

Serum S100B levels in patients with obstructive sleep apnea syndrome

Tıkkayıcı uyku apne sendromlu hastalarda serum S100B düzeyleri

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ABSTRACT

Objectives: Obstructive sleep apnea syndrome (OSAS) which is characterized by recurrent pharyngeal narrowing and breathing disturbances, can affect central nervous system (CNS). S100B, which exerts neurotrophic and gliotrophic features, is a calcium binding protein. The aim of this study was to evaluate serum levels of S100B in patients with OSAS.

Materials and methods: Clinical and laboratory assessment were performed in 26 patients (5 women and 21 men) with OSAS and 28 (8 women and 20 men) age-, body mass index (BMI)- and sex- matched healthy subjects served as controls. The patients included in the study had whole-night polysomnography (PSG) performed in the sleep laboratory. Measurements of S100B were done using the commercially available ELISA kit.

Results: Patients and controls were well matched with regard to demographic characteristics. Serum S100B concentrations were 149.4±84.5 ng/L and 139.2±70.7 ng/L for patients with OSAS and control subject, respectively. There was no statistically significant difference in serum S100B concentrations between OSAS and control groups (p>0.05).

Conclusions: Serum S100B protein level in serum did not significantly higher in patients with OSAS compared to the healthy subjects. However, further investigations are required, particularly in the area of biochemical markers of mild cerebral damage in patients with OSAS. *J Clin Exp Invest* 2012; 3 (3): 345-349

Key words: Obstructive sleep apnea syndrome, serum S100B, brain, polysomnography

ÖZET

Amaç: Tekrarlayan faringeal daralmalar ve nefes almada zorlukla karakterize olan obstrüktif uyku apne sendromu (OSAS) santral sinir sistemini etkileyebilir. Nörotrofik ve gliotrofik özellikler gösteren S100B kalsiyum bağlayıcı bir proteindir. Bu çalışmanın amacı, OSAS bulunan hastalarda serum S100B'nin düzeylerini araştırmaktır.

Gereç ve yöntem: Klinik ve laboratuvar değerlendirmeler için OSAS'lı 26 hasta (5 kadın ve 21 erkek)' çalışmaya dahil edildi. Yaş, cinsiyet ve vücut kitle indeksi uygun 28 (8 kadın ve 20 erkek) sağlıklı gönüllü kontrol grubu olarak alındı. Hastalara uyku laboratuvarında tüm gece polisomnografi uygulandı. Serum S100B ölçümü ticari bir ELISA kiti kullanılarak yapıldı.

Bulgular: Hasta ve sağlıklılar demografik özellikleri bakımından birbirleriyle uyumlu. Serum S100B konsantrasyonları hastalar ve sağlıklı bireyler için sırasıyla, 149.4±84.5 ng/L ve 139.2±70.7 ng/L olarak bulundu. Obstrüktif uyku apne sendromu ve kontrol grubu arasında S100 B konsantrasyonlar bakımından anlamlı farklılık yoktu (p>0.05).

Sonuç: Serum S100B protein düzeyinde OSAS'lı hastalar kontrol grubu ile karşılaştırıldığında anlamlı fark bulunmadı. Daha sonraki çalışmalarla, OSAS'lı hastalarda özellikle küçük serebral hasarı gösteren biyokimyasal belirteçler alanında yapılacak ileri araştırmalara ihtiyaç vardır.

Anahtar kelimeler: Tıkkayıcı uyku apne sendromu, serum S100B, beyin, polisomnografi

INTRODUCTION

Sleep is natural part of human life.¹ However, sleep disorder such as obstructive sleep apnea syndrome affect sleep quality and cause clinical symptoms which are mostly neuropsychiatric.² Obstructive

sleep apnea syndrome (OSAS) is very common disorder in adult population.³

Obstructive sleep apnea syndrome, which is defined by the occurrence of at least five obstructive apnea, hypopneas, or per hour during sleep, is characterized by recurrent pharyngeal narrowing

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and breathing disturbances.⁴ The severity of OSAS is measured by apnea hypopnea index (AHI), which is classified three levels: mild (AHI=5 to 15 events per hour); moderate (AHI=15 to 30 events per hour); and severe (AHI=>30 events per hour).⁵

