Evening and morning plasma levels of protein S100B in patients with obstructive sleep apnea

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Abstract

OBJECTIVE: Responsible for sleep brain perfusion changes, obstructive sleep apnea (OSA) constitutes a cardiovascular risk. To find out about any diffuse damage to the brain tissue, we studied the S100B protein, whose serum level is known to rise in stroke and craniocerebral trauma.

METHODS: 60 men (mean age 51.7±11.8 years) referred to us for OSA without any major comorbidity were examined polygraphically. S100B was determined with electrochemiluminiscence immunoassay (ECLIA) from evening and morning blood samples.

RESULTS: All sixty men were diagnosed with OSA. The difference between the evening level of S100B 0.068±0.030 μg/l and the morning level 0.059±0.029 μg/l was significant (p=0.0004). Patients with mild OSA were found to have the evening S100B 0.063±0.023 μg/l, the morning level 0.042±0.012 μg/l, the difference being significant (p=0.00051). In moderate OSA the difference between the evening –0.070±0.017 μg/l and morning levels –0.055±0.025 μg/l was less significant (p=0.043). In severe OSA no difference was found between the evening and morning concentrations of S100B (0.070±0.036 μg/l and 0.070±0.031 μg/l respectively). The difference between the evening and morning S100B levels correlated negatively with AHI and ODI and positively with basal saturation and average minimal oxygen saturation.

CONCLUSIONS: Sleep with signs of severe OSA influences S100B protein release.

ORIGINAL ARTICLE

INTRODUCTION

Obstructive sleep apnea (OSA) results from recurrent episodes of increased upper airways resistance in the course of sleep, leading to spells of apnea or hypopnea which again are responsible for nocturnal sleep fragmentation. OSA is regarded as an independent risk factor of hypertension and coronary artery disease. OSA is believed to increase the risk of stroke. OSA predisposes to arteriosclerosis, and is seen as part of the metabolic syndrome. As some studies suggest, the cardiovascular risk of OSA depends on the gravity of OSA. The OSA-related cardiovascular risk can be reduced by adequate treatment with the method of continuous positive airway pressure (CPAP). OSA causes excessive daytime sleepiness as well as cognitive deficit, though these as mostly reversible [1,2]. The still unanswered question is whether or not OSA can cause diffuse damage to the brain tissue. This prompted us to focus attention on comparisons between evening and morning plasma levels of the S100B protein in patients with OSA on the assumption that any diffuse brain tissue damage would lead to its elevation during exposure to nocturnal symptoms of OSA.

The S100B protein is a Ca^{2+}, Cu^{2+} and Zn^{2+} binding member of the S100-calmodulin-troponin superfamily, and is primarily found in high abundance within the nervous system. S100B is a protein synthesised mainly by astrocytes, but also by oligodendrocytes, Schwann´s cells and other cells of the brain including neurons [27]. S100B constitutes about 0.2% of the total brain protein. Outside the nervous system, only some 5% of it is found in adipocytes, chondrocytes, in striated and cardiac muscle and in the liver. Intracellular functions of the S100B comprise regulation of phosphorylation, enzyme activity, calcium homeostasis, cell proliferation and differentiation [12].

However, S100B is not only implicated in the regulation of intracellular processes, it is also a secretory protein and exhibits cytokine-like activities, which mediate the interactions among glial cells and between glial cells and neurons. These effects are induced, in part, by S100B interaction with the receptor for advanced glycation end products, a multiligand receptor that has been shown to transduce inflammatory stimuli and effects of several neurotrophic and neurotoxic factors [8]. S100B secretion from astrocytes is stimulated under metabolic stress (oxygen, serum and glucose deprivation), and suppressed by glutamate [10,29]. S100B acts in a dose-dependent manner: low levels stimulate neurite growth and promote neuronal survival. However, high levels result in opposite effects and can even induce neuronal apoptosis, leading to the induction of pro-inflammatory cytokines such as interleukin 1 beta or tumour necrosis factor alpha, and inflammatory stress-related enzymes such as inducible nitric oxide synthase [13,15].

In clinical practice the S100B protein is used for rating the therapeutic effect and for estimating the prognosis of patients with malignant melanoma [17,20]. S100B levels rise in reaction to various intensive traumatic brain damage and to minor head injuries [3,4,7,16]; indeed, its serum levels were found to depend on the number of headers per soccer match [26], and to rise in cases of ischemic stroke [5,23,16] and acute brain infection [16,30]. Increased cerebrospinal fluid levels of S100B were found in Alzheimer´s disease [22]. The protein serum concentrations were elevated in Parkinson´s disease [25]. Serum S100B is increased in schizophrenics, and found declining in response to adequate treatment [19]. S100B levels also rise in acute alcohol intoxication [9].

