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Relationship Between Serum Substance P Levels and Daytime Sleepiness in Obstructive Sleep Apnea Syndrome*

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Objective: **We hypothesized that intermittent hypoxia might influence serum substance P levels, and that this effect might in turn contribute in excessive daytime sleepiness (EDS) in patients with obstructive sleep apnea syndrome (OSAS).**

Patients and methods: **Fifty-five patients with newly diagnosed OSAS and 15 age-matched nonapneic control subjects were enrolled in this study. Full polysomnography was performed in all patients. Single blood samples were drawn between 8:00 AM and 9:00 AM after the sleep study. Substance P levels were analyzed with a competitive enzyme immunoassay (substance P EIA kit; Cayman Chemical; Ann Arbor, MI).**

Results: **There were no significant differences in age, gender, body mass index, smoking habit, and snoring between the two groups. Serum substance P levels in the OSAS group were significantly lower than that in the control group ($p < 0.0001$). Serum substance P levels were positively correlated with rapid eye movement sleep ($r = 0.330$, $p = 0.049$) and slow-wave sleep ($r = 0.324$, $p = 0.049$) phases. Serum substance P levels were negatively correlated with Epworth sleepiness scale score ($r = -0.253$, $p = 0.048$), number of total apneas during the night ($r = -0.247$, $p = 0.036$), number of respiratory events during the night ($r = -0.266$, $p = 0.024$), apnea-hypopnea index ($r = -0.287$, $p = 0.015$), respiratory arousal index ($r = -0.267$, $p = 0.026$), time spent in apnea and hypopnea ($r = -0.307$, $p = 0.01$), average oxygen desaturation ($r = -0.265$, $p = 0.026$), and oxygen desaturation index ($r = -0.254$, $p = 0.031$).**

Conclusion: **We concluded that EDS seen in some of the OSAS patients might be associated with various pathophysiologic mechanisms including substance P levels.** ***(CHEST 2007; 131:1400–1405)***

Key words: obstructive sleep apnea syndrome; sleepiness; substance P

Abbreviations: AHI = apnea-hypopnea index; BBB = blood-brain barrier; BMI = body mass index; EDS = excessive daytime sleepiness; ESS = Epworth sleepiness scale; NK-1 = neurokinin-1 receptor; OSAS = obstructive sleep apnea syndrome; REM = rapid eye movement; TST = total sleep time

Obstructive sleep apnea syndrome (OSAS) is a commonly seen disorder characterized by repeated episodes of upper airway obstruction during sleep leading to intermittent hypoxemia or arousal.[1] OSAS is associated with daytime sleepiness, fatigue, and significant mortality due to resulting accidents and cardiovascular events.[1–3] The prevalence of OSAS in adults aged 20 to 100 years has been reported in a community-based study[4] to be 3.9% in men and 1.2% in women.

Substance P is an undecapeptide that belongs to the neurokinin (tachykinin) family of peptides. Sub-

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stance P is colocalized with other neurotransmitters and has important neuromodulatory effects.[5] Substance P exerts its effects by binding to the neurokinin-1 receptor (NK-1) and activates phospholipase C.[6] Substance P mediates various biological functions, such as smooth-muscle contraction, neuronal excitation, pain transmission, and mood regulation.[7–9] It has been supposed to be involved in the etiopathology of psychiatric disorders, anxiety disorders, schizophrenia, affective disorders, and social phobia.[10] Substance P also influences sleep physiology, and NK-1 may also be implicated in the control of sleep/wake behavior.[11,12] Increased levels of substance P in the brainstem have been observed under experimentally induced hypoxemia.[13]

In the present study, we hypothesized that intermittent hypoxia might influence levels of substance P, and this effect might in turn contribute in excessive daytime sleepiness (EDS) in patients with OSAS. In order to evaluate this hypothesis, we compared serum substance P levels between OSAS patients and age-matched control subjects.

Materials and Methods

Subjects

Fifty-five consecutive patients with newly diagnosed OSAS and 15 age-matched, nonapneic control subjects were enrolled in this study. Exclusion criteria were use of any other medications, personal or family history of psychiatric disorders, history of alcohol and drug abuse, and any other significant medical illnesses such as cancer, pulmonary, or neuromuscular disease. A questionnaire was administered to each patient in the presence of the bed partner. The questionnaire inquired about the presence of any history of snoring, witnessed apnea, EDS, and Epworth sleepiness scale (ESS). Demographic information (age, gender, smoking habits) and anthropometric measurements (height, weight, body mass index [BMI; weight/height squared]) were obtained on presentation to the sleep center. All subjects gave informed consent, and the protocol was approved by the Ethics Committee of Uludağ University Hospital.

