Inflammatory Cytokines and Sleep Disturbance in Patients with Temporomandibular Disorders

Ji Woon Park, DDS, PhD
Assistant Professor
Orofacial Pain Clinic
Department of Oral Medicine and Oral Diagnosis
School of Dentistry and Dental Research Institute
Seoul National University
Seoul, Korea

Jin Woo Chung, DDS, PhD
Professor
Orofacial Pain Clinic
Department of Oral Medicine and Oral Diagnosis
School of Dentistry and Dental Research Institute
Seoul National University
Seoul, Korea

Correspondence to:
Dr Jin Woo Chung
Orofacial Pain Clinic, Department of Oral Medicine and Oral Diagnosis
School of Dentistry and Dental Research Institute, Seoul National University
28 Yunkeun-Dong, Chongro-Ku, Seoul 110-749, Korea
Fax: +82-2-744-9135
Email: jwchung@snu.ac.kr

Aims: To assess the degree and interrelationship of sleep disturbance and plasma cytokine levels in temporomandibular disorder (TMD) pain patients. **Methods:** Forty female TMD patients and 20 age-, sex-, and body mass index (BMI)–matched healthy subjects were enrolled. TMD was diagnosed using the Research Diagnostic Criteria for TMD. The TMD patients were classified as having low or high disability according to Graded Chronic Pain Scale findings. The Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS) were used to measure sleep quality. Plasma concentrations of interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) were measured from blood samples collected between 9 am and noon. Statistical analyses included Kruskal-Wallis and one-way analysis of variance tests to compare results between different groups and multivariate general linear models to evaluate the effect of sleep status on cytokine levels. **Results:** The high-disability group had the highest PSQI and ESS scores ($P < .001$). Plasma levels of IL-1β, IL-6, IL-10, and TNF-α were significantly higher in the patient groups, with the high-disability group exhibiting the highest values ($P \leq .001$). The plasma cytokine levels were significantly correlated with PSQI scores ($P < .05$). Plasma levels of IL-10 and TNF-α were significantly associated with the disability level after adjusting for both sleep indices (both $P < .05$). **Conclusion:** Patients with TMD, especially those with high disability, had elevated plasma cytokine levels and increased ESS and PSQI scores suggestive of sleep disturbance. *J Oral Facial Pain Headache 2016;30:27–33. doi: 10.11607/ofph.1367*

Keywords: *cytokine level, pain, sleep disturbance, temporomandibular disorders*

Sleep was once considered a passive state with low physiologic importance but is now recognized as a dynamic and active state that is essential for the normal functioning of an individual.[1] Furthermore, the association between disturbed sleep and chronic pain syndromes, including fibromyalgia, myofascial pain, and tension-type headache, is now well known.[2,3] A study comparing patients with temporomandibular disorders (TMD), fibromyalgia, or chronic fatigue syndrome showed that sleep disturbances were reported by approximately two-thirds of TMD patients, more than half of whom reported fatigue lasting longer than 6 months.[4] Moreover, TMD patients had a high degree of primary insomnia and associated hyperalgesia outside the orofacial region. These results suggest that disturbed sleep may eventually increase pain levels in patients with pain disorders by means of central sensitization.[5]

The mechanisms by which abnormal sleep affects pain are still unclear. Decreased sleep time impairs the immune response, and immune reactions affect sleep time and quality.[6] Changes in sleep duration alter the level of inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β, IL-5, and IL-6.[7–9] Furthermore, elevated levels of CRP and IL-6 after prolonged sleep deprivation have been found to increase pain sensitivity in healthy subjects,[10] and patients with disorders highly associated with sleep problems (eg, fibromyalgia, chronic low back pain, chronic fatigue syndrome) have been found to have altered cytokine levels.[11–13] These findings suggest

that chronic inflammation could be the underlying mechanism responsible for the association between abnormal sleep and chronic pain.

