SERUM MIDKINE LEVELS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA

HAKAN TANRIVERDI[1], FIRAT UYGUR[1], MURAT CAN2, FATMA ERBOY[1], BÜLENT ALTINSOY[1], TACETTIN ÖRNEK[1], FÜRUZAN KÖKTÜRK[3], MÜGE MELTEM TOR[1], FIGEN ATALAY[1]
[1]Bülent Ecevit University Faculty of Medicine Department of Chest Diseases and Tuberculosis, Zonguldak - [2]Bülent Ecevit University Faculty of Medicine Department of Biochemistry - [3]Bülent Ecevit University Faculty of Medicine Department of Biostatiscal Sciences, Turkey

ABSTRACT

Introduction*: Obstructive sleep apnoea (OSA) is a highly prevalent disease characterised by obstruction of the airway resulting in reduced oxygen saturation. Hypoxia and blood re-oxygenation episodes called "intermittent hypoxia" (IH) initiates the inflammatory response. Midkine (MK), is a heparin-binding protein and plays a key role in angiogenesis and inflammation. Expression of MK increases under hypoxic conditions. Thus, we hypothesised that serum MK levels would in increase in patients with OSA.*

Materials and methods: *Patients who underwent full polysomnography with suspicion of OSA were eligible for the study. Serum MK and C-reactive protein (CRP) levels were compared between OSA and control groups.*

Results*: Twenty-four control and 53 patients with OSA were enrolled in the study. Median serum MK level was significantly lower in the OSA group than that in the control group (85.93 vs. 131.86 pg/mL, respectively; p = 0.001). No significant correlation was detected between OSA severity and serum MK level. Median serum CRP level tended to be higher in the OSA than that in the control group (2.8 vs. 2.05 mg/L, respectively; p = 0.075), but the difference was significant only in patients with severe OSA compared with those in the control group (4.3 vs. 2.05 mg/L, respectively; p < 0.01).*

Conclusion: *Unexpectedly lower serum MK levels were found in patients with OSA than those in the control group, but the mechanism underlying this condition is unclear. Rapid endocytosis, internalisation of serum MK or a different inflammatory response to IH may be responsible for these results.*

Key words*: Apnoea, Midkine, Hypoxia, Inflammation.*
DOI:10.19193/0393-6384_2016_1_20

Received *May 30, 2015;* ***Accepted*** *January 02, 2016*

Introduction

Obstructive sleep apnoea (OSA) is a clinical syndrome characterised by recurring episodes of upper airway obstruction that lead to markedly reduced (hypopnoea) or absent (apnoea) airflow through the nose/mouth. These episodes are usually accompanied by loud snoring and hypoxaemia[(1)]. Major symptoms of OSA are snoring, witnessed apnoea and excessive daytime sleepiness[(2)]. The prevalence of OSA is approximately 3 to 7% in adult males and 2 to 5% in adult females[(3)] and 1.5-3 times higher in males than females[(2)]. In population-based longitudinal studies, 5 years incidence of moderate OSA (AHI≥15) was found 7.5-11.1% in males and 4.9% in females[(4,5)]. The strongest risk factor for the development of OSA is obesity. The patients with OSA have an increased cardiovascular morbidity and mortality. An independent association have been reported between OSA and type 2 diabetes, depression, stroke, hypertension, congestive heart failure and coronary artery disease[(2,3)].

The hypoxia that occurs in patients with OSA is termed intermittent hypoxia (IH) and is charac-

terised by repetitive short cycles of desaturation and rapid re-oxygenation. IH is likely to be a major factor initiating the inflammatory response[6] and plays a critical role in OSA pathophysiology[7]. The detailed molecular responses to IH remain poorly understood. The hypoxia-inducible factor-1 (HIF-1) is a master hypoxia-sensing regulator that integrates an adaptive response via gene expression. Transcription of genes encoding proteins activated by HIF-1 either increases oxygen delivery or results in metabolic adaptations under hypoxic conditions[8].

