 29 and ≥30 are categorized as control, mild, moderate and severe, respectively.

Results: One hundred and thirty-seven patients (76 women, 61 men) with a mean age of 55.75±10.13 years who underwent polysomnography were included in the study. Of 118 patients diagnosed with OSA, 19 (16.1%) had mild OSA, 39 (33.1%) moderate OSA and 60 (50.8%) severe OSA. Nineteen cases formed the control group. Apnea/hypopnea index and oxygen desaturation index (ODI) values were significantly higher in moderate and severe non-alcoholic fatty liver disease (NAFLD) compared to the non-NAFLD group. Mean nocturnal SpO_2 values were significantly lower in mild NAFLD and severe NAFLD compared to the non-NAFLD group. Lowest O_2 saturation ($LaSO_2$) was found low in mild, moderate and severe NAFLD compared to the non-NAFLD group in a statistically significant manner.

Conclusions: We assessed polysomnographic parameters of AHI, ODI, $LaSO_2$ and mean nocturnal SpO_2 levels, which are especially important in the association between NAFLD and OSAS. We think that it is necessary to be attentive regarding NAFLD development and progression in patients with OSA whose nocturnal hypoxia is severe.

Keywords: Sleep Apnea, Obstructive, Non-Alcoholic Fatty Liver Disease, Hypoxia

1. Background

Non-alcoholic fatty liver disease (NAFLD) has a broad spectrum from a simple fatty infiltration to severe fibrosis, cirrhosis or even hepatocellular cancer. NAFLD is one of the most frequent causes of chronic liver disease. Today, the frequency of disease is increasing rapidly, especially in western societies. The estimated prevalence of NAFLD in the world is 20% - 30% and up to 57% - 74% in obese patients (1, 2). The primary risk factors for NAFLD are obesity, diabetes mellitus, dyslipidemia and insulin resistance. Although the NAFLD pathogenesis is not fully understood, insulin resistance, oxidative stress and excess cytokine production are the key mechanisms (3).

Obstructive sleep apnea (OSA) is a disease characterized by upper airway obstruction throughout sleep and resultant oxygen desaturation. A severity classification is made according to apnea/hypopnea index (AHI). In normal individuals, AHI score is below 5. Patients with sleep apnea and AHI scores of 5 - 14, 15 - 29 and ≥ 30 are categorized as mild, moderate and severe, respectively. OSA prevalence in the general population is 14% in men and 5% in women when AHI is accepted to be present at scores ≥ 5. In obese individuals, this ratio increases up to 60% (4). In OSA there are many predisposing factors like obesity, male sex, endocrine disorders, smoking, fluid retention and advanced age (4, 5).

OSA causes accumulation of fatty acids in the liver and inflammation as a result of recurrent nocturnal hypoxia, insulin resistance, oxidative stress and dysregulation of

adipokines. It has been shown that nocturnal hypoxia shows correlation with development and progression of NAFLD in OSA patients. Studies regarding the effect of the degree of nocturnal hypoxia on the severity of NAFLD are scarce. In studies with OSA patients, it was found that nocturnal hypoxia causes NAFLD development and progression (6, 7).

2. Objectives

In our study, we assessed the effect of severity of hypoxia on hepatic steatosis levels in OSA patients in the light of biochemical tests and ultrasonographic and polysomnographic data.

3. Patients and Methods

All patients who performed an overnight polysomnography test in the sleep laboratory due to sleep apnea symptoms between December 2011 and December 2013 were included in the study. Of 180 patients, 43 were excluded as they had not an abdominal ultrasonography. This study was approved by the ethics committee of Cumhuriyet university, faculty of medicine. All subjects gave an informed consent for their participation in the study.

The clinical and laboratory data of all patients were reviewed. Patients with a history of alcohol consumption, diabetes mellitus or dyslipidemia were identified. Patients with excess alcohol consumption (identified as > 20 g/day for men and > 10 g/day for women), current use of hepatotoxic drugs, viral hepatitis and other chronic liver diseases were excluded. Height and weight of all patients were measured. Body mass index (BMI) was calculated by dividing the body weight into the square of height (kg/m²). The liver function tests, blood glucose level, total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride values of each patient were obtained from the records.

