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Inflammatory cytokine level in patients with obstructive sleep apnea and treatment outcome of oral appliance therapy

폐쇄성 수면무호흡증 환자의 염증성 cytokine 농도와 구강내장치 치료 효과

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ABSTRACT

Inflammatory cytokine level in patients with obstructive sleep apnea and treatment outcome of oral appliance therapy

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The aims of this study were to analyze the association between inflammatory cytokine and obstructive sleep apnea (OSA), and to evaluate treatment outcome and changes of plasma inflammatory cytokine levels after oral appliance therapy.

Twenty-seven subjects who visited the Snoring and Sleep Apnea Clinic, Department of Oral Medicine in Seoul National University Dental Hospital were performed nocturnal polysomnography and analyzed plasma C-reactive protein (CRP), interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α levels. Each subject was evaluated with Pittsburgh Sleep Quality Index (PSQI), and Epworth Sleepiness Scale (ESS). The subjects were classified into 12 OSA

patients (Apnea-Hypopnea Index, AHI>5) and 15 control (AHI≤5) groups. The OSA group was treated with mandibular advancement device (MAD) for 3 months and re-evaluated nocturnal polysomnography and plasma inflammatory cytokine levels.

The obtained results were as follows,

1. Plasma TNF-α, IL-10, and IL-6 levels were significantly higher in OSA patients compared to controls. ($p<0.05$) There were no significant differences in plasma CRP and IL-1β levels between the two groups.

2. Total AHI showed significant positive correlations with plasma IL-6 and TNF-α levels ($p<0.01$). Percentage time of $SpO_2<90$ and lowest SpO_2 were significantly correlated with plasma TNF-α level ($p<0.05$). ESS showed significant positive correlation with plasma IL-10 level ($p<0.01$). There were no significant correlations between PSQI or BMI and plasma cytokines levels.

3. Total AHI, percentage time of $SpO_2<90$, lowest SpO_2, and mean SpO_2 were significantly improved after the MAD therapy. ($p<0.05$)

4. Plasma TNF-α level was significantly decreased after MAD therapy ($p<0.01$).

There were no significant changes in plasma levels of CRP, IL-1β, IL-6 and IL-10 after MAD therapy.

Key words: Obstructive sleep apnea, Mandibular advancement device (MAD), C-reactive protein, Interleukin, TNF-α

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Inflammatory cytokine level in patients with obstructive sleep apnea and treatment outcome of oral appliance therapy

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I. INTRODUCTION

Inflammatory cytokines are various intercellular signaling proteins which are involved in the regulation of local and systemic inflammatory responses. They carry out many important functions in biological processes by binding to specific surface receptors of target cells. [1)] Inflammatory cytokines are produced in peripheral lymphocytes, macrophages, and somatic cells and are not produced in specific glands unlike endocrine hormones. Most cytokines act in autocrine or paracrine fashion, so only small amounts are detected in the blood of healthy individuals.

Inflammatory cytokines were generally known to exist in the peripheral immune systems, but recently cytokines and their receptors have also been found in the brain, and have been discovered to be produced in the neurons and glial cells. It is known that cytokines take part in central nervous system processes including the regulation of arousal state, modulation of mood, feeding, thermoregulation, and sexual behavior, so they are considered as mobile brain.

Sleep regulation is one of the multiple functions of inflammatory cytokines. During the past two decades of studies, it has been reported that interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 are strongly involved in physiologic sleep regulation without stimuli causing immune responses. [2)]

Sleep is not a passive state of reduced neuronal cell activity, but an active process of diverse recovery processes for an individual’s health. Sleep deprivation not only

causes excessive daytime sleepiness but also increases the risk of weight gain, diabetes, cardiovascular disease, and hypertension. An absolute lack of sleep or low quality of sleep cause body changes such as general fatigue and sleepiness, and changes in the secretion of inflammatory cytokines. Reversely, changes in inflammatory cytokines secretion also affect sleep quality. [3-4)]

Snoring is a common symptom affecting about 20% of adult men over 40 years old. Snoring is the noise caused by vibration of the pharyngeal wall and surrounding soft tissue during respiration, and causes repeated partial or complete closure of the upper airway during sleep. When the upper airway is closed partially or completely during sleep, the patient's lung cannot be supplied with air, and this is called obstructive sleep apnea (OSA). These sleep-related breathing disorders cause serious health problem. [5-10)]

The symptoms of OSA are frequent awakenings and repeated hypoxia during sleep. The patients with OSA complain of daytime sleepiness, fatigue, feeling of not being refreshed in the morning, and cognitive functional disorder. Most patients usually want to be treated because of their social problems caused from severe snoring, but sleep apnea and associated problems should be the major cause of treatment. Habitual and chronic primary snoring only reflects mild sleep disorder which does not threaten health, but sleep apnea can be a risk factor of physiologically important diseases such as hypertension, heart failure, and stroke. According to a previous epidemiological study, the cumulative survival rate of OSA

patients with an AHI over 20 is about 70 % of that of patients with an AHI under 20.

Etiological factors of OSA are obesity, maxillofacial deformity such as mandibular retrognathism and micrognathia, large tongue and large tonsil. Age is also correlated with the prevalence of OSA. The airflow via the upper respiratory airway during sleep is related to the size and stiffness of the upper respiratory tract, and the neuronal control of pharyngeal muscles. In recent studies, a strong association between inflammatory cytokines and OSA has been reported as we have mentioned above. [11-12)]

For mild OSA, lifestyle changes such as sleep posture change, weight control, cessation of alcohol, and regular exercises can be a therapeutic option, but in severe OSA these may not be effective. For the treatment of moderate to severe OSA, pharyngeal or orthognathic surgeries, and continuous positive airway pressure (CPAP) have been suggested. Recently, many oral appliances have been developed and effectively used in dental clinics for the treatment of OSA because these treatment modalities are reversible and relatively simple.