In addition to their main location in the peripheral immune system, cytokines and their receptors also reside in the brain. These brain cytokines are known to regulate various processes including sleep.[14] Considerable evidence also shows that cytokines play an important role in the modulation of nociceptive processes in both peripheral tissues and the central nervous system. Proinflammatory cytokines including IL-1β induce cyclooxygenase-2 and prostaglandin E synthase from cells of the blood-brain barrier, leading to central hypersensitivity.[15] An animal study showed that increased peripheral production of proinflammatory cytokines TNF-α and IL-1β and decreased anti-inflammatory cytokine IL-10 levels may mediate granulocyte-colony stimulating factor-induced hyperalgesia.[16] Moreover, a study reported that proinflammatory cytokine levels in pain patients significantly increased and were positively correlated with the patients' pain intensity.[17]

The aim of the present study was to assess the degree and interrelationship of sleep disturbance and plasma cytokine levels in TMD pain patients.

Materials and Methods

Subjects

The study involved 40 consecutive female TMD pain patients (mean age ± standard deviation [SD], 32.9 ± 13.3 years) who attended the Seoul National University Dental Hospital and 20 age-, sex-, and body mass index (BMI)–matched healthy subjects, 10 of whom were dental school students (mean age ± SD, 32.4 ± 12.0 years)

TMD was diagnosed according to the Research Diagnostic Criteria for TMD.[18] The clinical examination was performed by two specialists in TMD and orofacial pain (J.W.P. and J.W.C.) who had more than 5 years of clinical experience. The patients were classified as having low or high disability according to Graded Chronic Pain Scale (GCPS) findings.[19] The characteristics and demographic information of both groups are shown in Table 1.

Exclusion criteria for patients and control subjects included history of other pain disorders in the previous 6 months, history of psychiatric, cardiovascular, kidney, liver, endocrine, immune, rheumatologic diseases or primary sleep disorders, intake of medication in the previous 4 months that could affect the results (eg, oral or local corticosteroids, nonsteroidal anti-inflammatory drugs, anticytokine antibody therapy, analgesics, hormones, sedatives, antipsychotics), history of trauma (diagnosed from history and radiographic examination), an acute or chronic active inflammation or infection in other body parts, and positivity for rheumatoid factor and fluorescent antinuclear antibody. Control subjects were also excluded if they had a history of TMD or if they had sought medical help for any other type of pain in the 6 months before the study. Exclusion criteria were assessed during a comprehensive screening, and blood tests including complete blood cell count with differential, erythrocyte sedimentation rate, and blood chemistry were performed before the TMD examinations.

The study was reviewed and approved by the institutional review board of Seoul National University Dental Hospital (CRI 09011). Informed consent was obtained from each subject.

Evaluation of Sleep Quality

Sleep quality was evaluated by means of the Pittsburgh Sleep Quality Index (PSQI)[20] and daytime sleepiness by means of the Epworth Sleepiness Scale (ESS).[21] 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 have excessive daytime sleepiness. The internal consistency of the PSQI is 0.83 (Cronbach alpha),[20] and that of the ESS is 0.75 (Cronbach alpha).[21]

Collection of Plasma

Plasma samples of all subjects were obtained from the antecubital vein and stored in lavender tubes coated with ethylenediaminetetraacetic acid (Becton Dickinson Vacutainer System). All samples were collected between 9 am and noon. The plasma was immediately centrifuged (2,000 rpm) for 10 minutes at 4°C and stored at −70°C before analysis.

Quantification of CRP and Inflammatory Cytokines

The plasma concentrations of proinflammatory cytokines IL-1β, IL-6, TNF-α, and the anti-inflammatory cytokine IL-10 were measured by means of Procarta cytokine assays (Panomics). The assays are multiplex immunoassays based on xMAPtechnology (Luminex). Each cytokine-specific antibody was coupled with 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 the specimens were washed with assay wash buffer, diluted biotinylated secondary antibody was added and they were incubated at 500 rpm for 30 minutes. After 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) to assess the concentration of the cytokines. Plasma samples were