3.1. Ultrasound

Abdominal ultrasound was performed by operators who did not know clinical and laboratory characteristics of patients using 3.5 MHz curvilinear probe (Aplio 300, Toshiba, Japan). Both subcostal and intercostal scans were performed. The sonographic findings of hepatic steatosis were diffuse increase in echogenicity, increased hepatorenal index and vascular opacity. Patients with ultrasonographic liver and renal cysts, vascular malformation and renal changes were not included in the study. The severity of fatty liver was measured with liver/renal echogenicity ratio (hepatorenal index), which is a simple and reliable method, and categorized as mild, moderate and severe (8).

3.2. Polysomnographic Evaluation

Overnight polysomnography (Embla System, model S4500, Colorado, USA), was performed with recording the following parameters; electrocardiography, electroencephalography, bilateral electrooculography, submental and anterior tibialis electromyography, air flow using a thermistor, respiratory effort recorded using chest and abdomen impedance belts, snoring using tracheal microphone, arterial oxyhemoglobin saturation using a pulse oximetry and parameters of the body position recorded using a sensor. Polysomnography was started around 11.00 pm and ended around 7.00 am. The sleep stage was scored according to the criteria of the American Academy of Sleep Medicine's manual for the Scoring of Sleep and Associated Events; Rules, Terminology and Technical Specifications, published in 2007 (9). Apnea was identified as reduction in ventilation by $\geq 90\%$ from baseline for more than ≥ 10 seconds. Hypopnea was defined as a reduction in ventilation by $\geq 50\%$ within ≥ 10 seconds that resulted in a decrease in oxyhemoglobin saturation of 4%. Apnea-hypopnea index was obtained by calculation of the apnea and hypopnea numbers at each hour of sleep. Oxygen desaturation index was defined as the number of episodes of $\geq 4\%$ oxygen desaturations at each hour of total sleep. In addition, lowest oxygen saturation and mean nocturnal oxygen saturation parameters were recorded. OSA was categorized in three groups as mild when AHI was 5-14 events/hour, moderate when 15 - 29 events/hour and severe when ≥ 30 events/hour (10).

3.3. Statistical Analysis

Data was analyzed using SSPS (version 22.0) program and Variance Analysis, Tukey test, and Chi-square test were used when the parametric test assumptions were achieved (Kolmogorov-Smirnov) in the evaluation of data. For all analysis, P value < 0.05 was accepted as significant.

4. Results

A total of 137 cases entered the study of whom 76 were women and 61 men. Average age of patients was 56 ± 10 years (range, 32 - 82). BMI (Body Mass Index), clinical, biochemical, ultrasonographic and polysomnographic results of all patients were obtained from the records. Obstructive sleep apnea (AHI ≥ 5 events/h) was found in 118 patients. Nineteen of these patients had mild OSA (AHI 5 - 14 events/h), 39 moderate OSA (AHI 15 - 29 events/h) and 60 severe OSA (AHI ≥ 30 events/h). Nineteen cases formed the control group. The patients were grouped as mild with 53 cases, moderate with 39 cases and severe NAFLD with 22 cases as well as non NAFLD in 23 cases.

Metabolic and polysomnographic characteristics of patients are given in Table 1. There were no significant differences between patient groups for age, sex and BMI. When the metabolic and liver function parameters were evaluated, there was an increase as a result of diabetes mellitus frequency and OSA severity (P < 0.05). In patients with OSA, alanine aminotransferase (ALT) level was 19% higher and aspartate aminotransferase (AST) level was

9.5% higher compared to the control group. Among the patient groups with OSA, ALT and AST values of mild and moderate level OSA groups were similar to the control group, but significantly higher in severe OSA group ($P < 0.05$). In severe AHI group, ultrasonographic hepatosteatosis was higher ($P < 0.05$). Regarding AHI and oxygen desaturation index (ODI) values of the polysomnographic parameters, there was a significantly strong association between mild, moderate and severe OSA groups ($P < 0.001$). As the severity of OSA increased, AHI and ODI values were significantly higher. Regarding LaSO$_2$ and mean nocturnal SpO$_2$, there was a significantly strong association between mild, moderate and severe OSA groups ($P < 0.001$). As the severity of OSA increased, the lowest O$_2$ saturation (LaSO$_2$) and mean nocturnal SpO$_2$ values were significantly lower.