There are several studies reporting on the changes of inflammatory cytokines after CPAP and pharyngeal surgery, but we could not find a study on the changes of plasma cytokine levels after oral appliance treatment.

The aims of this study were to analyze the association between inflammatory cytokine and obstructive sleep apnea (OSA), and to evaluate treatment outcome and changes of plasma inflammatory cytokine levels after oral appliance therapy.

II. REVIEW OF LITERATURES

1. Overview of sleep apnea

Obstructive sleep apnea (OSA) describes irregular breathing during sleep due to intermittent partial or complete collapse of the upper airway. [13)] The prevalence of OSA in adults is 24% in men and 9% in women and increases dramatically with obesity. Risk factors for OSA also include male gender, older age, and higher neck circumference. [12)] During an apnea or hypopnea, the patients experiences intermittent hypoxia and may also develop hypercapnia.

The severity of OSA is usually described using the Apnea-Hypopnea Index (AHI). The AHI measures the number of events per hour which breathing ceases for at least 10 seconds (apnea), or 90% decreases of airflow with at least 3% decrease of hemoglobin oxygen desaturation during sleep. [14)] During the apnea and hypopnea, the sleeper undergoes intermittent hypoxia and could also develop hypercapnia. Futile effort to breathe against the obstructive upper airway can also cause autonomic activation [15)] and alterations in intrathoracic pressure. [16)] OSA can be alleviated or completely abolished with lifestyle changes such as weight loss. [17)]

Though in most cases, active therapies are required to improve upper airway patency. Continuous positive airway pressure (CPAP) is most common type of

treatment, and works to maintain the airway open with pressured air. But it is limited by difficulty with adherence. [18, 19)]

Mandibular advancement devices are designed to lift soft tissue from the site of airway collapse, can be used effectively for OSA treatment. [20)] Surgery could be used to resolve the anatomical problem or used as an alternative treatment for patients who are not adaptable to use CPAP. [21)]

Health impacts of OSA have received growing attention from the public and healthcare community. Sleep fragmentation, intermittent hypoxia, hypercapnia, and ineffectual breathing efforts account for the majority of physiological derangements in OSA. [22)] The typical symptom of OSA is excessive daytime sleepiness. Sleepiness from OSA decreases quality of life and increases risks from activities such as driving. [23)] OSA is also associated with cardiovascular disease (CVD) and chronic risk factors for CVD such as hypertension, [24)] insulin resistance, [25)] atherosclerosis, and hyperlipidemia. [26)] A higher incidence of CVD and cerebrovascular disease [27)] is found in patients with OSA. However, the relationship between OSA and CVD is complex. The underlying obesity that predisposes to both OSA and CVD may account for at least some of the increased risks of CVD. In addition, OSA may itself confer risks for development of CVD. Some of the proposed pathways linking OSA to CVD include SNS activation, metabolic dysfunction, and inflammation. [28, 29)]

2. Cytokines and sleep regulation

Cytokines are mediators of immune system responses with multiple biologic actions on several target tissues. Cytokines are a diverse group of non-antibody intercellular signaling proteins that regulate local and systemic immune and inflammatory responses as well as many other biologic processes. These proteins may have an important role in regulation of sleep. [1)]

Evidences from investigation in animal and human suggest the existence of an interaction between the immune system and the neuroendocrine system in sleep-wake behavior. Cytokines may represent a link between these systems. [30, 31)] Early researches of the pathogenesis of acute phase response to infection showed that cytokines are major mediators of somnolence and fatigue. [31, 32)]

Sleep disturbance is common disease in modern society. Abnormal sleep is found in conditions such as insomnia, sleep loss, depression, chronic diseases, and sleep disorders Excessive daytime sleepiness (EDS) is a common symptom affecting about 5−15% of the general population and is often caused by sleep deprivation. EDS is the major complaint of the patients visiting sleep disorders centers. [33)] Obstructive sleep apnea (OSA), narcolepsy and idiopathic hypersomnia are the most common sleep disorders associated with EDS. [34)] There is evidence that cytokines may be involved in the pathophysiology of sleep disorders associated with EDS. [35)]

In healthy people, obesity is related to increased daytime sleepiness. [36)] There is

also a high prevalence of obesity in OSA patients. [37)] Adipose tissue produces numerous pro-inflammatory and anti-inflammatory factors that have been implicated in the pathogenesis of sleepiness, the development of insulin resistance and the pathogenesis of cardiovascular disease, which are commonly observed in OSA and obesity. [37-39)]

IL-1β and TNF-α have been known as important sleep-promoting substances. Animal studies have shown that central administration of these cytokines induces non-rapid eye movement (NREM) sleep, while their inhibitors block this response. [3)] Moldofsky et al. have found that endogenous IL-1 activity in the cerebrospinal fluid of a cat increases during sleep in comparison to wake. [40)] Detection of TNF-α in the lymphatic system of experimental animals after injection in their cerebral ventricles suggests a direct communication between CNS and the peripheral immune system. [41)]

Several neuroendocrine hormones such as glucocorticoids, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone, growth hormone-releasing hormone (GHRH) and growth hormone (GH) influence sleep–wake behavior either by promoting or inhibiting sleep. [42)] GHRH enhances NREM sleep in several animal species and humans either after central or peripheral administration. [43)] There is also evidence of a close relationship with IL-1β actions. [3)] IL-1β enhances GHRH and GHRH receptor mRNA production. CRH central administration in rats decreases NREM sleep after inhibition of IL-1β production. GHRH, CRH and IL-