Table 1 Characteristics of the Study Population

Disability group	Age (y)[a] Mean ± SD	BMI[a] Mean ± SD	Sleep time (h)[b] Median (IQR)	Pain duration (d)[b,c] Median (IQR)	Pain intensity (VAS score)[b,d] Median (IQR)
High (2) (n = 20)	33.00 ± 12.17	20.56 ± 2.44	5.00 (4.13–7.00)	112.50 (30.00–318.72)	60.00 (50.00–70.00)
Low (1) (n = 20)	32.85 ± 14.50	20.67 ± 2.30	5.25 (5.00–6.75)	30.00 (11.00–303.75)	20.00 (12.50–37.50)
Control (0) (n = 20)	32.35 ± 12.02	20.69 ± 2.00	6.00 (6.00–7.38)	0.00 (0.00–0.00)	0.00 (0.00–0.00)
P value	.99	.98	.10	.00	.00
Post hoc comparison[e]	–	–	–	–	0 vs 1* 0 vs 2* 1 vs 2*

[a]Results were obtained using one-way analysis of variance.
[b]Results were obtained using the Kruskal-Wallis test.
[c]Sleep time was time spent sleeping the night before blood collection (reported by patient).
[d]Pain was measured on a visual analog scale (VAS) ranging from 0 to 100.
[e]Post hoc analyses for multiple comparisons between disability and control groups were performed using the Tukey test: *$P < .001$.
BMI = body mass index; IQR = interquartile range; SD = standard deviation.

diluted three-fold with assay diluents. In each plate, the standards and a quality control pool were tested three times, and the 60 samples were tested twice.

Plasma concentrations of CRP were analyzed by means of a highly sensitive immunoturbidimetric assay autoanalyzer (Hitachi 7180, Hitachi High-Technologies). The person conducting the measurements was blinded to subject information.

Statistical Analyses

The Kolmogorov-Smirnov test was used to check for normality of the data. Nonparametric tests were applied when data were not normally distributed. The Kruskal-Wallis test was used to compare sleep time, pain duration, pain intensity, CRP, and cytokine level (IL-1β, IL-6, IL-10, and TNF-α) findings between the three groups. One-way analysis of variance was used to compare age, BMI, ESS, and PSQI between the different groups. Post hoc analyses for multiple comparisons were performed by means of the Tukey post hoc test. The Fisher exact test was used to compare the number of subjects with an ESS score ≥ 10 or PSQI score ≥ 6 between the different groups. Correlations between CRP and cytokines as well as the sleep scores (ESS, PSQI) were analyzed using the Spearman rank correlation coefficient. To evaluate the effect of sleep status on cytokines, multivariate general linear models were used involving disability group (normal, low, and high as an independent variable and CRP and cytokine levels as dependent variables) after adjusting for the effect of the ESS and PSQI scores. Because of positive skewed distribution, the values of plasma IL-1β, IL-6, IL-10, and TNF-α were normalized using log-transformation to approximate a normal distribution before being entered into the models. A probability level of $P < .05$ was considered statistically significant.

Results

Clinical Characteristics and Demographic Features

Age, BMI, and hours of sleep during the night before blood collection did not vary significantly between the groups ($P > .05$). The low-disability group comprised 20 patients, 10 with both arthrogenous and myogenous pain, 3 with myogenous pain, and 7 with arthrogenous pain. The high-disability group comprised 20 patients, 15 patients with arthrogenous and myogenous pain, 2 with myogenous pain, and 3 with arthrogenous pain. The disability groups did not significantly differ in TMD diagnoses ($P > .05$), but pain duration and intensity were significantly different between the groups ($P < .001$). These results are shown in Table 1.

Sleep Scores

The ESS and PSQI scores significantly differed between the groups (both $P < .001$), with the high-disability group exhibiting the highest scores for both measures. The difference of ESS scores was significant between the high- and low-disability groups ($P < .01$). The ESS score of the control group was higher than that of the low-disability group, but the difference was not statistically significant ($P > .05$). The differences in PSQI scores were significant between the disability groups and the control group ($P < .05$ and $P < .001$, respectively) and also between the low- and high-disability groups ($P < .001$).

The number of subjects who scored ESS ≥ 10 or PSQI ≥ 6 differed between the groups ($P < .001$ for both indices), with the high-disability group having the highest number of subjects with these scores (55% with an ESS > 10 and 95% with a PSQI > 6). The results are shown in Table 2.