Midkine (MK) is a heparin-binding growth factor which is rich in basic amino acids and cysteine. MK was originally reported during embryogenesis as a product of a retinoic acid-responsive gene. MK promotes migration of inflammatory cells, particularly macrophages and neutrophils, during the inflammatory response[9]. Experimental studies showed that MK is involved in the early stages of muscle regeneration[10] and partially prevents anoxia-induced apoptosis in mesenchymal stem cells[11]. Increased serum levels of MK have been reported in patients with hepatocellular carcinoma[12], ulcerative colitis[13], rheumatoid arthritis[14]. Hypoxia induces the expression of MK through the binding of HIF-1 to a hypoxia responsive element in the MK promoter[15]. However, the source of the soluble MK in the vascular system during hypoxia is unknown[16].

OSA is an inflammatory disease characterised by repeated hypopnoea and apnoea episodes resulting in hypoxia. Serum MK levels increase in inflammatory diseases and during hypoxic conditions. Thus, we hypothesised that serum MK levels increase in patients with OSA compared to those in patients without OSA as a control group due to inflammation and repeated hypoxic episodes during sleep. Thus, we compared serum MK and C-reactive protein (CRP) levels in patients with OSA and controls. To the our best knowledge this is the first study to assess serum MK levels in patients with OSA.

Material and methods

Patient selection

Patients who were admitted to sleep disorder center of Bülent Ecevit University Hospital with one of the cardinal symptoms of OSA (snoring, excessive daytime sleepiness or witnessed apnoea) were eligible for the study. All patients underwent full-night 12-channel polysomnography that includes electroencephalogram, electrooculogram, submental and bilateral leg electromyograms, and electrocardiogram. Airflow and snoring were measured using an oral thermistor with a nasal transducer; thoracic and abdominal wall movements and body position by inductive plethysmography. PSG was scored in accordance with the standard criteria of the American Academy of Sleep Medicine (AASM)[17]. Fifty-three newly diagnosed patients with OSA and 24 age, gender and body mass index (BMI) matched controls (apnoea-hypopnoea index [AHI] < 5) were enrolled in this study. OSA severity was classified according to the AHI as follows: 5 ≤ AHI < 15, mild; 15 ≤ AHI < 30, moderate; and AHI ≥ 30, severe. Patients with other sleep disorders (periodic leg movement syndrome, narcolepsy or central sleep apnoea syndrome), congestive heart failure, cerebrovascular disease or a lung disease with hypoxaemia (chronic obstructive pulmonary disease), were excluded. The study protocol was approved by local ethics committee and written informed consent was obtained from all subjects before enrollment.

MK assay

Blood samples were obtained between 08:00 and 09:00 am after completing the PSG recording and stored at -80°C until assay. MK levels were determined by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available human plasma kit (Boster Biological Technology, Pleasanton, CA, USA), according to the manufacturer's instructions. This particular immunoassay utilises a capture antibody coated to the bottom of each well that is specific for a particular human MK epitope. The test samples were added to the wells, an MK-specific biotinylated polyclonal antibody was added and the wells were washed. An avidin-biotin-peroxidase complex was added, the unbound conjugate was washed off and the 3, 3', 5, 5'-tetramethylbenzidine substrate was added allowing for a colourimetric reaction. The reaction was catalysed by peroxidase, and a blue color was produced, which was representative of the antigen concentration. After sufficient color development, the reaction was terminated by adding an acidic solution, which turned the solution yellow. Absorbance was measured at 450 nm using an Elx800 automatic ELISA reader (BioTek, Winooski, VT, USA). A standard curve was prepared from eight human MK standards, and MK

sample concentrations were determined from the standard curve. The intra and inter-assay coefficients of variation were 4.6-7.3%.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 19.0 software (SPSS Inc., Chicago, IL, USA). Normality of the data was assessed with the Shapiro-Wilk test. Continuous variables are presented as means ± standard deviations or medians (interquartile range), and categorical variables are presented as frequencies and percentages. Categorical variables were compared using Pearson's chi-squared or Fisher's exact chi-square tests. Continuous variables between two groups were compared by the independent sample t-test or the Mann–Whitney U-test. The differences among three or more groups were detected by using analysis of variance (ANOVA) or the Kruskal–Wallis test. If the ANOVA or Kruskal-Wallis test was statistically significant, Tukey's or Dunn's test, respectively, was used for the post hoc analysis. Spearman's correlation analysis was conducted to determine the relationship between continuous variables. Analysis of covariance (ANCOVA) and a partial correlation analysis were used to check for confounding factors. A p-value < 0.05 was considered to indicate significance for all tests.