The repetitive hypoxia and reoxygenation cycle in OSAS is thought to resemble ischemia-reperfusion injury.⁶ The central neural system(CNS) is vulnerable to these hypoxic condition and neurocognitive manifestation of OSAS, which includes daytime sleepiness alteration in personality, impairment in concentration, perception, communication, learning and memory . OSAS affect not only CNS but also other system such as the cardiovascular system,⁷ bone metabolism,⁸ the eyes⁹ and the endocrine system.¹⁰

S100B, which exerts neurotrophic and gliotrophic features, is a 21kDa calcium binding protein. It is produced and released mainly by astrocyte in CNS.¹¹ S100B is also expressed in melanocytes, adipocytes, chondrocytes, and in smaller amounts in several other cell types both in the nervous system (e.g., some neuronal populations and microglia cell lines) and outside the nervous system (e.g., renal cells, myoblasts, skeletal muscle cells, skin Langerhans cells).¹²

The purpose of this study was to evaluate potential diagnostic utility of serum S100B in predicting brain damage caused by OSAS.

MATERIALS AND METHODS

Subjects and ethics

Clinical and laboratory assessment was performed in 26 patients (5 women and 21 men) with OSAS clinically evaluated. 28 (8 women and 20 men) age-, body mass index (BMI)- and sex- matched healthy subjects served as controls.

We excluded subject with any of the following condition in this study: central sleep apnea; history of any cardiovascular, cerebrovascular, pulmonary disease, autoimmune disease , malignancy, migraine, thyroid disease, psychiatric disorder, renal failure, dysfunction of liver metabolism, acute infections, major surgery or trauma within one month. Subjects who participated in the study underwent a detailed cardiac and neurological examination. Subjects with risk factors for cardiovascular disease such as high total cholesterol and low density lipoprotein cholesterol and high triglyceride levels were also excluded from the study. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height.

This study was approved by the local ethics committee of Ankara Diskapi Yildirim Beyazit Education and Research Hospital. The experiments were performed in accordance with the principles enumerated in the Helsinki Declaration of 1975. Informed consent was obtained from all subjects.

Sleep analysis

The patients included in the study had whole-night polysomnography (PSG) in the sleep laboratory. Their 4-channel electroencephalograms (EEG) were recorded with Alice 5 equipment. Electromyogram (EMG) was recorded bilaterally from anterior tibial muscles in order to determine leg movements. Chin EMG was also recorded. Nasal airflow was recorded with a nasal cannula. Abdominal and thorax movements, lying position, oxygen saturation, and electrocardiogram (ECG) were also recorded. The records obtained during the sleep study were scored manually according to AASM 2007 criteria by ENT specialists who had theoretical and practical education on sleep medicine and had their certificates.

Measurement of S100B

In the morning, blood samples were obtained from all patients and control subjects. The samples was centrifuged at 5000 X g for 10 minutes, the serum was separated and stored at -80°C until assessment of S100B. Measurements of S100B were performed in an EPOCH system (USA) using the commercially available ELISA kit (Bio Vendor Research and Diagnostic Products, Czech Republic). Briefly, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-cow S100B antibody. After 120 minutes incubation and washing, biotin labeled monoclonal anti-human S100B antibody is added to the wells and incubated for 60 minutes with captured S100B. After another washing, Streptavidin- HRP Conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the Substrate solution. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of S100B. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve. S100B levels are expressed as ng/L (mean±SD). The samples were carried out together in the same experiment. Intra-assay and the inter-assay variation coefficients were 3.8% and 5.2%, respectively.

The assay range of the S100B protein, ELISA kit was 50-2000 ng/L, The samples were carried out together in the same experiment.

Statistical Analysis

Results were presented as means plus minus standard deviation. Data were analyzed by using a commercially available statistics software package (SPSS vs. 17). Data normality was assessed using the Kolmogorov-Smirnov test. To compare variables between OSAS and control groups we performed Student's t-test for normally distributed variables, Mann-Whitney's U-test when the distribution was skewed. Categorical data were analyzed by X² test. Statistical significance was considered for p<0.05.