Sleep Study

Full polysomnography was performed in all patients (Compumedics P-series Sleep System; Compumedics Sleep; Melbourne, Australia). All participants reported to the sleep laboratory at approximately 8:30 PM, and polysomnography was initiated at approximately 10:30 PM. Polysomnographic recordings included two EEG channels (C3/A2 and O2/A1), two electrooculogram channels, one submental electromyogram channel, and one ECG channel. Ventilatory monitoring included recording of oronasal airflow (with an oronasal thermistor), hemoglobin oxygen saturation by pulse oximetry (oxygen saturation measured via a finger oximeter), respiratory movement (with an inductive plethysmography) including chest and abdomen, and body position.

Sleep staging was performed according to the standard criteria of Rechtschaffen and Kales. To assess ventilation during sleep, nasal airflow was analyzed carefully. Apnea was defined as episodes lasting at least 10 s with airflow cessation. Hypopnea was defined as episodes lasting at least 10 s with reductions of thermistor signal amplitude by at least 50% and associated fall of at least 3% in oxygen saturation or an arousal. The sum of time spent in apnea and hypopnea was divided by the total sleep time (TST) to obtain by the apnea-hypopnea index (AHI). Subjects with AHI ≥ 5 were considered to have OSAS. Subjects with AHI < 5 were included in the control group.

Determination of Serum Substance P Levels

Single blood samples were drawn between 8:00 AM and 9:00 AM after the sleep study. Blood samples were centrifuged within 30 min at 4°C at 3,000*g* for 10 min. Serum samples were stored at − 80°C until performance of assay. Substance P level was measured by competitive enzyme immunoassay (Substance P EIA kit; Cayman Chemical; Ann Arbor, MI) with a working range of 3.9 to 500 pg/mL.

Statistical Analysis

Statistical analysis was performed using statistical software (SPSS for Windows, version 13.0; SPSS; Chicago, IL). Comparisons of data between OSAS and control groups were performed with Student *t* test, χ^2 test, and the Mann-Whitney *U* test. Concordance of normal distribution of all variables were calculated by Shapiro-Wilk test before compared between OSAS and control groups. If the data were not normally distributed, we used nonparametric tests for dependent variables. Relationship between serum substance P levels and ESS, number of total apneas, number of total respiratory events, time spent in apnea and hypopnea, average oxygen desaturation, oxygen desaturation index, rapid eye movement (REM) sleep, slow-wave sleep, and respiratory arousal index were calculated using Pearson correlation analysis; $p < 0.05$ was considered statistically significant.

Results

Baseline Characteristics

There was no significant difference in age, gender (male/female ratios, 45/10 in the OSAS group, 10/5 in the control group; $p = 0.098$), BMI, smoking habit, and snoring between the two groups. In the OSAS group, EDS and ESS scores were significantly higher than those of the control group ($p < 0.001$). There were no significant differences in the TST, sleep efficiency, and baseline oxygen saturation between the two groups. Significant differences in sleep stages 3 and 4, arousal (per hour), AHI, duration of apnea-hypopnea, oxygen desaturation index, average oxygen saturation during sleep, average oxygen desaturation, and length of time spent with an oxygen saturation $< 90\%$ were noted when the OSAS group was compared to control group ($p < 0.001$). Baseline clinical and polysomnographic characteristics of the OSAS and control groups are shown in Table 1.