1β affect each other by way of a feedback mechanism. [3, 43] The interaction of somnogenic cytokines with the hypothalamic–pituitary–adrenal (HPA) is important in sleep–wake behavior. Research in animals has suggested a possible role of cytokines in sleep regulation in humans. Early studies in humans have shown that sleep onset was associated with increased activity of IL-1 followed by elevations of IL-2 that appeared to be related to a decline in plasma cortisol and the appearance of slow wave sleep (SWS). [44] IL-1 activity was greater during SWS, suggesting its role in sleep regulation. In another study, negative feedback was found between IL-1 and CRH during stress, leading to lower IL-1 during sleep. [45] In another study, circulating IL-2 had been found to be increased after one hour of sleep onset, but during the night its levels were similar to waking levels and there was no association between IL-2 levels and sleep. [46] Thus, circulating cytokine levels may not reliably reflect the relationship between the sleep–wake cycle and cytokines.

3. Biomarkers related to OSA and morbidities

For diagnosis for OSA, the overnight polysomnography (PSG) has been considered as ‘gold standard’. However PSG results are poor predictor of OSA-associated morbidities**.** [47] In other words, two patients with similar PSG results may present with different clinical phenotypes. These diverse phenotype features provoked exploration of biomarkers which could enable the identification of the prognosis of OSA, therefore would help the therapeutic interventions. And these

studies explored the opportunity to identify the morbidity biomarkers. The search for appropriate biomarkers becomes critical. Studies for biomarkers of OSA-associated morbidity provide information of prognosis and response of treatment.

Several biomarkers have been proposed for OSA over last two decade. The majority of studies evaluated blood biomarkers. To assess the magnitude of the systemic inflammatory response, as measured by C-reactive protein (CRP) serum levels, that may identify children with OSA at higher risk for cognitive morbidity. Gozal et al. [48] concluded that. CRP levels are higher in children with OSA and particularly in those who develop neurocognitive deficits, suggesting that the magnitude of the inflammatory responses elicited by OSA is a major determinant of increased risk for neurocognitive dysfunction.

Blood-based biomarkers accounted for the majority of the studies, and most of the explored approaches did not identify definitive biomarkers of OSA morbidity. IL-6 and CRP appear to exhibit a favorable profile as biomarkers aiming to discriminate OSA patients with and without morbidity in adults.

4. Inflammatory markers and OSA

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention. [49] In case of OSA, useful biomarker should help making diagnosis, assessing disease severity, and evaluating

the response to treatment. [50] Furthermore a biomarker should reflect the pathogenesis of disease and should respond the therapy, and should be easy to evaluate.

Inflammatory biomarkers of OSA are CRP, TNF-α, interleukin, and so on. OSA is considered as important risk factor of cardiovascular disease. [27] Sleep deprivation and hypoxia are postulated to contribute the etiology of cardiovascular events. [51, 52] OSA-induced hypoxia could increase the circulating inflammatory biochemical mediators such as CRP, TNF-α, interleukin-6, and interleukin-8.

CRP is a marker of inflammation that is mainly produced by the liver in response to IL-6. Increased CRP levels are identified as consequence of trauma or infection. CRP has an active role in the atherogenesis and future cardiovascular events. [53, 54] Repetitive hypoxemia was correlated with CRP. CPR levels were also correlated with nocturnal hypoxia which means hypoxia is probably the contributor of inflammation in OSA. [55] CRP levels were associated with AHI positively. Serum CRP levels were significantly higher in OSA patients. [56] AHI was and independent predictor for CRP. CRP levels in OSA patients were decreased after CPAP therapy [57] but other study showed no CRP level changes. [58]

The increased serum CRP levels in OSA patients and its association with disease severity may reflect the risk of developing cardiovascular events or the presence of subclinical atherosclerosis. Therefore, therapeutic interventions that aim at decreasing serum CRP levels may have a protective role for future cardiovascular

and cerebrovascular complications.

TNF-α is a pro-inflammatory cytokine produced by monocytes, macrophages in acute inflammation. [59] TNF-α participate in a signaling events leading to necrosis or apoptosis. [59] TNF-α is also correlated with excessive daytime sleepiness, nocturnal sleep disturbance, and hypoxia. [60] Several studies have confirmed the relationship between increased serum TNF-α levels and OSA. [60-62]

Serum concentration of TNF-α was associated with duration of hypoxia during total sleep time, and they were decreased after one month of CPAP application. [27] Moreover, prior evidence demonstrated that increased levels of serum TNF-α can be attenuated after CPAP treatment. [63, 64]

However, there is also evidence that there may be no difference in serum TNF-α among OSA patients compared with controls, before and after therapy with CPAP. [65, 66] Obesity may be the primary factors affecting the level of TNF-α, because TNF-α is secreted by macrophage of adipose tissue. [38]

There may be a role for OSA in promoting inflammation among obese individuals possibly mediated by TNF-α. But this interaction is complex and need more studies. Increased pro-inflammatory cytokines may affect the treatment outcomes, especially about increased frequency of cardiovascular and cerebrovascular complications associated with OSA.