Table 2 Sleep Indices of the Study Population

Disability group	ESS[a] Mean ± SD	ESS ≥ 10[b] n (%)	PSQI[a] Mean ± SD	PSQI ≥ 6[b] n (%)
High (2) (n = 20)	9.74 ± 1.42	11 (55)	11.40 ± 3.42	19 (95)
Low (1) (n = 20)	5.74 ± 1.39	3 (15)	6.60 ± 3.35	11 (55)
Control (0) (n = 20)	6.35 ± 0.87	3 (15)	3.75 ± 2.15	4 (20)
P value	.00	.00	.00	.00
Post hoc comparison[c]	1 vs 2**	–	0 vs 1* 0 vs 2*** 1 vs 2***	–

[a]Results were obtained using one-way analysis of variance.
[b]Results were obtained using the Fisher exact test.
[c]Post hoc analyses for multiple comparisons between disability and control groups were performed using the Tukey test: *$P < .05$, **$P < .01$, ***$P < .001$.
ESS = Epworth sleepiness scale; PSQI = Pittsburgh sleep quality index.

Table 3 Median (IQR) Cytokine Levels of the Groups[a]

Disability group	CRP (mg/dL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
High (2) (n = 20)	0.07 (0.03–0.13)	0.75 (0.44–1.77)	0.88 (0.50–1.75)	1.55 (0.92–4.67)	4.55 (1.55–10.71)
Low (1) (n = 20)	0.05 (0.04–0.07)	0.43 (0.33–1.28)	0.54 (0.33–0.92)	1.00 (0.78–4.42)	1.86 (0.17–13.46)
Control (0) (n = 20)	0.06 (0.04–0.13)	0.35 (0.00–0.42)	0.23 (0.00–0.41)	0.76 (0.24–0.88)	0.11 (0.00–1.11)
P value	.61	.00	.00	.00	.00
Post hoc comparison[b]	–	–	0 vs 2*	0 vs 2*	0 vs 1* 0 vs 2**

[a]Results were obtained using the Kruskal-Wallis test.
[b]Post hoc analyses for multiple comparisons between disability and control groups were performed using the Tukey test: *$P < .01$, **$P < .001$.
CRP = C-reactive protein; GCPS = Graded Chronic Pain Scale; IQR = interquartile range; IL = interleukin; TNF = tumor necrosis factor.

Table 4 Spearman Rank Correlations of Inflammatory Cytokine Plasma Levels and Sleep Indices

Sleep index	CRP	IL-1β	IL-6	IL-10	TNF-α	ESS
ESS	−0.208	0.312*	0.154	0.349**	0.161	–
PSQI	−0.180	0.494**	0.449**	0.304*	0.430**	0.426**

*$P < .05$, **$P < .01$.
CRP = C-reactive protein; IL = interleukin; ESS = Epworth Sleepiness Scale; PSQI = Pittsburgh Sleep Quality Index; TNF = tumor necrosis factor.

Inflammatory Cytokine Levels

The CRP values were highest in the high-disability group, but between-group differences were not statistically significant ($P > .05$).

The levels of the proinflammatory cytokines IL-1β, IL-6, and TNF-α differed significantly between the groups ($P \leq .001$ for all). The high-disability group had the highest levels of all tested proinflammatory cytokines. Significant differences were found between the high-disability group and the control group for IL-6 ($P < .01$) and between the disability groups and the control group for TNF-α ($P < .01$ and $P < .001$, respectively). The between-group differences were not statistically significant for IL-1β. Anti-inflammatory cytokine IL-10 levels were significantly different between the groups ($P < .001$), with the high-disability group having the highest plasma level. The difference in IL-10 levels among groups was significant between the high-disability and control groups ($P < .01$). The results are shown in Table 3.

Correlations of Inflammatory Cytokine Plasma Levels and Sleep Indices

The IL-1β ($P < .05$) and IL-10 ($P < .01$) levels were significantly associated with the ESS scores, whereas the IL-1β ($P < .01$), IL-6 ($P < .01$), IL-10 ($P < .05$), and TNF-α ($P < .01$) levels showed a statistically significant association with the PSQI scores. ESS and PSQI scores were significantly correlated ($P < .01$). The results are shown in Table 4.