Measurements of Serum Substance P Levels

Results of measurements of serum substance P levels are presented in Figure 1. Serum substance P

Table 1—*Baseline Clinical and Polysomnographic Characteristics of the OSAS and Control Groups

Characteristics	OSAS	Control	p Value
Age, yr	49.8 ± 1.3	48.5 ± 3.2	0.520
Male/female gender	45/10	10/5	0.098
BMI, kg/m^2	31.8 ± 0.9	29.8 ± 1.9	0.087
Smoker/nonsmoker	35/20	11/4	0.780
Smoking habit, pack-yr	13.7 ± 2.6	19.9 ± 5.8	0.410
ESS score	11.2 ± 2.3	6.5 ± 1.1	< 0.001
TST, h	6.2 ± 0.9	6.9 ± 1.1	0.680
Sleep efficiency, %	81.5 ± 1.3	75.6 ± 4.1	0.087
Stages 3,4, % of TST	10.0 ± 1.0	21.9 ± 3.9	0.002
REM, % of TST	15.1 ± 0.8	23.1 ± 3.8	0.01
AHI, /h	35.2 ± 3.4	2.6 ± 0.3	< 0.0001
Duration in apnea-hypopnea, min	111.1 ± 14.3	5.3 ± 0.3	< 0.0001
Arousal, /h	35.3 ± 2.5	15.2 ± 1.7	0.001
Baseline oxygen saturation, %	92.7 ± 0.5	93.7 ± 0.8	0.278
Average oxygen saturation, % in sleep	88.2 ± 1.0	92.5 ± 1.4	0.002
Average desaturation, %	9.5 ± 0.7	3.6 ± 0.4	< 0.0001
Oxygen saturation < 90%, length of time, min	81.8 ± 14.7	24.1 ± 11.5	< 0.0001
Oxygen desaturation index, /h	17.2 ± 2.84	56.9 ± 3.48	< 0.0001

*Data are presented as mean ± SEM or No.

levels of the OSAS group were significantly lower than those of the control group (2.39 ± 1.56 pg/mL vs 5.09 ± 2.37 pg/mL, $p < 0.0001$) [mean ± SD]. There were 34 sleepy OSAS patients (61.8%) with ESS scores > 10. There was no significantly difference in serum substance P levels between sleepy and nonsleepy OSAS patients (2.78 ± 1.98 pg/mL vs 3.45 ± 2.28 pg/mL, $p > 0.05$). There were 20 patients (36.3%) with mild OSAS (AHI < 15), 7 patients (12.7%) with moderate OSAS (AHI, 15 to 30), and 28 patients (50.9%) with severe OSAS (AHI > 30). There were no significantly differences in serum substance P levels among patients with mild, moderate, and severe OSAS (2.98 ± 1.96 pg/mL, 2.36 ± 1.77 pg/mL, and 2.26 ± 1.30 pg/mL, respectively; $p > 0.05$).

Serum substance P levels were negatively correlated with ESS ($r = -0.253$, $p = 0.048$), number of total apneas during the night ($r = -0.247$, $p = 0.036$), number of respiratory events during the night ($r = -0.266$, $p = 0.024$), and AHI ($r = -0.287$, $p = 0.015$) [Fig 2], time spent in apnea and hypopnea ($r = -0.307$, $p = 0.01$), respiratory arousal index ($r = -0.267$, $p = 0.026$), average oxygen desaturation ($r = -0.265$, $p = 0.026$), and oxygen desaturation index ($r = -0.254$, $p = 0.031$) [Fig 3]. Serum substance P levels were positively correlated with REM sleep ($r = 0.330$, $p = 0.049$) and slow-wave sleep ($r = 0.324$, $p = 0.049$) [Fig 4].

Discussion

The results of our study showed that the circulating levels of substance P in the OSAS group were

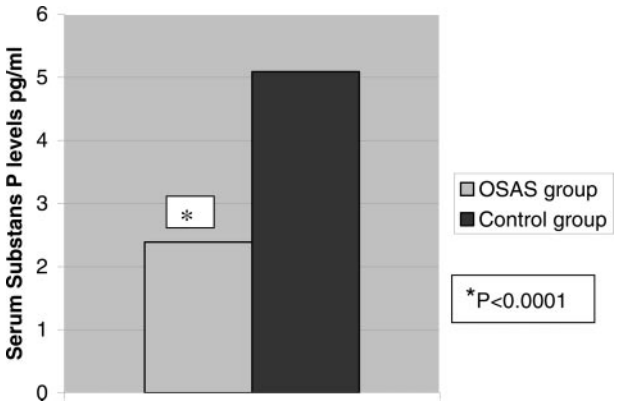

Figure 1. Serum substance P levels in OSAS patients and control subjects.