IL-6 is circulating cytokine produced by various cells, including macrophages and lymphocytes. [67] It is a major initiator of the acute phase response and the

regulator of the hepatic CRP production. IL-6 levels of OSA are conflicting. Some studies reported IL-6 levels in OSA patients were higher in comparison with normal group, while other studies reported that obesity per se rather than OSA was associated with systemic inflammation. [55, 68, 63, 65)] 15-30% of the circulating IL-6 is originated from fat tissue. A positive correlation was found between the IL-6 level and AHI, as well as percentage of oxygen saturation. [62)] Maeder et al. [69)] found higher IL-6 serum levels with moderate/severe OSA than mild/moderate OSA. About IL-6 levels after CPAP treatment, some studies reported that IL-6 levels were lowered after treatment, other studies reported no changes were found. [70)] Increased IL-6 levels are associated with OSA severity and are often correlated with higher serum CRP levels, both cytokines being involved in the pathogenesis of cardiovascular and cerebrovascular disease.

In patients with OSA, the rhythm of circadian release of TNF-α is altered with a reduction of the normal nocturnal peak and presence of an additional daytime peak. Treatment with nasal CPAP for three months had no effect on TNF-α circadian production. [71)] It has been speculated that TNF-α may play a role in the pathogenesis of OSA.

The examination of plasma levels of TNF-α in 27 OSA patients, seven patients with chronic tonsillitis and four healthy controls, evaluated the effect of surgical treatment on TNF-α levels. [72)] The authors showed that TNF-α levels are higher in OSA patients compared to healthy subjects or patients with chronic tonsillitis. There

was no correlation between TNF-α and AHI, and it was speculated that inflammation in the upper airway and tonsils explains the absence of a relationship between AHI and levels of cytokine. TNF-α has been suggested to be the mediator of somnolence and fatigue and also promotes pharyngeal inspiratory muscle dysfunction, [73] leading to a vicious cycle of worsening sleep apnea events during sleep. TNF-α may be involved in the pathogenesis of sleep apnea and may not merely be a metabolic effect of hypoxia and oxidative stress present in OSA.

5. Cytokine levels changes after sleep apnea treatment

In patients with OSA, the rhythm of circadian release of TNF-α is altered with a reduction of the normal nocturnal peak and presence of an additional daytime peak. Treatment with nasal CPAP for three months had no effect on TNF-α circadian production. [72] It has been speculated that TNF-α may play a role in the pathogenesis of OSA Increased circulating TNF-α levels may promote pharyngeal inspiratory muscle dysfunction and lead to aggravation of sleep apnea in a vicious cycle. [73, 74]

CPAP treatment improves insulin sensitivity but not the adiponectin levels because of the effect of increased body fat in OSA patients. The increase of the insulin sensitivity has been shown to be more pronounced in patients with the highest adiponectin levels before CPAP application, after adjustment for body fat. [75] The interaction of secretory activity of adipose tissue and insulin sensitivity may

be important in cardiovascular disease in OSA.

The examination of plasma levels of TNF-α in 27 OSA patients, seven patients with chronic tonsillitis and four healthy controls, evaluated the effect of surgical treatment on TNF-α levels. [72] The authors showed that TNF-α levels are higher in OSA patients compared to healthy subjects or patients with chronic tonsillitis. There was no correlation between TNF-α and AHI, and it was thought that inflammation in the upper airway and tonsils explains the absence of a relationship between AHI and levels of cytokine. It has been shown that inflammation in upper airway is mainly related to obesity. [76] TNF-α has been suggested to be the mediator of somnolence and fatigue and also promotes pharyngeal inspiratory muscle dysfunction, [73] leading to a vicious cycle of worsening sleep apnea events during sleep. TNF-α may be involved in the pathogenesis of sleep apnea and may not merely be a metabolic effect of hypoxia and oxidative stress present in OSA. This study is the first showing that surgical removal of inflamed tissue in OSA patients leads to decreased levels of TNF-α. The decrease of TNF-α was significant only in patients with severe OSA, and some patients still had high levels one week after the operation. This may reflect the effect of surgical wound or insufficient improvement of apnea symptoms. The measurement was done one week after the operation and this time interval could be not enough for TNF-α decrease. The administration of etanercept, a TNF-α antagonist, to obese patients with severe OSA resulted in reduction of objectively measured sleepiness that remained significant after

adjustment for placebo values. [77] This supports the suggestion that pro-inflammatory cytokines are mediators of excessive sleepiness and fatigue in humans. Patients who received etanercept showed a small reduction in AHI. This supports the hypothesis that inflammation may play, at least partially, a role in the pathogenesis of OSA. There was also a significant reduction of IL-6 levels. Thus, the increased secretion of IL-6 is possibly a secondary consequence of TNF-α activation.

Infliximb is a chimeric monoclonal antibody to TNF-α with proven efficacy in the treatment of rheumatoid arthritis. In a small study on patients with rheumatoid arthritis, it was shown that quality of sleep and daytime performance in these patients were improved after administration of infliximb. [78] These studies provide more evidence on the role of TNF-α in the pathogenesis of OSA. Further research on this field is needed. In summary, cytokines are mediators of sleepiness and implicated in the pathogenesis of OSA symptoms. Adipocyte-derived cytokines are involved in local and systemic inflammation, visceral fat obesity, and insulin resistance and seem to be important in the pathogenesis of the cardiovascular complications in OSA. The recognition of their role may lead to novel therapeutic approaches.

6. Further research perspectives

Cytokines are mediators of sleepiness and implicated in the pathogenesis of OSA

symptoms. Adipocyte-derived cytokines are involved in local and systemic inflammation, visceral fat obesity, and insulin resistance and seem to be important in the pathogenesis of the cardiovascular complications in OSA. The recognition of their role may lead to novel therapeutic approaches.