The biological half-life of endogenous S100B in melanoma is estimated at 30minutes [11] and at 97 minutes in minor head trauma [28].

MATERIAL AND METHODS

Enrolled in the study were adult men indicated for nocturnal polygraphy for suspected OSA. Enrollment was preceded by detailed examination with special respect to the subjects´ history of neurological involvement and sleep disorders after the elimination of the following diseases, past and present: stroke, craniocerebral injury, major cardiological pathologies including cardiac insufficiency, pulmonary arterial hypertension and coronary artery disease, treatment with antipsychotics, drug or alcohol abuse, and abnormal neurological physical findings. The patients rated their sleepiness according to the Epworth sleepiness scale. Between 18 and 19 hours before polygraphy, the patients had blood samples taken from the cubital vein for assaying their serum levels of S100B. During the night (23:00 to 06:00 hours), they were examined using the method of nocturnal polygraphy (air flow, chest and abdomen movements, heart rate, hemoglobin oxygen saturation). At 07:00 the following day, each patient had another sample of peripheral blood taken for the estimation of the basic biochemical and hematological parameters and the S100B morning level.

The nocturnal polygraphic record was scored visually. The following parameters were processed: the apnoea/hypopnea index (AHI – average number of spells of apnea and hypopnea per hour of subjective sleep duration), the oxygen desaturation index (ODI – average number of oxygen hemoglobin saturation drops of 3% and more per hour of subjective sleep duration), and average oxygen saturation. The patients were classified into three subgroups of AHI-rated OSA gravity: mild OSA – OHI 5–14, moderate OSA – AHI 15–19, and severe OSA – AHI of 30 and more. S100B concentration was determined with the electrochemiluminiscence method (ECLIA) using the Modular E170 (Roche) analytical system.

The study was approved by the local ethics committee, and the patients gave their written consent each to being enrolled in the study.

Since the data were not normally distributed, we employed non-parametric statistical tests. A logistic regression was used to test for possible predictors (such

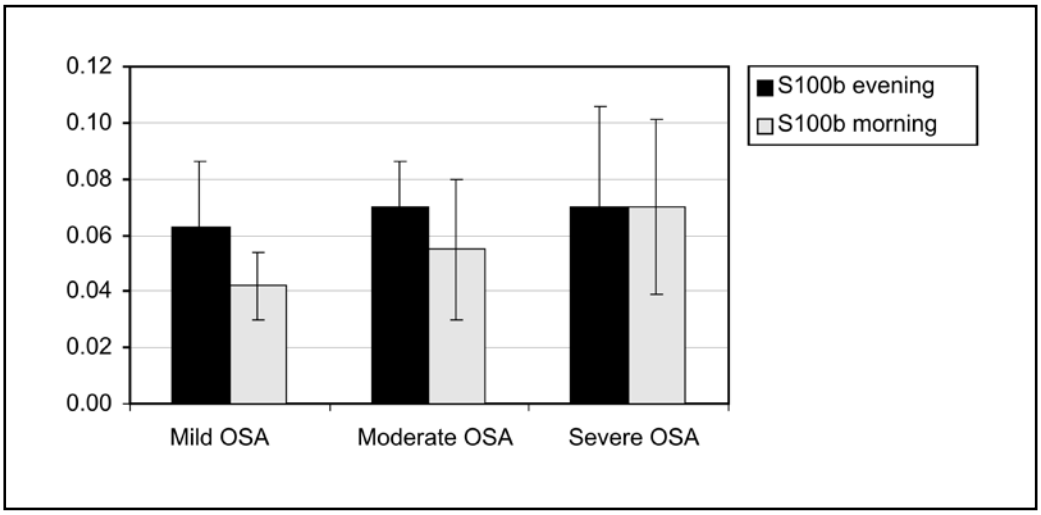

Figure 1. Evening and morning average S100B protein levels (μg/l) in patients with mild (n=18), moderate (n=12) and severe OSA (n=30) (±SD). The difference between the evening and morning values was significant in mild OSA (p=0.00051) and moderate OSA (p=0.043).

as hypertension or severity of OSA) of S100b morning and evening levels and their difference.

RESULTS

A total of 60 men aged 22–74 years (mean age 51.7±11.8 years, mean BMI 30.4±6.3) were studied. All 60 were diagnosed with OSA as defined in the International Classification of Sleep Disorders Second Edition – ICSD 2 of 2005 [1]. The average AHI was 35.6±26.3), the average ODI 36.9±29.0). The mean hemoglobin oxygen saturation was 92.3±4.0%. The ESS value in the entire cohort was 9.3±5.6. 18 patients were included in the mild OSA subgroup, 12 in the moderate OSA, and 30 in the severe OSA subgroups.