The association between polysomnographic parameters of patients and NAFLD severity is given in Table 2. Regarding AHI and ODI values, a strong association was found between moderate NAFLD ($P < 0.05$) and severe NAFLD ($P < 0.001$) groups compared to the non-NAFLD group. Regarding LaSO2 value, a strong association between mild NAFLD ($P < 0.001$) moderate NAFLD ($P < 0.05$) and severe NAFLD ($P < 0.001$) was found compared to the non-NAFLD group. Regarding mean nocturnal SpO$_2$ values, a significantly lower association was found between mild NAFLD ($P < 0.05$) and severe NAFLD ($P < 0.05$) compared to the non-NAFLD group. There was a significant association between polysomnographic parameters of AHI, ODI, LaSO$_2$ and mean nocturnal SpO$_2$ values and NAFLD severity. However, a very strong association was found between LaSO$_2$ value and NAFLD severity.

Table 1. Association Between Metabolic and Liver Function Parameters and the Severity of Obstructive Sleep Apnea[a,b]

	Controls (n = 19)	Mild OSA (n = 19)	Moderate OSA (n = 39)	Severe OSA (n = 60)	P Values
Gender (M/F), No.	9/10	9/10	11/28	32/28	0.102
Age, y	49.1 ± 8.7	55.7 ± 9.7	54.9 ± 10.7	56.2 ± 9.9	0.058
Body mass index, kg/m^2	33.2 ± 6.2	34.4 ± 5.2	34.6 ± 5.6	34.6 ± 5.9	0.817
Diabetes mellitus, %	0	15.8	20.5	35	0.017
ALT, U/L	18 ± 5.6	24.2 ± 10.6	24 ± 9.8	27.7 ± 12.6	0.012
AST, U/L	18 ± 4.7	18.7 ± 5.5	21.2 ± 6.4	23.4 ± 8.9	0.014
Total cholesterol, mg/dL	185 ± 34.7	191.7 ± 42.7	207.5 ± 46.8	204.2 ± 46.3	0.225
LDL-C, mg/dL	119.7 ± 35.9	135.6 ± 37.8	141.2 ± 43.2	142.3 ± 40.6	0.187
HDL-C, mg/dL	56.1 ± 31.1	45.7 ± 9.1	51.3 ± 25.1	46.5 ± 18.9	0.313
Triglycerides, mg/dL	126.8 ± 46.4	175.1 ± 100.6	173.8 ± 79.7	199.9 ± 89.2	0.012
AHI	4.6 ± 0.2	11.2 ± 2.2	21.2 ± 5.2	48.2 ± 19.3	0.001
ODI	9.1 ± 2.9	21.1 ± 5.1	25.1 ± 10.6	57.2 ± 57.6	0.001
LaSO$_2$, %	80.8 ± 5.7	78.7 ± 8.9	73.9 ± 10.3	67.5 ± 11.6	0.001
Mean nocturnal SpO$_2$, %	91.7 ± 2.3	90.6 ± 2.9	89 ± 5.1	86.1 ± 7.9	0.001

[a]Abbreviations: AHI, apnea-hypopnea index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein-cholesterol; LaSO2, lowest O2 uration; LDL, low density lipoprotein-cholesterol; ODI, oxygen desaturation index.
[b]Normally distributed data were expressed as mean + SD. Categorical variables were expressed as number (percentage).