Cytokines play an important role in the interaction between peripheral immune signals and brain responses. Sleep–wake behavior is related to energy balance regulation, with the cytokine network and neuroendocrine system acting in close relationship. Eventually their actions may be involved in the pathogenesis of sleepiness and other ‘‘sickness’’ symptoms that characterize sleep loss in many situations. Cytokines may be the central player in the evolution of important health hazards like the aging process, depression, sleep deprivation, obesity and cardiovascular disease in conditions of sleep disturbance like OSA. A better understanding of their role may give rise to novel treatment targets in clinical medicine.

III. METHODS

1. Subjects

Twenty-seven subjects who visited the Snoring and Sleep Apnea Clinic of the Department of Oral Medicine, Seoul National University Dental hospital were

examined by nocturnal polysomnography and their serum inflammatory cytokine levels were analyzed.

The subjects were classified into 12 OSA patient (AHI>5) and 15 control (AHI≤5) groups according to the polysomnography results. The OSA group was treated with mandibular advancement device (MAD) for 3 months and re-evaluated nocturnal polysomnography and plasma inflammatory cytokine levels. Exclusion criteria of the subject were infection, injury, or surgical operation within 6 months; collagen, hematological, allergic, cardiovascular, respiratory, or malignant disease; and any medication affecting plasma cytokine level within 1 month before the baseline examination.

The study was approved by the institutional review board of Seoul National University Dental Hospital.

2. Evaluation of sleep quality

Sleep quality was evaluated by means of the Pittsburgh Sleep Quality Index (PSQI) and daytime sleepiness by means of the Epworth Sleepiness Scale (ESS). Subjects with a PSQI score of more than 6 are considered to have poor-quality sleep and those with an ESS score of more than 10 to suffer of excessive daytime sleepiness.

3. Polysomnography

Multi-channel recordings of electroencephalogram (EEG), submental and leg electromyogram (EMG), electrocardiogram (ECG), nasal thermistor, nasal pressure transducer, thoracic and abdominal piezoelectric belts, and oxygen saturation were performed using level I polysomnography (Alice 5, Respironics, Pittsburgh, USA). Body position was also confirmed through direct observation of the patient by the technician using a low light camera and simultaneous digital recording with a posture tag at the thoracic piezoelectric belt.

Sleep was staged and respiratory events were scored using the standard criteria of the American Academy of Sleep Medicine. Briefly, obstructive sleep apnea was defined as a reduction in airflow greater than 90% with a duration of at least 10 sec in which there was persistent respiratory effect, whereas hypopnea was defined as a reduction of airflow by 30% for more than 10 sec accompanied by oxygen desaturation $\geq$ 3%.

4. Collection of plasma

Plasma samples of all controls and patients were obtained from the antecubital vein and stored in Lavender tubes coated with ethylenediaminetetraacetic acid (Becton Dickinson Vacutainer System, Rutherford, NJ, USA). All samples were collected between 9:00 a.m. and 12:00 noon. The plasma was immediately centrifuged (2000 rpm) for 10 minutes at 4°C, and stored at –70°C before analysis.

5. Quantification of CRP and inflammatory cytokines

The plasma concentrations of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and the anti-inflammatory cytokine IL-10 were measured by means of the Procarta cytokine assays (Panomics, Fermont, CA, USA). The assays are multiplex immunoassays based on xMAP® technology (Luminex, Austin, TX, USA). Each cytokine-specific antibody was coupled to a different microsphere labeled with a unique fluorescent dye through covalent bonding. All specimens were incubated in a 96-well microtiter filter plate with the microspheres at 500 rpm for 60 minutes at room temperature. After washing with assay wash buffer, diluted biotinylated secondary antibody was added then incubated at 500 rpm for 30 minutes. Following washing, streptavidin-phycoerythrin was added and incubated for 30 minutes. After another washing, the plate was evaluated with Bio-Plex 200 analyzer (BIO-RAD Laboratories Inc., Hercules, CA, USA) to decide the concentration of the cytokines. Plasma samples were diluted 3-fold with assay diluents. In each plate, the standards and a quality control pool were tested in triplicate, and the 60 samples were tested in duplicate.

Plasma concentrations of CRP were analyzed by means of a highly sensitive immunoturbidimetric assay autoanalyzer (Hitachi 7180, Hitachi High-Technologies Corp., Tokyo, Japan).

The person conducting the measurements was blind to the identity of the subjects.

6. Mandibular advancement devices (MADs)

All appliances used to advance the mandible and were custom made. The SNU appliance was used in this study. The appliances were made by the respective laboratories and the degree of mandibular advancement was set to 60% of the patient's maximum protrusion at the time the impressions were made. For titration, incremental anterior adjustments of the mandible were made until the maximum comfortable limit was reached. An additional sleep study was performed with the MAD after 3 months' of oral appliance therapy to determine treatment efficacy.

7. Statistical analysis

Comparison of all measures of apnea severity and the effect of MAD between the control and OSA group was performed by t-test. The relationships between polysomnography parameters and plasma cytokine were evaluated using Pearson's correlation test.

The effect of 3-month treatment of MAD on polysomnography parameters and plasma cytokine levels were evaluated using paired t-test.

IV. RESULTS

1. Subject

Anthropometric features, total AHI, PSQI, and ESS of the two groups are shown in Table 1. Comparing OSA patients and control groups, there were no significant differences in age, BMI, PSQI, and ESS scores. There were significant differences in total AHI between two groups.

2. Baseline plasma cytokine levels

Baseline levels of plasma cytokine of the OSA and control group are shown in Table 2. There were significant differences in plasma TNF-α, IL-10, and IL-6 levels between OSA and control groups. Plasma TNF-α, IL-6, and IL-10 levels were higher in OSA patients than controls. There were no significant differences in plasma CRP and IL-1β levels between the two groups.