Results

A total of 77 subjects (24 controls and 53 patients with OSA) were enrolled. Mean age was 48.2 ± 10.7 years. There was no significant difference between the OSA and control groups in terms of age, sex, BMI, presence of comorbidities or smoking status (Table 1). Median serum MK level was significantly lower in patients with OSA than that in the control group (85.93 vs. 131.86 pg/ml, respectively; p = 0.001) (Table 2 and Figure 1).

Serum MK level remained significant after ANCOVA and partial correlation analysis were performed to exclude the effects of age, sex, smoking status, and BMI (data not shown). Median serum CRP level tended to be higher in the OSA group than that in the control group, but the difference was not significant (2.8 vs. 2.05 mg/L, respectively; p = 0.07).

However, a subgroup analysis revealed that serum CRP level was significantly higher in patients with severe OSA than that in the control group, whereas no difference was detected between patients with mild/moderate OSA and the control group.

	Control	**OSA**	**p**
	n=24	n=53	
Age (years) mean (SD)	44.2±13.4	49.9±8.8	0.751
Gender male n (%)	21 (87.5)	45 (84.9)	0.533
Smoking status, current smoker n (%)	13 (54.2)	26 (49.1)	0.284
Presence of comorbidity n (%)	13 (54.8)	26 (49.6)	0.43
Presence of cardiovascular diseases n (%)	11 (45.3)	24 (45.5)	0.573
BMI (kg/m2)	29.4±4.6	31.6±5.2	0.081
AHI	*1.73±1.2*	27.5±22.7	<0.001
Mean SaO2	93.4±2.2	87.5±7.4	<0.001
Lowest SaO2	87.5±4.8	75.1±13.6	0
Total sleep time minute	381±64	374±62	0.68
Stage I, %TST	4.1±2.9	4.5±4.6	0.991
Stage II, %TST	60.1±11.2	67.3±10.7	0.01
Stage III, %TST	19.6.±9.8	13.8±9.8	0.007
REM, %TST	16.2±6.7	12.4±5.7	0.024
Arousal index	18.6±13.7	30.4±21.2	0.004

Table 1: Comparison of baseline characteristics and polysomongraphy results of patients with OSA and control group. There was no significant difference between OSA and control group except polysomongraphy findings.

Data presented as means ± SD, SD: Standart deviation, AHI: Apnea hypopnea index, BMI: Body mass index, IQR: Interquartile range, SaO2: Haemoglobin oxygen saturation, TST: Total sleep time, REM: rapid eye movements

	Control	**OSA**			p
		Mild	Moderate	Severe	Total	
	n=24	n=20	n=15	n=18	n=53	
CRP mg/L	2.05 (3.38)	2.85 (4.58)	2.85 (8.07)	4.3 (3.05)*	2.8 (3.8)*	0.132
MK pg/ml	126.9 (151.8)	84.6 (40.8)*	82.6 (40.4)*	91.2 (45.9)*	85.9 (41.6)*	0.016

Table 2: Comparison of median serum CRP and MK levels of patients with OSA groups and control group. Median MK levels were significantly lower in all OSA group than control group but there was no significant difference among OSA groups. CRP was significantly higher only in severe OSA than control group.