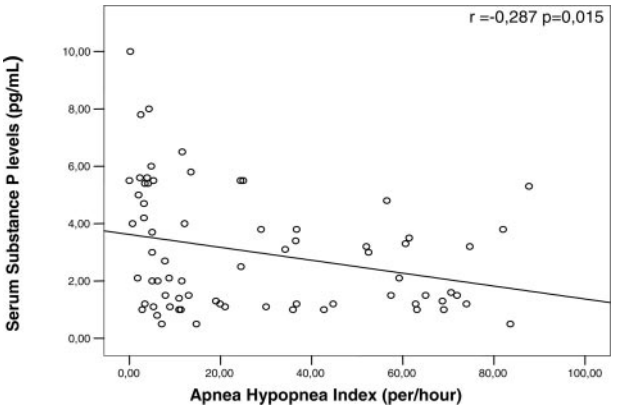

Figure 2. Correlation between serum substance P levels and AHI.

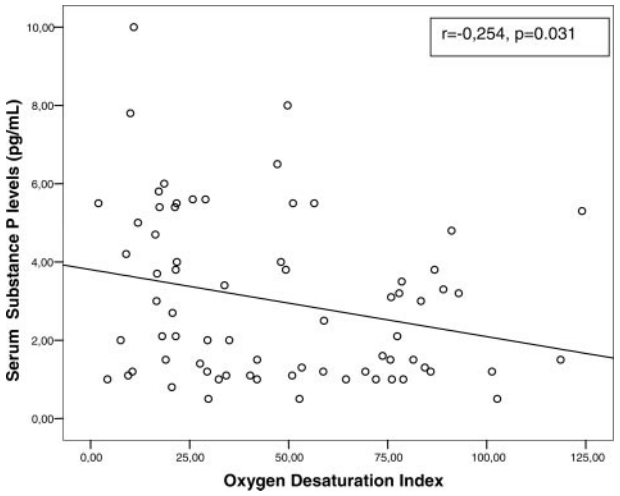

FIGURE 3. Correlation coefficient between serum substance P and oxygen desaturation index.

significantly lower than those in the control group. There was a significant negative correlation between serum levels of substance P and ESS, number of total apneas during the night, number of respiratory events during the night, AHI, time spent in apnea-hypopnea, respiratory arousal index, average oxygen desaturation, and oxygen desaturation index. There was a significant positive correlation between serum levels of substance P and REM sleep and slow-wave sleep.

Substance P is the most abundant neurokinin peptide in mammals and is widely distributed in the central, peripheral, and enteric nervous systems of many species. Substance P has been suggested to be implicated in the regulation of autonomic and physiologic functions such as nociception and pain transmission, respiration, thermoregulation, cardiovascular function, and modulation of many behavioral and

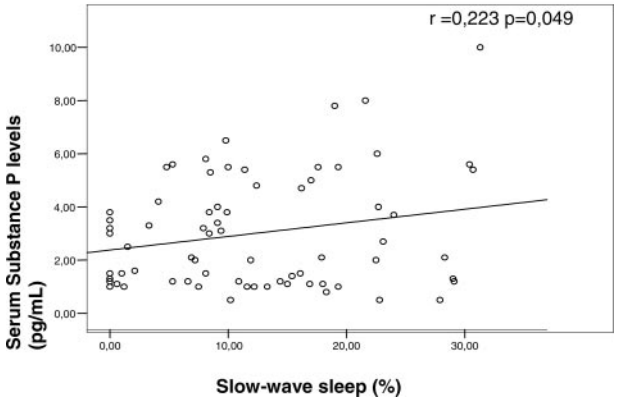

FIGURE 4. Correlation coefficient between serum substance P and slow-wave sleep.

cognitive functions including sleep, emotional, and anxiety-related behaviors.[7–12] Lieb et al[14] reported that IV infusion of substance P in healthy young men caused a significant worsening of the mood of the subjects, increased REM latency, time spent awake during the substance P infusion intervals, and stage 1 sleep in the first part of the night, and decreased REM density in the second part of the night. Furthermore, they indicated that substance P infusion increased cortisol and decreased growth hormone levels as well as the worsening of mood.

