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RESEARCH ARTICLE

Liver enzymes in obstructive sleep apnoea syndrome

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ABSTRACT

Background: Obstructive sleep apnea syndrome (OSAS) is a common form of sleep disordered breathing. OSAS is associated with the cluster of metabolic abbreations that comprise the metabolic syndrome, including nonalcoholic fatty liver disease. **Aims and Objectives:** We investigated the effects of OSAS and its treatment with short term nasal continuous positive airway pressure (CPAP) therapy on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. **Materials and Methods:** We studied 20 adult males and postmenopausal female aged 50-60 years with OSAS. None had hepatitis B antigen or C antibody positive, autoimmune disease, an alcohol intake higher than 20 g/day or on regular use of hepatotoxic drugs. Abdominal ultrasound was done to establish the presence of fatty liver. Serum levels of AST and ALT were determined at baseline and after nasal CPAP treatment. **Results:** The baseline ALT and AST values were within normal limits. There was no significant change in ALT (25.9 ± 4.7 vs. 26.2 ± 3.4 after CPAP, $P > 0.05$) and AST (27.5 ± 2.0 vs. 24.6 ± 1.8, $P > 0.05$) values after one night of CPAP treatment. **Conclusion:** Serum aminotransferase may have limited use in assessing liver damage in the OSAS patients. Short term CPAP therapy doesn't seem have beneficial effects on serum aminotransferase levels in patients of OSAS.

KEY WORDS: Obstructive Sleep Apnea Syndrome; Continuous Positive Airway Pressure; Nonalcoholic Fatty Liver Disease; Serum Aminotransferease

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a breathing disorder during sleep that is characterized by repetitive episodes of upper airway occlusion leading to periods of apnea along with loud, frequent snoring and excessive day sleepiness.[1] With the increasing obesity epidemic past decade has seen a rapid increase in the number of patients being referred for OSAS. Indeed in many centers, possible OSAS is now among the most common respiratory referrals and is a common outpatient respiratory diagnosis.[2] The prevalence of OSAS is estimated to be 3-7% in men and 2-5% in women.[3] In a recent survey OSAS is estimated to be present in 41% of patients with a body mass index (BMI) >28.[4] This prevalence is similar in magnitude to the prevalence of some diseases considered to be major public health issues such as diabetes mellitus and asthma.[1] One recent study estimated that 93% of women and 82% of men with moderate to severe OSAS remain undiagnosed emphasizing the importance of vigilant evaluation for clinical signs and symptoms of OSAS.[5] It is well established that patients of OSAS exhibit various metabolic abnormalities like insulin resistance, systemic hypertension, dyslipidemia, obesity and oxidative stress including non-alcoholic fatty liver disease (NAFLD).[6-9] NAFLD is the most common chronic liver disease. NAFLD is a spectrum, the mildest form

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being simple hepatic steatosis without inflammation, next is non-alcoholic steatohepatitis (NASH) and more severe form of NAFLD is cirrhosis and less frequently hepatocellular carcinoma. It now affects 30% of the general population and 60-70% of patients who have diabetes or are obese.[10,11] In OSA, there occurs chronic intermittent hypoxia (CIH) during sleep; CIH induces lipid peroxidation and inflammation in the livers of mice on a high-fat, high-cholesterol diet.[12] It is seen that hepatocytes from fatty livers have increased sensitivity to anoxia.[13] and frequent hypoxic episodes in patients with OSA could hinder hepatic function. Thus hypoxia in OSA plays a role in pathogenesis of NAFLD by predisposing to liver injury. Savransky et al.[12] demonstrated CIH induced oxidative stress in the livers of mice model of OSA. Hence OSA and NAFLD may be considered related based on fact that they share common clinical predictors like central obesity, dyslipidemia and insulin resistance.[14,15]

Numerous treatment options are being considered for NAFLD, but, with a possible exception of a moderate benefit from antioxidant vitamin E, effective therapy is still lacking.[16,17] Continuous positive airway pressure therapy (CPAP) remains the first line of treatment in OSAS. Studies have shown that metabolic derangements like insulin resistance and dyslipidemia induced by OSAS can be reversed by CPAP.[18,19] Studies on the effect of CPAP treatment on liver enzymes are very limited and inconclusive.

Thus we aimed at measuring the levels of liver enzymes serum alanine transaminase (ALT) and aspartate transaminase (AST) in patients of OSAS and to assess the effect of short term CPAP treatment on liver enzymes.