RESULTS

The characteristics of the patient and control groups are listed in Table 1. Patients and controls were well matched with regard to demographic characteristics. Data were normally distributed for patient age, BMI and S100B. Data of sleep analyze in patients with OSAS are shown in Table 2. S100B concentrations were similar in both study groups.

Table 1. Characteristic of obstructive sleep apnea syndrome (OSAS) and control subjects ^a

Variable	OSAS (n=26)	Control (n=28)	p ^b
Age (years)	56 ±10	56±11	0.995
Body mass index (kg/m ²)	28.8± 3.1	27.7±2.5	0.135
Gender (M/F)	21/5	20/8	0.422
S100B (ng/L)	149.42±84.53	139.16±70.73	0.630

^a Data are given as mean± SD or number

^b t-Test for continuous variables and X²-test for categorical variables.

Table 2. Data of sleep analyze in patients with obstructive sleep apnea syndrome (OSAS) (mean±SD)

Variable	OSAS (n=26)
Time asleep (min)	356 ± 48
AHI (event/hour)	51± 39
Lowest oxygen saturation (%)	76± 8

AHI: Apnea-Hipopnea Index

DISCUSSION

Epidemiological studies showed that OSAS had a high frequency in general population with a prevalence of 2-4%.¹³

Some cerebrospinal fluid (CSF) markers, including S-100B protein, may serve as sensitive markers of the extension of brain damage. CSF concentrations of S100B protein have positively correlated with serum concentrations of S100B protein in traumatic brain injury.¹⁴ However, a lumbar puncture is an invasive procedure which must be performed by an experienced doctor. For these reasons, in serum, a biochemical marker indicating cerebral damage would be of benefit in patients with OSAS.

Repeated apnoeic episodes during sleep in patients with OSAS may lead to changes in cerebral metabolism. Hypoxic brain damage and fragmentation of sleep are generally thought to be causes of excessive daytime sleepiness, emotion and cognitive deficiencies.¹⁵ The evidence that intermittent hypoxia is associated with increased oxidative stress is based on findings from cell culture studies and animal models, and from controlled studies in humans.⁶ Neurons are particularly susceptible to oxidative damage as brain is enriched with polyunsaturated fatty acids.^{16,17} The nervous system also has low level of defense repair mechanisms and very high oxygen consumption against oxidative injury.¹⁸ It has been showed that astrocytes respond to intermittent hypoxia exposure with reactive gliosis after only one day of intermittent hypoxia exposure in rats.³ The glial protein S100B is secreted by reactive astrocytes.¹⁹ Reactive astroglia, increased S100B levels.³ S100B level in serum can increase not only glial damage but also solely by opening of the blood brain barrier.²⁰

S100B's effect is depend on its concentration inducing neuronal survival or death. In a low concentration of S100B has a potent effect on neuritis extension and enhances neuronal survival after injury.²¹ while in the high concentration, S100B has been shown to exert neurotoxic effects.²²

Obstructive sleep apnea syndrome might be an important risk factor for stroke.²³ Brouns et al.²⁴ have analyzed S100B concentrations in CSF of acute ischemic stroke patients and control subjects. In the recent study, S100B concentrations were similar in both groups. However, S100B correlated with stroke severity and outcome.

In line with some previous studies^{25,26} showed that serum S100B level of patients with OSAS was significantly higher than control group. Jordan et al.²⁷ have also investigated biochemical markers, including NSE, S100B and beta-trace proteins, of brain damage in OSAS patients just before and after sleep. However, their results were showed that no difference between controls and patients. Simi-

larly, in this study serum S100B levels were not statistically higher in the OSAS group compared to the control group.

Ethnicity is an important contributing factor in sleep disordered breathing.²⁸ In addition to this, older patients are more likely to suffer brain damage due to OSAS than are younger people.²⁹ Therefore, it has been shown that a negative correlation between blood S100B and age in the first 20 years of life; after age 20, S100B has not appear to vary with age.³⁰

These different results could be explained by the ethnic difference, medications, cerebral areas affected or characteristics of population.⁵

In conclusion, in our study, S100B protein level in serum did not significantly higher in patients with OSAS compared to the control group. However, further investigations are required, particularly in the area of biochemical markers of small cerebral damage in patients with OSAS, to monitor progression and efficacy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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