Data presented as median (interquartile range)

*OSA: obstructive sleep apnoea, MK: Midkine, CRP: C -reactive protein(normal serum range of CRP is 0-8 mg/L), *: Difference is significant vs control*

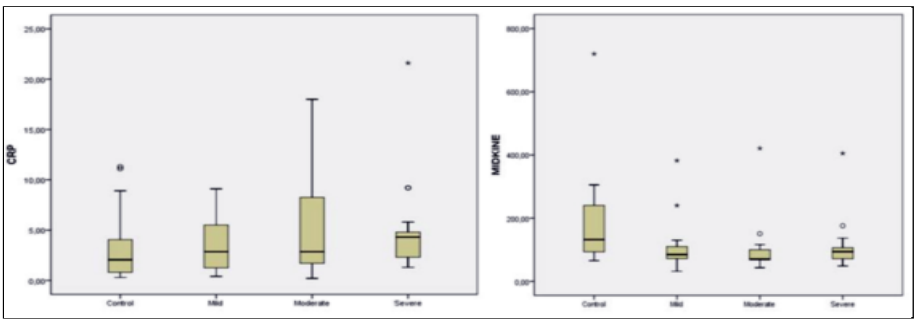

Figure 1: Box and whisker plots for subjects with obstructive sleep apnoea (OSA) and control group for serum midkine and C-reactive protein. The horizontal bar represents the median; the box length represents the interquartile range. Median midkine levels are significantly lower in patients with OSA than control group. Median C-reactive protein level was significantly higher in severe OSA patients than control group.

	Age		BMI		AHI		Lowest SaO2		Mean SaO2	
	r	p	r	p	r	p	r	p	r	p
MK	0.03	0.84	0.148	0.291	0.09	0.523	-0.211	0.129	-0.091	0.251
CRP	-0.138	0.344	0.348	0.014	0.208	0.152	-0.263	0.068	-0.258	0.086

Table 3: Correlation analysis among serum CRP and MK levels and continous variables in patients with OSA. CRP significanty correlated with BMI.

MK: Midkine CRP: C -reactive protein, DSI: Desaturation index, AHI: Apnoea hypopnea index, BMI:Body mass index, SaO2: Hemoglobin oxygen saturation

No correlation was observed between serum MK level and AHI, BMI, desaturation index, age, sex, arousal index or the presence of comorbidities (Table 3).

Interestingly, serum CRP level was significantly correlated with BMI ($r = 0.382$, $p < 0.01$).

Discussion

We found that serum MK levels were significantly lower in patients with OSA than those in the control group. However, no correlation was observed between OSA severity and serum MK level.

OSA is characterised by repeated episodes of apnoea and hypopnoea that result in hypoxia and increased levels of inflammatory mediators and cytokines. The pathophysiology of OSA is complex and not fully understood[1]. MK expression increases in patients with inflammatory diseases and under hypoxic conditions. Thus, we expected to find higher serum MK levels in patients with OSA than those in the control group due to hypoxia and inflammation; however, we found significantly lower serum MK levels in the patients with OSA than in the controls.

The basic mechanisms underlying the inflammatory process in patients with OSA remain unclear. Hypoxia that occurs in patients with OSA is called IH and is characterised by repetitive short cycles of desaturation followed by rapid re-oxygenation. IH plays a significant role initiating inflammation. The intermittent re-oxygenation that occurs in patients with OSA distinguishes IH from sustained hypoxia (SH)[18]. Hypoxia enhances HIF-1 activity which induces transcription of downstream genes. Reynold et al.[15] showed that MK expression increases markedly in the lungs of hypoxia-sensitive CAST/eiJ mice after 4 weeks of hypoxia exposure. However, MK was not induced when adult hypoxia-resistant FVB/N mice were exposed to hypoxia. Thus, those authors suggested that HIF-1 directly activates MK expression mediated by a HIF-1 response element located in the MK gene promoter[18]. However, Ryan et al.[18] utilised an IH cell culture model and demonstrated that HIF-1 was not activated by IH when compared to the same degree of SH. Thus, they suggested that IH leads to selective and preferential activation of nuclear factor kappa B (NF-κB)-mediated inflammatory pathways over adaptive HIF-1-dependent pathways, which contrasts with SH[18]. Similarly, Xu et al. (19) reported that both NF-κB and monocyte chemoattractant protein 1 expression are upregulated by chronic IH or SH in the aorta and left ventricle. However, another cell culture study by Yuan et al.[20] demonstrated that low to moderate periods of IH do not lead to a significant HIF-1 activation response, and that the response occurs only with additional IH exposure. Thus, the initial sensing and signalling events that occur in response to IH remain to be determined.