The mean evening S100B concentration was 0.068± 0.030 μg/l), the mean morning level – 0.059±0.029 μg/l), the difference being statistically significant at p=0.0004 (Sign Test).

The mild OSA group´s mean S100B level was 0.063±0.023 μg/l in the evening and 0.042±0.012 μg/l in the morning, the difference being highly significant (p=0.00051). The moderate OSA group had the evening value of S100B equal to 0.070±0.016 μg/l and the morning value to 0.055±0.025 μg/l; the difference between the two values was also significant, albeit less so (p=0.043). The mean evening and morning values of the protein S100B in the severe OSA group showed no statistically significant difference, standing at 0.070±0.036 μg/l and 0.070±0.031 μg/l respectively. Indeed, 9 patients of this particular subgroup showed an increase in the S100B level during the night. The mean morning level of the S100B protein in the severe OSA subgroup was significantly higher than in the mild OSA subgroup (Figure 1).

The difference between the evening and morning levels of S100B was found negatively correlated with the AHI (p=0.0014), with the ODI (p=0.0048), and with the length of sleep at haemoglobin saturation below 90% (p=0.0046). The difference between the evening and morning levels of S100B was found positively correlated with basal saturation (p=0.0047) and average minimal saturation (p=0.011).

The evening and morning levels of S100B and the difference between them were found in no way correlated with age, BMI, systolic or diastolic blood pressure or ESS either. Logistic regression did not yield any positive results.

DISCUSSION

As evident from the results of measurements, the plasma level of the S100B protein is influenced by the presence of OSA, and appears to depend on sleep and/or circadian rhythm. This effect on S100B levels is documented by the outcome in the three subgroups of OSA patients and by the difference in the mean morning levels seen in patients with mild and severe OSA, but also by all the demonstrable correlations suggesting that the more significant are the parameters of OSA intensity, the smaller is the difference between the evening and morning levels of S100B. This finding is consistent with the hypothesis and consequently unsurprising. What is surprising and as yet unaccounted for is the discovery of an increased evening concentration of S100B, an elevation prominent in the mild OSA subgroup. This would suggest an S100B level decline in the healthy population during sleep or in night-time. This suspicion will have to be verified on a cohort of healthy subjects.

The results actually obtained are no coincidence because the difference was the most significant in the mild OSA subgroup and practically absent from the severe OSA subgroup. Jordan *et al.* [14] found no such

difference, which may be put down to a lower number of patients and to an insufficiently sensitive method of S100B assaying because in 15 out of 19 patients the protein concentration was below the level of measurability. Recently Braga *et al*. [6] reported elevated serum S100B levels in 29 patients with OSA compared with control subjects, and a significant S100B correlation with the number of years from the onset of snoring, which indirectly reflects OSA chronicity. The difference between the morning levels found in healthy subjects and in patients tallies with the difference which we found between the morning protein levels in patients with mild and severe OSA. The patients enrolled in that study were younger than those in our own cohort.

The moot question is why should S100B rise or at least not abate during nighttime/sleep with apneic pauses. OSA patients´s comorbidities such as hypertension or diabetes mellitus are unlikely to elevate the S100B concentration; nor do the results of logistic regression in our study support this hypothesis. A connection can be sought with cerebral hypoperfusion similarly as elevated S100B is accounted for in critical illness without brain damage [24]. Another plausible explanation would relate to a disorder at the level of brain cell membranes such as will induce S100B protein passive output from the cells into extracellular space. This is how Mussack *et al*. account for S100B elevation during carotid stenting or during carotid endarterectomy [21]. Or there may have been a change in blood-brain barrier permeability, which Larsson *et al*. [18] believe is responsible for elevated S100B after endotoxemic shock. However, the S100B increase may just as well be a physiological reaction to OSA-induced changes in the brain. That would be consistent with the idea that S100B secretion is a physiological active response to a metabolically conditioned stress stimulus in the brain [10].

Since our study admittedly falls short of accounting for increased or declined S100B levels during night-time/sleep with manifestations of severe OSA, more research is necessary. The small range of S100B level variation and the assumption that this is really just a discontinuation of the trend of changes in mild OSA would suggest that rather than a consequence of simple brain parenchyma damage and passive S100B departure from the cells (such as is likely in stroke and cerebral trauma), this is in actual fact an active, physiologically substantiated process.

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