

ORIGINAL ARTICLE



Sleep quality and depression in patients with ankylosing spondylitis and their associations with clinical parameters: A cross-sectional, case-control study

Ankilozan spondilitli hastalarda uyku kalitesi, depresyon ve bunların klinik parametrelerle ilişkisi: Kesitsel, vaka-kontrol çalışması

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Summary

Objectives: This study aimed to explore sleep quality in patients with ankylosing spondylitis (AS) and to reveal the clinical parameters that predict sleep quality and depression in AS.

Methods: This study included 100 AS patients and 100 age/sex-matched healthy individuals. The AS activity was assessed by Bath AS Disease Activity Index (BASDAI), AS Disease Activity Score (ASDAS-ESR, ASDAS-CRP), and Visual Analog Scale (VAS). The functional status was assessed by the Bath AS Functional Index (BASFI). AS Quality of Life Questionnaire was administered for the assessment of the disease-related quality of life. Pittsburgh Sleep Quality Index for sleep assessment and Beck Depression Inventory for psychological assessment were administered to all participants.

Results: Sleep problems and depressive symptoms were significantly higher in AS patients compared to healthy individuals. All of the AS clinical parameters were significantly higher in AS patients with poor sleep quality than in AS patients with good sleep quality. In the correlation analysis, it was determined that poor sleep quality, depressive symptoms and low quality of life were strongly correlated with each other and AS clinical parameters. The most effective predictor for sleep problems was higher BASDAI scores, followed by higher BASFI, VAS, ASDAS-ESH scores, and younger age. Higher BASFI and VAS scores were predictors for depressive symptoms.

Conclusion: The findings indicate that poor sleep, depressive symptoms and low quality of life may negatively affect the AS clinic, and therefore sleep quality and depression should not be ignored in the examinations of AS patients.

Keywords: Ankylosing spondylitis; depression; pain; sleep.

Özet

Amaç: Bu çalışmada, ankilozan spondilitli (AS) hastalarda uyku kalitesini araştırmak ve AS'de uyku kalitesi ve depresyonu öngören klinik parametreleri ortaya koymak amaçlandı.

Gereç ve Yöntem: Bu çalışmaya, 100 AS'li hasta ve hasta grubuyla yaş/cinsiyet açısından uyumlu 100 sağlıklı birey dahil edildi. AS aktivitesi, Bath AS Hastalık Aktivite İndeksi (BASHAİ), AS Hastalık Aktivite Skoru (ASDAS-ESH, ASDAS-CRP) ve Görsel Analog Skala (GAS) ile değerlendirildi. Fonksiyonel durum Bath AS Fonksiyonel İndeksi (BASFİ) ile değerlendirildi. Hastalığa bağlı yaşam kalitesinin değerlendirilmesi için Ankilozan Spondilit Yaşam Kalitesi Anketi (ASYKA) kullanıldı. Tüm katılımcılara uyku değerlendirmesi için Pittsburgh Uyku Kalitesi İndeksi (PUKİ) ve psikolojik değerlendirme için Beck Depresyon Envanteri (BDE) uygulandı. Bulgular: AS'li hastalarda uyku sorunları ve depresif belirtiler sağlıklı bireylere göre anlamlı derecede yüksekti. Uyku kalitesi kötü olan AS'li hastalarda AS klinik parametrelerinin tümü, uyku kalitesi iyi olan AS'li hastalara göre anlamlı olarak daha yüksekti. Korelasyon analizinde kötü uyku kalitesi, depresif belirtiler ve düşük yaşam kalitesinin birbirleriyle ve AS klinik parametreleriyle güçlü bir şekilde ilişkili olduğu belirlendi. Uyku sorunları için en etkili yordayıcı, yüksek BASDAİ puanlarıydı ve bunu yüksek BASFİ, GAS, ASDAS-ESH puanları ve genç yaş izledi. Yüksek BASFİ ve GAS skorları depresif belirtilerin yordayıcılarıydı. Sonuç: Bulgular, kötü uyku, depresif belirtiler ve düşük yaşam kalitesinin AS kliniğini olumsuz etkileyebileceğini ve bu nedenle AS'li hastaların muayenelerinde uyku kalitesi ve depresyonun göz ardı edilmemesi gerektiğini göstermektedir.