Association of Inflammatory Cytokines and GCPS After Adjusting for the Effect of Sleep Quality

IL-10 (low-disability group, $P = .01$; high-disability group, $P = .03$) and TNF-α (low-disability group, $P = .00$, high-disability group, $P = .02$) maintained a significant association with the disability groups after adjusting for the effect of ESS and PSQI scores. The results are shown in Table 5.

Discussion

The findings of this study suggest that sleep disturbance is common in TMD patients, especially in those with a high degree of disability. The mean PSQI score of both disability groups was ≥ 6. Moreover, in the high-disability group, 95% of patients had a PSQI score ≥ 6, and more than half (55%) had an ESS score of ≥ 10, which represents a high likelihood of having a primary sleep disorder and therefore a need for evaluation and possibly therapy. These findings emphasize the fact that sleep disturbance could be a more serious problem than generally thought, especially in TMD patients with a higher degree of disability, and how it is important for clinicians to evaluate sleep quality in the treatment of TMD patients.

Interestingly, although ESS scores did not differ significantly between the low-disability and control groups, PSQI scores did. Moreover, PSQI scores differed significantly

Table 5 Multivariate General Linear Models for Inflammatory Cytokine Plasma Levels According to the Different GCPS Disability Groups After Adjusting for the Effect of ESS and PSQI Scores

Dependent variable (cytokine level)	Independent variable (disability group)	Coefficient	β value	*P* value	*P* value for trend
CRP	Low	−0.278	0.757	.44	.37
	High	0.501	1.651	.29	
IL-1β	Low	0.393	1.481	.23	.40
	High	0.352	1.422	.38	
IL-6	Low	0.667	1.948	.11	.19
	High	0.691	1.996	.21	
IL-10	Low	0.902	2.463	.01	.03
	High	0.975	2.651	.03	
TNF-α	Low	1.862	6.437	.00	.03
	High	1.795	6.021	.02	

CRP = C-reactive protein; ESS: = Epworth Sleepiness Scale; GCPS = Graded Chronic Pain Scale; IL = interleukin; PSQI = Pittsburgh Sleep Quality Index; TNF = tumor necrosis factor.

between the disability groups. Thus, the PSQI may be a more sensitive tool than the ESS for diagnosing poor sleep quality in TMD patients. This observation may be explained by the fact that PSQI scores are more closely related to psychological disorders than are ESS scores.[22]

Both ESS and PSQI scores were higher than those in previous studies, which reported that 19% of TMD patients had excessive daytime somnolence.[23,24] The lower ESS and PSQI scores reported in these studies are likely a result of the fact that the patients were not separated according to their degree of disability, therefore leading to an underestimation of the seriousness of sleep problems in TMD patients with high disability.

The level of proinflammatory cytokines IL-1β, IL-6, and TNF-α were significantly elevated in the disability groups compared with the control group, thus confirming the findings of previous investigations that showed elevated levels of circulating concentrations of proinflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8, in patients with chronic pain syndromes compared with healthy control subjects.[25,26] In addition, the results of the present study showed a significant increase of IL-10, which corroborates the results of other studies showing an elevation of both pro- and anti-inflammatory cytokines in such conditions.[27–29] The increase in IL-10 levels may be related to the TMD patients' low quality of sleep, because anti-inflammatory cytokines are known to have a sleep-disrupting effect.[14] However, not all studies investigating the relationship between sleep duration and inflammatory markers have shown a significant increase in the same categories of cytokines that were examined.[7,10] This inconsistency may result from a number of factors: first, the lack of a gold standard to measure cytokine levels and the poor reliability among different assay methods.[30] In the present study, the Luminex technology was used, as it has been shown that this technique is the only one able to differentiate fibromyalgia patients from control subjects.[27] Second, the diurnal variations in cytokine expression may have contributed to the variable findings.[31] To reduce this variation source, all blood samples in the present study were collected between 9:00 am and noon. Third, cytokine levels are affected by a number of factors such as age, sex, medication intake, and pain duration. The present study was designed to keep such factors to a minimum by excluding patients who had taken medication that could affect test results and by age- and sex-matching the study groups before dividing them into disability groups according to GCPS. Because of such efforts, the present results provide a relatively more accurate comparison between different disability groups than previous studies.