MATERIALS AND METHODS

We studied 20 adult males and postmenopausal females (M = 13, F = 7) aged 50-60 years, attending the respiratory medicine Outpatient Department of VPCI, New Delhi with symptoms suggestive of OSAS. A detailed clinical data of the patients including their demographics and sleep habits was collected. History of hypertension, diabetes mellitus, or dyslipidemia was also recorded. All subjects were administered the Epworth sleepiness scale (ESS) questionnaire. A score of >10 on ESS was taken as evidence for excessive daytime sleepiness.[20] After getting the institutional ethical clearance, consent was obtained from all participants in the informed consent form. Patients with severe respiratory disease, forced expiratory volume 1 <50% predicted, respiratory failure on the basis of ABG analysis, consuming psychotropic drugs, on regular use of hepatotoxic drugs, already being treated for OSA, alcohol intake higher than 20 g/day, history of known liver disease, liver transplantation etc., hepatitis B antigen and hepatitits C antibody positive were excluded from the study. All subjects underwent overnight polysomnography (PSG) in the sleep laboratory for confirmation of diagnosis.

Anthropometry

Height and weight were measured using standard methods[21] and the BMI was calculated using the following formula-weight in kg/(height in m)^2. The circumference of the neck was measured at the cricothyroid membrane level. All the measurements were performed by the same observer.

Blood Pressure

Blood pressure readings were obtained as an average across 2 measurements with the patients in supine position using a manual cuff sphygmomanometer.[22]

PSG

A split-night (diagnostic and CPAP titration done on the same night) PSG was performed in all of the subjects. All subjects were acclimatized in the sleep lab one night prior to sleep study. PSG was done (RemlogicTM version 1.1, Embla N7000, Medcare, Netherland) with a standard montage of electroencephalogram, electro-oculogram and electromyogram signals, pulse oximetry, respiratory impedance, nasal airflow measurements, thoracoabdominal movements, limb movements, body position and electrocardiogram. Apneas were defined as decrements in air flow ≥90% from baseline for ≥10 sec. Hypopneas were defined as a ≥30% decrease in flow lasting at least 10 sec and associated with a ≥4% oxyhemoglobin desaturation. The number of apneas and hypopneas per hour of sleep was calculated to obtain the apnea-hypopnea index (AHI). OSA was defined as an AHI score of 5 or more events per hour. The data were scored manually by a sleep technician according to the recommendations of the American Academy of Sleep Medicine.[23] Following diagnosis and CPAP titration, patients were given short term CPAP treatment at prescribed pressures for one night.

Ultrasound

Abdominal ultrasound was performed by a trained radiologist who was unaware of the participant's characteristics. Normal liver parenchyma was seen as solid homogenous echo texture, which was midway between the renal cortex and pancreatic echogenicity. Hepatic steatosis included increased echogenicity and sound attenuation.[24] All subjects included in the study had evidence of fatty liver on ultrasound.

Biochemical Investigations

Venous fasting blood samples were collected early in the morning before the patient got out of bed for assessment of liver enzymes. Sampling was done twice- first baseline before sleep study during acclimatization and second after short term CPAP therapy. All samples collected were stored at - 80°C.

Liver Enzymes

Serum aminotransferases AST and ALT was measured at 340 nm using colorimetric method described by Reitman and

Frankel.[25] The reactions involved in the determination of AST activity are as follows, the oxaloacetate produced by the transaminase serves as substrate for malate dehydrogenase by which it is reduced to malate in the presence of dihydronicotinamide-adenine dinucleotide (NADH), which is simultaneously oxidized. NADH has an absorbance peak at 340 nm which is not shown by the oxidized form, and the decrease in absorbance at this wavelength provides a means for the measurement of the transaminase activity. Similar principles are employed in the measurement of ALT activity.

Statistical Analysis

All the data were expressed as mean ± standard error of the mean. Paired *t*-test with two tail significance was used to compare the changes in study parameters in the same patient before and after CPAP treatment. The tests were considered significant if they yielded $P < 0.05$.

RESULTS

The results presented here are the data collected from 20 OSAS patients who stayed for the full length of the study. All our subjects were middle aged, obese, had severe OSA and excessive daytime sleepiness. The complete anthropometric details including blood pressure and ESS are given in Table 1.

The baseline ALT and AST values were within normal limits. There was no significant change in ALT ($P > 0.05$, Table 2) and AST ($P > 0.05$, Table 2) values after one night of CPAP treatment.