Another mechanism to explain the lower levels of serum MK in patients with OSA may be rapid endocytosis of MK. MK binds to nucleolin, which is a nuclear protein located on the cell surface that functions as a shuttle to the nucleus[9]. The low-density lipoprotein receptor-related protein (LRP) is a component of the MK receptor that internalises bound MK[21].

Cytoplasmic MK is transferred to the nucleus by nucleolin and a laminin-binding protein precursor after internalisation. This nuclear transfer is important for cell survival induced by MK[22]. MK enters the nucleus immediately after rapid LRP1-mediated endocytosis and suppresses apoptosis[22]. Thus, MK may also act within the nucleus. Moreover, MK levels may be downregulated after internalisation by the LRP-1 endocytic receptor[22].

In an experimental study, Weckbach et al.[16] showed that MK expression increases substantially in human polymorphonuclear neutrophils (PMN), monocytes and vascular endothelial cells compared with that in a normoxic control after 4 h of hypoxia (1% O2). However, MK expression decreased after 6 and 20 h when compared with the peak at 4 h, indicating that MK expression was transiently upregulated by hypoxia. In the above-mentioned study, MK was detected in neither supernatants of PMN nor monocyte after 4 or 6 h of hypoxia, suggesting that substantial amounts of soluble MK are not released into the supernatant despite the marked upregulation of MK expression in PMN and monocytes during hypoxia[16]. Another finding of the study by Weckbach et al.[16] was the detection of MK in PMN and monocyte endocytotic vesicles, suggesting that MK is rapidly internalised from the cell surface by endocytosis. Overall, the findings suggest that MK is upregulated or over-expressed during acute hypoxia but the long-term effects of chronic hypoxia on MK expression and the potential source of MK remain to be clarified.

Another study[7] showed that rats exposed to IH for 8 weeks display higher levels of proinflammatory factors (tumour necrosis factor-α, interleukin [IL]-6, and IL-8) and NF-ϰB than those in their counter-parts exposed to SH or normoxia. However, production of the potent anti-inflammatory cytokine IL-10 decreased, suggesting that biphasic changes in pro- and anti-inflammatory factors reflect the dynamic changes and equilibrium between the "engine" and the "brake" of the inflammatory response induced by IH[7]. They certainly involve self-limitation of the organism to the inflammatory response and the compensatory mechanisms to such stressors in the long-term[7].

Experimental autoimmune encephalomyelitis (EAE) has been used widely as an animal model to study multiple sclerosis (MS), and MK expression is enhanced during induction and progression of EAE[23]. However, Shaygannejad et al.[24] reported that serum MK levels in patients with MS are significantly lower than those in healthy controls, and no significant correlation was detected between disease severity and serum MK level. The authors[24] suggested that these inconsistencies might be due to the effects of interferon-β treatment in these patients.

We also assessed serum CRP as an inflammatory marker and found that serum CRP level was significantly higher only in patients with severe OSA compared to those in the control group, and that serum CRP level was significantly correlated with BMI. Elevated serum CRP levels have been reported in patients with OSA in numerous studies[25-27] but not in others[28-30]. Similar to our results Taheri et al.[28] did not detect an independent association between serum CRP and OSA after adjusting for BMI. Thus, whether CRP levels increase in patients with OSA is debatable. Our data suggest that CRP levels are not related to OSA but are significantly correlated with BMI in accordance with a previous study, which suggested that elevated levels of CRP might be strongly linked to obesity[30].

Our study had some limitations. First, we had a small sample size. Second, we obtained blood samples when the patients were normoxic, as blood could not be obtained during apnoeic episodes or hypoxic conditions. Last, we assessed MK levels only at the time of the OSA diagnosis. Serum levels of MK and other inflammatory cytokines should be determined both before and after non-invasive positive airway pressure treatment.