Although this study did not analyze the ratio between pro- and anti-inflammatory cytokine levels, the increase in IL-10 level suggests that TMD patients show a shift in the balance between anti- and proinflammatory cytokines as in fibromyalgia patients.[32] Studies of the role of cytokines in TMD pain patients are limited to the analysis of temporomandibular joint synovial fluid.[33,34] Also, because a relationship seems to exist between chronic pain and increased levels of certain plasma cytokines, it cannot be excluded that the pain in chronic TMD pain patients may be modulated by systemic factors rather than localized inflammatory mediators. This hypothesis has yet to be proven but is supported by a previous study showing that the intensity of temporomandibular joint (TMJ) pain on jaw movements and the pressure pain thresholds of rheumatoid arthritis patients correlated only with systemic pain mediators.[35] Considering this possibility, the present results that are based on plasma cytokine levels may more accurately reflect the general impact of TMD pain compared with an analysis based on specimens obtained from areas limited to the TMJ.

The multivariate general linear model and correlation analysis showed that plasma TNF-α and IL-10 levels were significantly associated with the degree of GCPS disability even after adjusting for sleep indices. This finding implies that the plasma levels of certain cytokines may be more affected by pain intensity than by sleep. In fact, it has been postulated that increased proinflammatory cytokine levels may contribute to the establishment of chronic pain.[36] Cytokines interact with various systems including those involved in pain processing and facilitation.[37,38] In an animal study, exogenous administration of proinflammatory cytokines elicited pain and hyperalgesia, whereas their neutralization blocked it.[39] The positive association between the severity of headache and muscle pain occurring with infection and IL-6 levels expressed from peripheral mononuclear cells may also support the role of cytokines in the elicitation of pain.[40]

A complex and bidirectional interaction exists between sleep, systemic cytokine levels, and pain, although the cause and effect relationship between these factors is yet to be understood. Disturbed sleep alters systemic cytokine levels and vice versa.[6–9] Disturbed sleep is present in many disorders accompanied by pain[3,4] and can enhance pain sensitivity by itself by means of shared pathways.[5,10,41] In addition, cytokines may directly cause more pain,[42,43] and pain disturbs sleep.[44] So, it cannot be concluded solely from the results of the present study whether the disturbed sleep of TMD pain patients caused cytokine dysregulation or vice versa. However, the results of this study and many previous studies suggest a close and intertwined correlation between these three facets.

The present study had several limitations. First, the exclusion of subjects with a history of other pain disorders within 6 months of study initiation was based solely on the subjects' history and did not involve a thorough examination. Future studies should be more careful in diagnosing comorbidities, as the results may be affected by their presence and severity.[45,46] Second, the TMD patients were divided into low- and high-disability groups. Grouping the patients according to the origin of TMD pain may have yielded more insights into the effect of the origin of TMD pain on sleep and cytokine aberrance. Third, the study revealed no evidence that the measured peripheral cytokine levels reflected their levels in the central nervous system, which are more directly associated with central sensitization. Fourth, the duration of sleep disruption was not considered. It is possible that long-term sleep disturbance can further alter cytokine levels. Studies with grouping according to sleep disturbance duration could reveal more information.

This study was based on the hypothesis that disturbances in the cytokine-sleep network may play a role in TMD pain. The results showed that TMD pain patients with high disability had increased plasma cytokine levels and more sleep disturbance. This association, however, does not allow for the establishment of their role in TMD pain. Further studies are needed to investigate how and whether the increase in sleep problems and plasma levels of both pro- and anti-inflammatory cytokines are related to TMD pain patients with a high degree of disability and whether these factors should be considered in the treatment of TMD pain patients.

Conclusions

Patients with TMD, especially those with GCPS scores indicative of high disability, had elevated plasma cytokine levels and increased ESS and PSQI scores suggestive of sleep disturbance.

Acknowledgments

This study was supported in part by the Seoul National University Research Grant. The authors thank Mi Yeon Lee, a statistician of Kangbuk Samsung Hospital, for helping with the statistical analyses of data. The authors report no conflicts of interest related to this study.