Table 1: Baseline anthropometric, blood pressure and sleep characters

Subjects (n=20)	Mean±SEM
Age (years)	54.6±4.3
Sex	M=13, F=7
BMI (kg/m^2)	33.8±2.3
Systolic blood pressure (mmHg)	132.1±3.6
Diastolic blood pressure (mmHg)	86.4±2.1
NC (cm)	39.0±1.0
ESS	13.0±0.7
AHI (/h)	45.6±8.1
Sleep efficiency (%)	90.4±1.4
Oxygen desaturation events (/h)	44.6±8.1
Average snore episode duration (sec)	0.4±0.06

NC: Neck circumference, ESS: Epworth sleepiness score, AHI: Apnea-hypopnea index, CPAP: Continuous positive airway pressure, SEM: Standard error of the mean, BMI: Body mass index

Table 2: Comparison of liver enzymes before and after CPAP treatment

Liver enzymes	Before CPAP	After CPAP
Serum ALT (U/L)	25.9±4.7	26.2±3.4
Serum AST (U/L)	27.5±2.0	24.6±1.8

CPAP: Continuous positive airway pressure, ALT: Alanine transaminase, AST: Aspartate transaminase

DISCUSSION

The main outcome of the present study is that there is no elevation of liver enzymes in patients of OSAS. Several studies suggest that in patients of OSAS there occurs liver damage due to the nocturnal CIH. Oxidative stress and lipid peroxidation in the liver leads to inflammation that plays a key role in the progression of NAFLD.[26-28] Damage to the liver classically results in a leak of serum aminotransferases (AST and ALT) into the blood stream. Since serum levels of AST and ALT are the most commonly used as screening tools to identify fatty liver disease, in the present study we used these measurements as a surrogate marker for suspected NAFLD.[29] Although there is no single standard cutoff point for abnormal liver function tests levels, the most commonly used criterion is a value of ≥40 U/L for both ALT and AST.[30] Few authors like Polotsky et al.[31] and Aron-Wisnewsky et al.[32] similar to the present study reported normal range serum ALT and AST values in bariatric population with a BMI >45, regardless of the severity of OSA and NASH. It is worthy to note that liver biopsy of patients in both studies showed evidence of NASH with ballooning and liver fibrosis. Further, Tatsumi and Saibara.[33] reported a direct correlation between a serum marker of liver fibrosis, Type III pro-collagen, triglyceride and fasting plasma glucose, but not ALT or AST. The reason behind such an observation is not yet clear. Possible explanations could be that though serum ALT and AST are considered as desirable non-invasive biomarkers of NAFLD, they are neither sensitive nor specific to diagnose NAFLD and characterize its severity.[34] Also, Jun et al.[35] demonstrated that lipid peroxidation is increased in the liver due to CIH in animal modal of OSA, but serum aminotransferases remained within the normal range suggesting the possibility that histopathological changes in the liver are not always associated with a concomitant increase in biochemical markers. Moreover the NASH clinical research network currently recommends serum ferritin to identify NAFLD patients at risk for NASH than serum aminotransferases.[36] However, the sensitivity and specificity of ferritin for the diagnosis of NASH are relatively low[37] and liver biopsy remains the gold standard for diagnosis and staging of NAFLD.[34] We also investigated the effects of short term treatment of CPAP on liver enzymes. We did not observe any change in the liver enzymes following CPAP therapy. Our findings are in agreement with the only randomized placebo controlled study by Kohler et al.[38] who did not find any effect of therapeutic CPAP treatment for 4 weeks on liver enzymes compared to sham CPAP. Sivam et al.[39] in a randomized double-blinded sham controlled trial of CPAP for 8 weeks reported no effect of CPAP on liver enzyme. In contrast to

the present study Chin et al.,[40] demonstrated abnormal serum aminotransferase levels in 35% of obese OSAS patients included in the study. Before treatment, AST levels were higher in the morning than in the previous afternoon. The overnight mean increases in serum aminotransferase levels were less marked after the first night of CPAP treatment. Improvements in serum aminotransferase levels were maintained after 1 and 6 months of CPAP treatment. Several cross-sectional studies have reported elevated levels of liver enzymes in patients with OSAS. Shpirer et al.[41] demonstrated increased ALT, AST, and alakaline phosphate in adult patients with moderate to severe OSA. In a study by Gude et al.[42] serum gamma glutamine transferease levels directly correlated with a degree of nocturnal hypoxemia in OSA patients. However, all of the above studies lacked a control group. Few cross-sectional studies that compared OSA patients to control subjects like Kheirandish-Gozal et al.[43] reported significantly higher serum ALT levels in children with OSA compared to controls. Also, Jouët et al.[44] demonstrated a higher prevalence of abnormal liver enzymes in patients with OSA compared to those without OSA. Thus, overall evidence that OSAS has an effect on liver enzyme levels remains inconclusive.

Our study has several limitations. Firstly, the liver enzymes were not compared with normal controls. Secondly, the sample size was too small to draw conclusions. Thirdly, the duration for which CPAP therapy was administered was also short. Finally liver biopsy to confirm NAFLD was not performed.

CONCLUSION

In conclusion, our data show that oxidative stress in the circulation during CIH does not necessarily reflect organ damage and that serum markers may be of limited use in assessing liver damage in the OSAS patients. Also, short term CPAP therapy doesn't seem have beneficial effects on serum aminotransferase levels in patients of OSAS.

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