3. Plasma cytokines levels and sleep parameters

Spearman's correlation coefficients among plasma levels of CRP, IL-1β, IL-6, IL-10, TNF-α, and sleep parameters are shown in Table 3. There were no significant correlations among PSQI, BMI, and plasma cytokines levels. Total AHI showed significant positive correlations with plasma IL-6 and TNF-α levels ($p<0.01$). Percentage time of $SpO_2<90$ showed significant positive correlation with plasma TNF-α level ($p<0.05$). Lowest SpO_2 showed negative correlation with plasma TNF-α level ($p<0.05$). ESS showed significant positive correlation with plasma IL-10 level ($p<0.01$). (Figure 1)

4. Sleep parameters changes after MAD treatment

Table 4 shows the changes of sleep parameter after 3 months of MAD therapy. Total AHI ($p<0.01$), percentage time of $SpO_2<90$ ($p<0.05$), lowest SpO_2 ($p<0.01$), and mean SpO_2 ($p<0.01$) were improved significantly after 3-month MAD therapy. But ESS and PSQI scores were not significantly changed.

5. Plasma cytokine levels changes after MAD treatment

Table 5 shows the changes of plasma cytokine levels after 3-months' MAD therapy. There were no significant differences in levels of plasma CRP, IL-1β, IL-6, and IL-10 between baseline and after treatment. Plasma TNF-α level showed significant decrease after MAD treatment ($p<0.01$).

V. DISCUSSION

The pathogenesis of OSA complications is multifactorial, including systemic inflammation, oxidative stress, and metabolic perturbations. OSA treatment is maintaining airway patency during sleep through CPAP, oral appliance, surgery, and weight loss. Although considerable progress has been made, results are not always certain and long term follow-up is required. So, the usefulness of new biomarkers would be important.

In the present study, AHI index was dropped significantly after MAD therapy. Assessment of AHI index is one of the most generally used criteria evaluating the effectiveness of MAD therapy. Mandibular advancement is directly associated with the efficacy of MAD. Further improvement in inflammatory cytokine levels could also be related to the efficacy of treatment.

We observed that CRP and IL-1β were not significantly different between the OSA and control group, while IL-6, IL-10, and TNF-α were higher in OSA group. Our study demonstrated that TNF-α level, one of the known pro-inflammatory cytokines, increased significantly in patients with OSA than in normal subjects. This finding was in line with the result reported by Vgontzas et al. [35] Other pro-inflammatory cytokines such as IL-1β, IL-6, and CRP have been reported to increase in patients with OSA in comparison with normal subjects. [79, 57] But our study did not show the same results in CRP and IL-1β levels. Our study confirmed the fact that plasma IL-6, IL-10, and TNF-α levels are significantly higher in patients with OSA than the control group. [57, 80] Plasma CRP and IL-1β levels were not different between the two groups. In some studies CRP and IL-6 levels are elevated in OSA patients compared to the normal group. It has been suggested that elevated CRP level in OSA patients may be related to obesity. [81, 82] IL-6 level appears to be predictive of future cardiovascular disease and is elevated in patients with unstable angina compared to those with stable angina. Elevated IL-6 level is often found to correlate with CRP levels. [82]

IL-1β is a marker of systemic inflammation and activated innate immunity, and has been reported to be elevated in OSA patients. [83] In our study IL-1β level was higher in the OSA group than in the control group, but no significant difference was found.

We also observed increased IL-10, an anti-inflammatory cytokine, in OSA patients, which is an identical same result reported by Sahlman et al. [84] This finding might represent a compensatory mechanism aiming to reduce the inflammatory response. IL-10 level after 3-months' MAD therapy was slightly decreased but it was not significant. This result should be further studied.

In our study total AHI showed significant positive correlations with plasma IL-6 and TNF-α levels. SpO_2<90, and lowest SpO_2 showed significant correlations with plasma TNF-α levels. These results speculate that hypoxic damage can induce general inflammatory condition by increasing inflammatory plasma cytokines. OSA represents intermittent hypoxemia during sleep and these conditions can be the predisposing factors of cardiovascular disease such as pulmonary hypertension, ischemic heart disease, and cardiovascular accident.

Our study showed that an effective treatment with MAD for 3 months in patients with OSA altered plasma TNF-α inflammatory cytokine levels. But other plasma inflammatory cytokines levels such as IL-1β, IL-6, IL-10, and CRP were not improved within 3 months after MAD therapy. These results are similar with a previous research of CPAP therapy. [63] And another research reported that TNF-α

and IL-6 levels were decreased in OSA patient after surgical treatment and IL-6 level showed more improvement than TNF-α. [85] We thought longer period of studies and a larger sample size are needed to evaluate the changes of other inflammatory cytokines after oral appliance therapy. For example IL-10 is a cytokine with anti-inflammatory properties capable of modulating inflammatory responses by suppressing the production of pro-inflammatory cytokines such as IL-1β, TNF- α, IL-6, and IL-8.

Elevated TNF-α level predicts the incidence of cardiovascular event in several conditions like heart failure and acute coronary syndrome. TNF-α level has been shown to be a major predictor of mortality and heart failure in acute myocardial infarction. Increased plasma TNF-α is associated with increased mortality in a wide range of heart failure. The TNF-α level of OSA patient were lower than the TNF-α level of heart failure and ischemic patients, but higher than in normal individuals. OSA is thought to be a strong predisposing factor of heart disease in a view of plasma cytokines. So OSA patients with high levels of TNF-α should be examined and treated for the prevention of heart diseases.