Conclusion

Serum MK levels were significantly lower in patients with OSA, independent of disease severity, but the mechanisms underlying this condition remain unclear. This was a preliminary study; thus, further prospective large-scale studies are necessary to obtain additional data on the relationship between OSA and serum MK level and to determine the role of MK in OSA pathogenesis.

References

1) Deegan PC, Mc Nicholas WT. *Pathophysiology of obstructive sleep apnoea*. Eur Respir J 1995; 8: *1161-1178*.

2) Lee W, Nagubadi S, Kryger MH, Mokhlesi B. *Epidemiology of obstructive sleep apnea: a population-based perspective*. Expert Rev Respir Med 2008; 2(3): *349-64*. doi:10.1586/17476348.2.3.349.

3) Punjabi NM. *The epidemiology of adult obstructive sleep apnea*. Proc Am Thorac Soc 2008; 5: *136-43*. doi: 10.1513/pats.200709-155MG.
4) Tishler PV, Larkin EK, Schluchter MD, Redline S. *Incidence of sleep-disordered breathing in an urban adult population: the relative importance of risk factors in the development of sleep disordered breathing*. JAMA 2003; 289: *2230-7*.
5) Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T. *Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study*. Arch Intern Med 2005; 165: *2408-13*.
6) Ryan S, Mc Nicholas WT. *Intermittent hypoxia and activation of inflammatory molecular pathways in OSAS*. Archives of Physiology and Biochemistry 2008; 114(4): *261-6*.
7) Li S, Qian X, Zhou W, Zhang Y, Feng J, Wan N, Zhang Z, Guo R, Chen B. *Time-dependent inflammatory factor production and NFκB activation in a rodent model of intermittent hypoxia*. Swiss Med Wkly. 2011; 141: w13309.
8) Semenza GL. *O2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1*. J Appl Physiol 2004; 96: *1173-7*.
9) Muramatsu T. *Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases*. Proc Jpn Acad Ser B Phys Biol Sci 2010; 86(4): 410-*25*.
10) Sakakima H, Kamizono T, Matsuda F, Izumo K, Ijiri K, Yoshida Y. *Midkine and its receptor in regenerating rat skeletal muscle after bupivacaine injection*. Actahistochemica 2006; 108: *357-64*.
11) Zhao SL, Zhang YJ, Li MH, Zhang XL, Chen SL. *Mesenchymal stem cells with overexpression of midkine enhance cell survival and attenuate cardiac dysfunction in a rat model of myocardial infarction*. Stem Cell Research & Therapy 2014; 5: 37. doi:10.1186/scrt425.
12) Zhu WW, Guo JJ, Guo L3, Jia HL, Zhu M, Zhang JB, Loffredo CA, Forgues M, Huang H, Xing XJ, Ren N, Dong QZ, Zhou HJ, Ren ZG, Zhao NQ, Wang XW, Tang ZY, Qin XL, Ye QH. *Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma*. Clin Cancer Res 2013; 19: *3944-5*4. doi: 10.1158/1078-0432.CCR-12-3363.
13) Krzystek-Korpacka M, Neubauer K, Matusiewicz M. *Clinical relevance of circulating midkine in ulcerative colitis*. Clin Chem Lab Med 2009; 47(9): *1085-90*.
14) Maruyama K, Muramatsu H, Ishiguro N, Muramatsu T. *Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis*. Arthritis & Rheumatism. 2004; 50(5): *1420-9*.
15) Reynolds PR, Mucenski ML, Le Cras TD, Nichols WC, Whitsett JA. *Midkine is regulated by hypoxia and causes pulmonary vascular remodeling*. J. Biol. Chem 2004; 279: *37124-32*.
16) Weckbach LT, Groesser L, Borgolte J, Pagel JI, Pogoda F, Schymeinsky J, Müller-Höcker J, Sakibaei M, Muramatsu T, Deindl E, Walzog B. *Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis*. Am J Physiol Heart Circ Physiol 2012; 303: *429-38*.
17) Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C. Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Davidson Ward SL, Tangredi MM; *American Academy of Sleep Medicine. Rules for scoring respiratory events in sleep: Update of the 2007 AASM manual for the scoring of sleep and associated events: Deliberations of the sleep apnea definitions task force of the American Academy of Sleep Medicine*. Journal of Clinical Sleep Medicine : JCSM : Official Publication of the American Academy of Sleep Medicine (2012; 8(5) *597-619*. doi:10.5664/jcsm.2172.
18) Ryan S, Taylor CT, Mc Nicholas WT. *Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome*. Circulation 2005; 112: *660-7*.
19) Xu XM, Yao D, Cai XD, Ding C, Lin QD, Wang XL, Huang XY. *Effect of chronic continual- and intermittent hypoxia-induced systemic inflammation on the* cardiovascular system in rats. Sleep Breath. 2014 doi:10.1007/s11325-014-1075-9.
20) Yuan G, Nanduri J, RamanBhasker C, Semenza GL Prabhakar NR. *Ca2+/Calmodulin kinase-dependent activation of hypoxia inducible factor 1 transcriptional activity in cells subjected to intermittent hypoxia*. J. Biol. Chem.2005; 280(6): *4321-8*.
21) Chen S, Bu G, Takei Y, Sakamoto K, Ikematsu S, Muramatsu T, Kadomatsu K. *Midkine and LDL-receptor-related protein 1 contribute to the anchorage-independent cell growth of cancer cells*. Journal of Cell Science 2007; 120: *4009-15*.
22) Shibata Y, Muramatsu T, Hirai M, Inui T, Kimura T, Saito H, McCormick LM, Bu G, Kadomatsu K. *Nuclear targeting by the growth factor midkine. Molecular and Cellular Biology 2002*; 22(19): *6788-96*.
23) Wang J, Takeuchi H, Sonobe Y, Jin S, Mizuno T, Miyakawa S, Fujiwara M, Nakamura Y, Kato T, Muramatsu H, Muramatsu T, Suzumura A. *Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population*. Pnas2008; 105(10): *3915-20*.
24) Shaygannejad V, Montazeri S, Jamshidian A, Tahani S, Gharagozloo M, Ashtari F, Vesal S, Hasheminia SJ, Dehghani L. *Correlation of Midkine serum level with pro- and anti-inflamatory cytokines in multiple sclerosis*. Iran J Immunol 2014; 11(2): *134-8*.
25) Panoutsopoulos A, Kallianos A, Kostopoulos K, Seretis C, Koufogiorga E, Protogerou A, Trakada G, Kostopoulos C, Zakopoulos N, Nikolopoulos I. *Effect of CPAP treatment on endothelial function and plasma CRP levels in patients with sleep apnea*. Med Sci Monit 2012; 18(12): *CR747-5*1.
26) Köktürk O, Ulukavak Çiftçi T, Mollarecep E, Çiftçi B. *Elevated C-reactive protein levels and increased cardiovascular risk in patients with obstructive sleep apnea syndrome*. Int Heart J 2005; 46: *801-9*.
27) Yokoe T, Minoguchi K, Matsuo H, Oda N, Minoguchi H, Yoshino G, Hirano T, Adachi M. *Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure*. Circulation 2003; 107(8): *1129-34*.
28) Taheri S, Austin D, Lin L, Nieto FJ, Young T, Mignot E. *Correlates of serum C-reactive protein (CRP) - no association with sleep duration or sleep disordered*

breathing. Sleep 2007; 30(8): *991-6*.
29) Ryan S, Nolan GM, Hannigan E, Cunningham S, Taylor C, Mc Nicholas WT. *Cardiovascular risk markers in obstructive sleep apnoea syndrome and correlation with obesity*. Thorax 2007; 62: *509-14*.
30) Guilleminault C, Kirisoglu C, Ohayon MM. *C-Reactive protein and sleep-disordered breathing*. Sleep 2004; 27(8): *1507-17*.

Corresponding author
HAKAN TANRIVERDİ
Bülent Ecevit University Faculty of Medicine Department of Chest Diseases and Tuberculosis,
Bülent Ecevit University Sağlık Uygulama Kampüsü, Esenköy, 67600 Zonguldak
(Turkey)