Table 2. Association Between Polysomnographic Parameters and NAFLD Severity[a]

Polysomnographic Parameter	Non-NAFLD	Mild NAFLD	Moderate NAFLD	Severe NAFLD
Apnea-hypopnea index	16.5 (4.4-60.9)[b,c]	28.9 (4.1 - 120.4)	31.9 (4.1 - 81.1)[b]	49.8 (9.6 - 79.2)[c]
Oxygen desaturation index	16.9 (3 - 53.7)[b,c]	31.7 (3.8 - 132.9)	34.8 (6.4 - 86.1)[b]	58.4 (7.9 - 119.9)[c]
Lowest desaturation value, %	81.6 (71.0 - 90.0)[b,c,d]	71.5 (50.0 - 89.0)[d]	71.7 (50.0 - 88.0)[b]	65.0 (50.0 - 85.0)[c,d]
Mean nocturnal SpO$_2$, %	92.2 (86.9 - 84.7)[e,f]	87.7 (57.7 - 95.6)[f]	88.2 (72.4 - 94.4)	84.0 (72.5 - 92.4)[e]

[a]All values are expressed as median (range).
[b]The difference between non-alcoholic fatty liver disease (NAFLD) and moderate NAFLD groups is statistically significant ($P < 0.05$).
[c]The difference between non-NAFLD and severe NAFLD groups is statistically significant ($P < 0.001$).
[d]The difference between non-NAFLD and mild NAFLD groups is statistically significant ($P < 0.001$).
[e]The difference between non-NAFLD and severe NAFLD groups is statistically significant ($P < 0.05$).
[f]The difference between non-NAFLD and mild NAFLD groups is statistically significant ($P < 0.05$).

5. Discussion

We found that the severity of NAFLD increased as polysomnography parameters of AHI and ODI indices increased and $LaSO_2$, mean nocturnal SpO_2 levels decreased. There was a strong association between NAFLD severity and a decrease in $LaSO_2$ levels. We also found a strong association between elevated liver enzymes and increase in nocturnal hypoxia severity in OSA patients.

In this retrospective study, we detected an increase in the severity of NAFLD as the nocturnal hypoxia severity of OSA patients increased. Therefore, it was considered that the nocturnal hypoxia level has an important role in the pathogenesis of hepatic steatosis. NAFLD pathogenesis is extremely complex. NAFLD pathogenesis is described as a two-hit model. The "first-hit" is triglyceride accumulation in hepatocytes with contribution of insulin resistance and obesity. Insulin resistance and adipocyte tissue increase because lipolysis and free fatty acids increase as a result of increase in hormone sensitive lipase activity. These increased free fatty acids lead to triglyceride synthesis and accumulation with increase in liver intake. The "second hit" leads to liver inflammation and fibrosis hepatic steatosis progression. This is primarily caused by oxidative stress and abnormal cytokine production. In addition, anti-oxidant defense deficiency, early mitochondrial dysfunction, iron accumulation, gut-derived microbial products and some gene polymorphisms are among the factors playing a role in hepatic steatosis [11, 12].

Oxygen is very important in vital functions and regulation of the liver as in other tissues. Hypoxia causes narrowing of hepatic sinusoids in the liver, occurrence of substrate change due to the swelling of hepatocytes, liver Kupffer cell activation, cell degeneration and necrosis. Hypoxia decreases insulin sensitivity and increases the expression of lipogenic genes. With increase in the expression of lipogenic gene, lipolysis, lipogenesis, lipid uptake and lipid droplet formation also increase. In a hypoxic environment, there is an increase in lipid accumulation in inflammation, which results from increase of oxidative stress and cytokine [13]. Hypoxia also causes fibrosis with an increase in vascular endothelial growth factor and collagen in liver tissue. When the relation between cytokine generation and sleep in humans was analyzed, it was found that proinflammatory cytokines like tumor necrosis factor alpha and interleukin-1b generation increased more in recurrent nocturnal sleep apnea compared to the normal sleep process [14, 15]. In approximately a half of patients with OSA symptoms, oxygen desaturation in sleep was accepted as a risk factor in the development of NAFLD and steatohepatitis [16]. In the study conducted by Tanne et al. steatosis, necrosis and fibrosis were considerable in the liver biopsies in severe OSA patient group [17]. However, in some studies no association was found between hepatic steatosis histology and OSA [18, 19]. In the study of Mishra et al. [20] on OSA (AHI > 5/h) patients, of nocturnal hypoxia polysomnographic parameters, AHI, mean