In our studies MAD therapy ameliorated the level of TNF-α after 3-months of follow-up. Circulating levels of TNF-α have been reported to correlate with signs of early atherosclerosis amongst healthy middle-age men and are predictive of coronary heart disease. Moreover persistently increased levels of TNF-α after myocardial infarction are predictive of future coronary events. MAD therapy is a

very useful treatment for OSA and would reduce the risk of future cardiovascular problems. MAD therapy has some advantages over CPAP or surgical therapy. The significant decrease of plasma TNF-α level in OSA patients who complied with MAD use could lead us to the argument of the direct effect of OSA on systemic immunity. So it could be said that MAD therapy is a useful treatment for OSA and its general inflammatory sequelae.

There are several limitations to our study. Firstly, our study was based on relatively a small sample size and short duration of observation. Secondly, we did not apply MAD to the control group. Thirdly, we did not consider systemic diseases of the subjects. There is growing evidence that inflammatory cytokines play important roll in the pathophysiology of cardiovascular disease in patients with OSA. Comorbid disease variables should be considered based on a larger size of population and longer duration in future studies to elucidate mechanisms behind the observed changes in inflammatory mediators.

However, our study is the first study to investigate the effect of oral appliance therapy on plasma cytokine levels and showed that MAD can decrease plasma TNF-α level. Our results also showed associations between OSA and plasma inflammatory cytokine levels.

VI. CONCLUSIONS

In conclusion, plasma TNF-α, IL-10, and IL-6 levels were higher in OSA patients compared to controls. There was no significant difference in plasma CRP and IL-1β levels between the two groups. Total AHI showed significant positive correlations with plasma IL-6 and TNF-α levels. Percentage time of SpO_2<90 and lowest SpO_2 were significantly associated with plasma TNF-α level. There were no significant differences in levels of CRP, IL-1β, IL-6, and IL-10 between baseline and after treatment. TNF-α level showed significant reduction between baseline and after MAD treatment.

We suggest that MAD therapy is an effective treatment modality for patients with OSA and can decrease plasma cytokine level as effectively as CPAP and surgical management.

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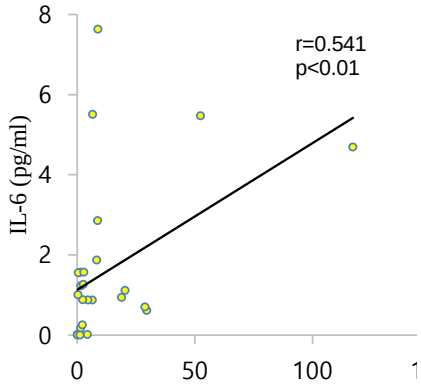
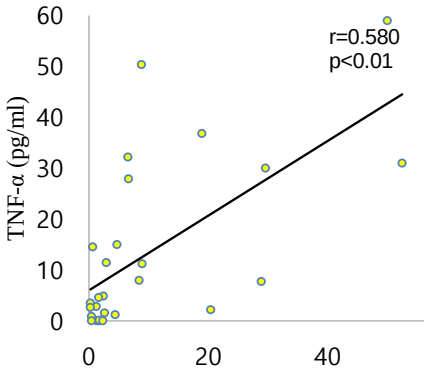
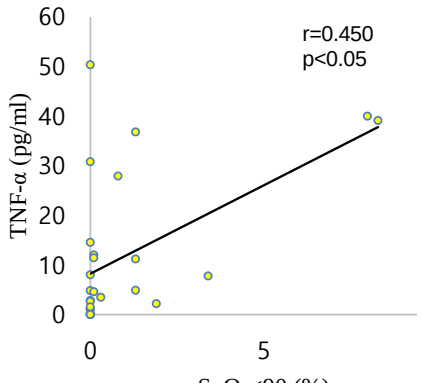


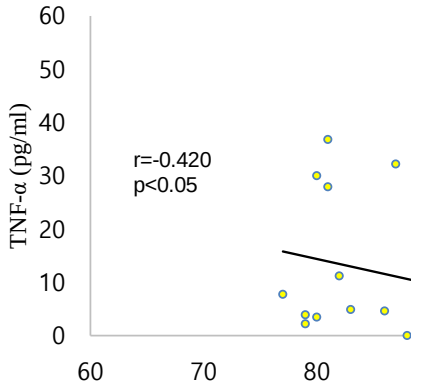
(a) Total AHI and plasma IL-6



(b) Total AHI and plasma TNF-α



(c) Percentage time of SpO_2<90 and plasma TNF-α



(d) Lowest SpO_2 and plasma TNF-α

Figure 1. Correlations among plasma levels of inflammatory cytokines and sleep parameters

Table 1. Characteristics of the study population

Group	Age	BMI (SI unit)	Total AHI (event/hr)	PSQI	ESS
OSA (n=12)	41.3 ± 3.0	25.9 ± 3.9	25.89 ± 31.95	6.55 ± 3.14	8.42 ± 4.31
Control (n=15)	40.8 ± 3.6	24.8 ± 1.5	1.59 ± 1.19	5.07 ± 2.69	5.60 ± 2.26
P-value	0.729	0.376	0.023	0.223	0.058

BMI: Body mass index

AHI: Apnea-hypopnea index

PSQI: Pittsburgh Sleep Quality Index

ESS: Epworth Sleepiness Scale

Results are shown as mean ± SD

p-values were obtained from independent T-test

Table 2. Comparisons of plasma cytokine levels between OSA and control groups.