References

1. Kalia M. Neurobiology of sleep. Metabolism 2006;55:S2–S6.
2. Bendtsen L, Jensen R. Tension-type headache: The most common, but also the most neglected headache disorder. Curr Opin Neurol 2006;19:305–309.
3. Goldenberg DL. Fibromyalgia, chronic fatigue syndrome, and myofascial pain syndrome. Curr Opin Rheumatol 1991;3: 247–258.
4. Aaron LA, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. Arch Intern Med 2000;160:221–227.
5. Smith MT, Wickwire EM, Grace EG, et al. Sleep disorders and their association with laboratory pain sensitivity in temporomandibular joint disorder. Sleep 2009;32:779–790.
6. Del Gallo F, Opp MR, Imeri L. The reciprocal link between sleep and immune responses. Arch Ital Biol 2014;152:93–102.
7. Patel SR, Zhu X, Storfer-Isser A, et al. Sleep duration and biomarkers of inflammation. Sleep 2009;32:200–204.
8. Taishi P, Chen Z, Obál F Jr, et al. Sleep-associated changes in interleukin-1beta mRNA in the brain. J Interferon Cytokine Res 1998;18:793–798.
9. Pérez de Heredia F, Garaulet M, Gómez-Martínez S, et al. Self-reported sleep duration, white blood cell counts and cytokine profiles in European adolescents: The HELENA study. Sleep Med 2014;15:1251–1258.