nocturnal SpO_2 and $LaSO_2$ were associated with liver damage and fibrosis. In patients with high AHI levels and low mean nocturnal SpO_2 and $LaSO_2$ levels, liver damage and fibrosis were greater. It was detected that $LaSO_2$ level was independently associated with NAFLD histology. However, in this study the association between the severity of OSA and liver damage and fibrosis was not reviewed [20]. We found an increase in the NAFLD development, progression and severity as a result of nocturnal hypoxia attacks. There was a significant association between polysomnographic parameters of AHI, ODI, $LaSO_2$ and mean nocturnal SpO_2 and NAFLD development, progression and severity. In particular, there was a very strong association between $LaSO_2$ value and NAFLD severity. The difference between our study and other studies was the correlation between OSA hypoxia severity and increased hepatic steatosis severity.

Abdominal ultrasound is the most frequently used non-invasive imaging technique in hepatosteatosis. Biopsy is the gold standard in the diagnosis, staging and prognosis of fatty liver disease. However, this method has limitations such as severe complications, unwillingness of patients, inability to reflect the whole liver tissue and errors in sampling and interpretation. Mishra et al. found an association between ultrasonographic findings and histological findings of liver fat infiltration [21]. In our study, there was an association between increase of hypoxia severity and hepatosteatosis severity in patients with OSA.

High fatty liver aminotransferase enzyme reflects hepatic steatosis, inflammation and fibrosis. In another study conducted on 109 OSA patients, nocturnal hypoxia severity was correlated with ALT and AST levels, but not with AHI [22]. In the study of Lin et al. on 85 patients, ALT and AST levels in moderate and severe OSA groups were significantly higher [23]. In the study of Turkay et al. there was no significant difference between liver function tests and OSA severity [24]. However in our study, in compliance with most of the literature, ALT and AST levels were significantly higher in the severe OSA group.

There was a correlation between the severity of hypoxia and hypertriglyceridemia and hypercholesterolemia in patients with OSAS [25]. In compliance with the literature, in our study, triglyceride level was found to be significantly higher in severe OSA group compared to the control group.

Our study had some limitations. We used ultrasonography to diagnose NAFLD instead of liver biopsy as the gold standard method. Liver biopsy could not be used because of patient's unwillingness and ethical reasons. In daily practice, ultrasonography has been used for screening NAFLD. Ultrasonography has some drawbacks, especially in staging NAFLD [26].

To summarize, our study was the largest case study to analyze the relation between nocturnal hypoxia and NAFLD. In our study, nocturnal hypoxia in OSA patients was an important risk factor for development and pro-

gression of hepatic steatosis. As the AHI and ODI values of polysomnographic parameters of nocturnal hypoxia increased and $LaSO_2$ mean nocturnal SpO_2 values decreased, the severity of NAFLD was observed to be increased. There was a very strong association between decrease of $LaSO_2$ value and increase of the severity of NAFLD. Therefore, it is necessary to be attentive regarding NAFLD development and progression in patients with OSA whose nocturnal hypoxia is severe.

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Footnote

Authors' Contribution: Study concept and design: Erol Cakmak, Faysal Duksal and Omer Tamer Dogan; Acquisition of data: Erol Cakmak, Faysal Duksal and Fettah Acibucu; Analysis and interpretation of data: Omer Tamer Dogan, Ozlem Yonem and Abdulkerim Yilmaz; Drafting of the manuscript: Erol Cakmak, Faysal Duksal and Omer Tamer Dogan; Critical revision of the manuscript for important intellectual content: Ozlem Yonem and Abdulkerim Yilmaz; Statistical analysis: Erol Cakmak and Omer Tamer Dogan; Administrative, technical and material support: Erol Cakmak and Faysal Duksal; Study supervision: Omer Tamer Dogan and Ozlem Yonem. Critical revision of the manuscript for important intellectual content: Engin Altinkaya, Ozlem Yonem and Abdulkerim Yilmaz.

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