Group	CRP (mg/dl)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)
OSA	0.11 ± 0.03	0.66 ± 0.14	2.76 ± 0.70	4.65 ± 1.41	81.16 ± 61.69
Control	0.05 ± 0.01	0.45 ± 0.03	0.64 ± 0.17	1.51 ± 0.23	3.21 ± 1.12
P-value	0.217	0.126	0.012	0.019	0.000

Results are shown as mean ± SEM

p-values were obtained from Kruskal-Wallis test

Table 3. Correlations among plasma levels of inflammatory cytokines and sleep parameters.

Sleep Parameters	CRP (mg/dl)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	TNF-α (pg/ml)
Total AHI (event/hr)	0.130	0.038	0.541**	0.373	0.560**
SpO_2<90 (%)	0.151	0.175	0.355	0.135	0.450*
Lowest SpO_2 (%)	-0.167	-0.256	-0.355	-0.160	-0.420*
Mean SpO_2 (%)	-0.274	0.072	-0.044	-0.211	-0.135
ESS	0.051	0.014	0.117	0.487**	0.112
PSQI	0.134	-0.231	-0.316	-0.217	-0.030
BMI (SI unit)	0.021	-0.330	-0.263	-0.294	-0.266

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

The appearing values are correlation coefficients of Spearman's correlation analysis.

Table 4. Changes of sleep parameters after MAD therapy

Sleep Parameters	Baseline	After MAD therapy	P-value
Total AHI (event/hr)	25.95 ± 9.22	13.87 ± 9.34	0.007
SpO_2<90 (%)	3.15 ± 1.62	1.44 ± 1.42	0.027
Lowest SpO_2 (%)	83.42 ± 1.51	90.08 ± 1.31	0.002
Mean SpO_2 (%)	95.17 ± 0.41	96.83 ± 0.41	0.001
ESS	8.64 ± 1.34	6.91 ± 1.30	0.197
PSQI	6.70 ± 1.03	5.40 ± 1.24	0.152

Results are shown as mean ± SEM

P-values were obtained from paired T-test

Table 5. Changes of plasma cytokine levels after MAD therapy.

Cytokine levels	Baseline	After MAD therapy	P-value
CRP (ml/dl)	0.11 ± 0.03	0.10 ± 0.03	0.766
IL-1β (pg/ml)	0.66 ± 0.14	0.63 ± 0.09	0.480
IL-6 (pg/ml)	2.76 ± 0.70	3.68 ± 2.94	0.099
IL-10 (pg/ml)	4.65 ± 1.41	4.06 ±1.80	0.937
TNF-α (pg/ml)	81.16 ± 61.69	8.18 ± 3.81	0.002

Results are shown as mean ± SEM

P-values were obtained from Wilcoxon Rank Sum test

국문초록

폐쇄성 수면무호흡증 환자의 염증성 cytokine 농도와 구강내장치 치료 효과

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오 재 탁

본 연구의 목적은 혈중 염증성 cytokine 농도와 폐쇄성 수면무호흡증과의 연관성을 분석하고, 구강내장치 치료에 따른 혈중 cytokine의 변화 양상을 알아보는데 있다.

서울대학교치과병원 구강내과 코골이 수면무호흡증 클리닉을 방문한 27명을 대상으로 야간수면다원검사를 시행하여 12명의 폐쇄성 수면무호흡증 환자군과 15명의 정상 대조군으로 나누었으며, 각각의 혈액을 채취하여 C-reactive protein (CRP), interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α 농도를 측정하였다. 각 대상자에게 Pittsburgh Sleep Quality Index (PSQI)와 Epworth Sleepiness Scale (ESS) 설문지를 작성하게 하여 수면의 질과 주간 졸리움을 평가하였다. 수면무

호흡증 환자군에 대하여는 하악전방위치장치 (mandibular advancement device, MAD) 치료를 3개월 간 시행하였으며, 치료 후 야간수면다원검사 시행, 혈중 cytokine 농도의 분석과 PSQI, ESS 설문을 시행하여 다음과 같은 결과를 얻었다.

1. 혈중 TNF-α, IL-10, IL-6의 농도는 수면무호흡 환자군에서 대조군에 비하여 통계적으로 유의하게 높게 나타났다 ($p<0.05$).

2. 총 수면무호흡-저호흡지수 (Apnea-Hypopnea Index, AHI)는 혈중 IL-6, TNF-α 농도와 유의한 양의 상관관계를 보였다 ($p<0.01$). Percentage time of $SpO_2<90$, lowest SpO_2 는 혈중 TNF-α 농도와 유의한 상관관계를 보였다 ($p<0.05$). ESS 수치는 혈중 IL-10 농도와 유의한 양의 상관관계를 보였다 ($p<0.01$). PSQI, BMI 와 혈중 cytokine 농도는 유의한 상관관계를 보이지 않았다.

3. 하악전방위치장치 치료 3개월 후 총 수면무호흡-저호흡지수, percentage time of $SpO_2<90$, lowest SpO_2, mean SpO_2 는 유의하게 개선되었다 ($p<0.05$). ESS와 PSQI는 하악전방위치장치 치료 후 유의한 변화를 보이지 않았다.

4. 혈중 TNF-α 농도는 하악전방위치장치 치료 3개월 후 치료 전에 비하여 유의하게 감소하였다 ($p<0.01$). 혈중 CRP, IL-1β, IL-6, IL-10 농도는 하악전방위치장치 치료 전후 유의한 차이를 보이지 않았다.

주제어: 폐쇄성수면무호흡증, 하악전방위치장치, C-reactive protein, Interleukin, TNF-α

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