10. Haack M, Sanchez E, Mullington JM. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. Sleep 2007;30:1145–1152.
11. Gur A, Karakoc M, Erdogan S, Nas K, Cevik R, Sarac AJ. Regional cerebral blood flow and cytokines in young females with fibromyalgia. Clin Exp Rheumatol 2002;20:753–760.
12. Heffner KL, France CR, Trost Z, Ng HM, Pigeon WR. Chronic low back pain, sleep disturbance, and interleukin-6. Clin J Pain 2011;27:35–41.
13. Lorusso L, Mikhaylova SV, Capelli E, Ferrari D, Ngonga GK, Ricevuti G. Immunological aspects of chronic fatigue syndrome. Autoimmun Rev 2009;8:287–291.
14. Kapsimalis F, Richardson G, Opp MR, Kryger M. Cytokines and normal sleep. Curr Opin Pulm Med 2005;11:481–484.
15. Samad TA, Moore KA, Sapirstein A, et al. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 2001;410:471–475.
16. Carvalho TT, Borghi SM, Pinho-Ribeiro FA, et al. Granulocyte-colony stimulating factor (G-CSF)-induced mechanical hyperalgesia in mice: Role for peripheral TNFα, IL-1β and IL-10. Eur J Pharmacol 2015;749:62–72.
17. Koch A, Zacharowski K, Boehm O, et al. Nitric oxide and pro-inflammatory cytokines correlate with pain intensity in chronic pain patients. Inflamm Res 2007;56:32–37.
18. Dworkin SF, LeResche L. Research Diagnostic Criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6: 301–355.
19. Von Korff M, Ormel J, Keefe FJ, Dworkin SF. Grading the severity of chronic pain. Pain 1992;50:133–149.
20. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. Psychiatry Res 1989;8:193–213.
21. Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 1991;14:540–545.
22. Buysse DJ, Hall ML, Strollo PJ, et al. Relationships between the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and clinical/polysomnographic measures in a community sample. J Clin Sleep Med 2008;4:563–571.
23. Collesano V, Segù M, Masseroli C, Manni R. Temporomandibular disorders and sleep disorders: Which relationship? Minerva Stomatol 2004;53:661–668.
24. Schmitter M, Kares-Vrincianu A, Kares H, Bermejo JL, Schindler HJ. Sleep-associated aspects of myofascial pain in the orofacial area among Temporomandibular Disorder patients and controls. Sleep Med 2015;16:1056–1061.
25. Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. J Transl Med 2009;7:96–103.
26. Zin CS, Nissen LM, O'Callaghan JP, Moore BJ, Smith MT. Preliminary study of the plasma and cerebrospinal fluid concentrations of IL-6 and IL-10 in patients with chronic pain receiving intrathecal opioid infusions by chronically implanted pump for pain management. Pain Med 2010;11:550–561.
27. Nakamura T, Schwander SK, Donnelly R, et al. Cytokines across the night in chronic fatigue syndrome with and without fibromyalgia. Clin Vaccine Immunol 2010;17:582–587.
28. Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. J Transl Med 2009;7:96–103.
29. Wang H, Moser M, Schiltenwolf M, Buchner M. Circulating cytokine levels compared to pain in patients with fibromyalgia—A prospective longitudinal study over 6 months. J Rheumatol 2008;35:1366–1370.
30. Pascal LE, True LD, Campbell DS, et al. Correlation of mRNA and protein levels: Cell type-specific gene expression of cluster designation antigens in the prostate. BMC Genomics 2008; 23:246–258.
31. Krueger JM, Obál FJ, Fang J, Kubota T, Taishi P. The role of cytokines in physiological sleep regulation. Ann N Y Acad Sci 2001;933:211–221.
32. Togo F, Natelson BH, Adler GK, et al. Plasma cytokine fluctuations over time in healthy controls and patients with fibromyalgia. Exp Biol Med (Maywood) 2009;234:232–240.
33. Hamada Y, Kondoh T, Holmlund AB, Sakota K, Nomura Y, Seto K. Cytokine and clinical predictors for treatment outcome of visually guided temporomandibular joint irrigation in patients with chronic closed lock. J Oral Maxillofac Surg 2008;66:29–34.
34. Lee JK, Cho YS, Song SI. Relationship of synovial tumor necrosis factor alpha and interleukin 6 to temporomandibular disorder. J Oral Maxillofac Surg 2010;68:1064–1068.
35. Alstergren P, Fredriksson L, Kopp S. Temporomandibular joint pressure pain threshold is systemically modulated in rheumatoid arthritis. J Orofac Pain 2008;22:231–238.
36. Poole S, Woolf CJ. Cytokine-nerve growth factor interactions in inflammatory hyperalgesia. In: Watkins LR, Maier SF (eds). Cytokines and Pain. Basel: Birkhäuser, 1999:89–132.
37. Schaible HG. Nociceptive neurons detect cytokines in arthritis. Arthritis Res Ther 2014;16:470.
38. Schaible HG, von Banchet GS, Boettger MK, et al. The role of proinflammatory cytokines in the generation and maintenance of joint pain. Ann N Y Acad Sci 2010;1193:60–69.
39. McMahon SB, Bennett DLH, Bevan S. Inflammatory mediators and modulators of pain. In: McMahon SB, Koltzenburg M (eds). Textbook of Pain. London: Elsevier, 2005:49–72.
40. Vollmer-Conna U, Fazou C, Cameron B, et al. Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans. Psychol Med 2004;34: 1289–1297.
41. Fine L. Sleep: Important considerations in management of pain. Phys Med Rehabil Clin N Am 2015;26:301–308.
42. Wei XH, Yang T, Wu Q, et al. Peri-sciatic administration of recombinant rat IL-1β induces mechanical allodynia by activation of src-family kinases in spinal microglia in rats. Exp Neurol 2012;234:389–397.
43. Wei XH, Zang Y, Wu CY, Xu JT, Xin WJ, Liu XG. Peri-sciatic administration of recombinant rat TNF-alpha induces mechanical allodynia via upregulation of TNF-alpha in dorsal root ganglia and in spinal dorsal horn: The role of NF-kappa B pathway. Exp Neurol 2007;205:471–484.
44. Smith MT, Haythornthwaite JA. How do sleep disturbance and chronic pain inter-relate? Insights from the longitudinal and cognitive-behavioral clinical trials literature. Sleep Med Rev 2004; 8:119–132.
45. Visscher CM, Ligthart L, Schuller AA, et al. Comorbid disorders and sociodemographic variables in temporomandibular pain in the general Dutch population. J Oral Facial Pain Headache 2015;29:51–59.
46. Koutris M, Visscher CM, Lobbezoo F, Naeije M. Comorbidity negatively influences the outcomes of diagnostic tests for musculoskeletal pain in the orofacial region. Pain 2013;154:927–932.