Substance P is found predominantly in the limbic system, raphe nuclei, locus coeruleus, medial nucleus of amygdale, caudate putamen, cortex, and hypothalamus. Locus coeruleus is a major area involved in the regulation of waking. Previous studies suggest that administration of substance P in different brain areas induce distinct effects on the sleep-wake cycle. Zhang et al[15] indicated that microinjection of substance P into the ventrolateral preoptic area promoted sleep. Andersen et al[16] found that administration of substance P into the intracerebral ventricle produces disturbance in sleep. Substance P exerts its effects by binding to the NK-1 and activates phospholipase C. Three types of neurokinin receptors have been identified in mammals: NK-1, neurokinin-2 receptor, and neurokinin-3 receptor. These receptors can be effectively blocked by specific neurokinin antagonists.[17]

Substance P works in concert with several hormones and other endogenous substances such as luteinizing hormone, angiotensin II, and serotonin, thus modulating their actions. Many of these effects require the selective passage of substance P across the blood-brain barrier (BBB). Substance P serves as a model compound for the study of the transport of tachykinins and other neuropeptides across the BBB.[18] Chappa et al[19] demonstrated that the carrier involved in substance P permeation across the BBB is NK-1. This newly identified carrier could serve as a target for drug delivery of SP conjugates to the brain.

Since the discovery of the first nonpeptide NK-1 antagonist, CP 96,345, several groups have produced structurally diverse, highly selective antagonists. Neurokinin receptor antagonist may be involved in the control of sleep/wake behavior. Kramer et al[20] reported that the side effect of the NK-1 antagonist is EDS in patients with major depression, suggesting an inverse relation between the reduced concentration of substance P and augmentation of sleep.

EDS is a common problem. Bixler et al[21] reported in their original population study that prevalence of 8.7% for EDS with approximately equal prevalence for men and women. There are many causes of EDS, but the most widely seen and treatable cause is sleep

apnea. EDS is commonly considered to be a cardinal sign of OSAS; however, the mechanism underlying this association is unclear. Sleep apnea-induced sleep fragmentation and hypoxemia produce decrements in frontal lobe functioning that lead to impaired daytime functioning.[22] However, the correlation between ESS and apnea severity is relatively weak. Population-based studies[22,23] suggest that there are no complaints of EDS in the majority of the individuals with SDB. Comorbid conditions including respiratory disease, sleep restriction, insomnia, and nocturnal leg complaints are important risk factors for EDS in patients with moderate-to-severe OSAS.[23]

To our knowledge, there is only one study in the literature about the relationship between substance P and OSAS: Gislason et al[24] reported that the amount of substance P-like immunoreactive material in the cerebrospinal fluid was higher in untreated patients with sleep apnea syndrome than in control subjects, and the levels remained high after the performance of operation in the group treated surgically. In our study on the contrary, circulating levels of substance P in the OSAS group were significantly lower than those of the control group. Strittmatter et al[25] measured the concentration of substance P, somatostatin, homovanillic acid, vanyl mandelic acid, and 5-hydroxyindolacetic acid in the cerebrospinal fluid of six patients with narcolepsy and 12 age- and gender-matched control patients. They indicated that substance P and somatostatin were significantly decreased in narcolepsy patients compared to control patients, and that the substance P correlated with the intensity of EDS. They suggested that reduced levels of substance P affecting serotonin release may be responsible for the diminished release of serotonin, which in turn might affect sleep cycles. Results of our study also indicate that circulating substance P correlates negatively with ESS, number of total apneas during the night, number of respiratory events during the night, AHI, time spent in apnea-hypopnea, respiratory arousal index, and average oxygen desaturation.

Potential limitations of this study merit consideration. Firstly, we measured circulating substance P level, although this approach has been used widely in previous studies. Secondly, we used oronasal thermistor for measured oronasal airflow. Thirdly, this is cross-sectional study, and our sample size is limited, and our control group subjects were not healthy people from the population.

It has been determined in previous studies[10,20] that NK-1 antagonists have EDS side effects and that there was a relationship between the levels of substance P and excessive sleepiness in subjects with narcolepsy. In our study, substance P levels were found to be significantly decreased in patients with OSAS as compared to control subjects, and significant correlations were established between the levels of substance P, and ESS and polysomnography parameters of OSAS severity. We concluded that EDS seen in some OSAS patients might be associated with various pathophysiologic mechanisms, including substance P levels. Further studies are needed to clarify the complex relation between OSAS, EDS, and substance P.

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