Anahtar sözcükler: Ankilozan spondilit; depresyon; ağrı; uyku.

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Introduction

Ankylosing spondylitis (AS) is a chronic, systemic and inflammatory disease, which primarily affects the spine, sacroiliac and peripheral joints, causing varying degrees of structural, and functional disorders, restriction in the mobility of the spine and low back pain.^[1] Although the exact etiology of AS remains unclear, it is known to be associated with HLA-B27 antigen, genetic and environmental factors. The prevalence of the AS ranges from approximately 0.4% to 1.4%. AS affects males approximately 2 times more frequently than females, and the initial manifestations usually emerge around the second decade of life.^[2,3] AS has caused a series of significant problems, such as pain, stiffness, fatigue, mobility restriction, functional impairment, and sleep problems.^[4,5]

Sleep problems have been reported to be more common in AS patients than that in a general population and other rheumatological diseases.^[6-8] The prevalence of sleep disorders in these patients is between 50% and 64.5%. Patients often complain about the difficulty in initiating sleep, difficulty awakening, nighttime pain, morning stiffness, poor sleep quality, and obstructive sleep apnea syndrome.^[9] Sleep problems negatively affect daily life by increasing fatigue, pain, aggravating the disease symptoms, and impairing psychological health. Neckelmann et al.^[10] indicated that sleep problems were a significant risk factor for depression. There have been only a few studies about sleeping problems and the related factors for AS patients in the literature. Poor sleep quality was associated with increased disease activity, more severe depressive symptoms and impaired functional status in patients with AS.^[11] Moreover, Deodhar et al.^[12] suggested that sleep problems were significantly associated with reduced quality of life, increased pain and disease activity in patients with AS.

Symptoms such as pain, stiffness, and fatigue appear in young adulthood, which is the most active years of life in patients with AS. The chronic and progressive nature of the disease causes limitations in physical activities, work, and social life. This situation facilitates the emergence of depressive symptoms.^[13] There have been studies suggesting that the incidence of depression in AS increases

significantly due to the burden of disease compared to the general population.^[14] The risk of depression is 2.21 times higher in patients with AS compared to the general population.^[13]

Contrary to other disorders related to the musculoskeletal system, there has been little information on the relationship between AS disease prognosis, clinical signs, and depression in the literature. Studies on AS often focused on investigating the physical aspects of the disease, such as functional disabilities and disease activity. However, information regarding sleep and mental health, which is extremely substantial for patients to adjust to social life and how it is related to the characteristics of the disease, is scarce. The overarching aims of this study are to investigate sleep quality in patients with AS and to reveal the clinical parameters that predict sleep quality and depression in AS.

Material and Methods

Study Group

This study, which was carried out between June 15 and October 15, 2019, had a cross-sectional design. A hundred patients between ages of 18 and 63 who presented to the Health Sciences University İstanbul Training and Research Hospital, Physical Medicine, and Rehabilitation Clinic outpatient clinic and were diagnosed with definite AS according to the Modified New York Criteria^[15] were recruited in this study. The control group consisted of a hundred healthy volunteers who were between ages of 20 and 63. After both groups were informed about the study, they were asked to sign informed consent forms if they agreed to participate in the study. Patients with any other inflammatory articular diseases, diseases of the central nervous system, substance abuse, and severe mental disorders that impeded decision-making besides the AS were excluded from the study. This study was approved by the Ethics Committee of Health Sciences University Istanbul Training and Research Hospital (21.12.2018/1588) and conducted in accordance with the Declaration of Helsinki Ethical Principles.

Demographical and clinical characteristics of the patients, medications (non-steroidal anti-inflammatory drugs [NSAIDs], disease-modifying antirheumatic drugs [DMARDs], anti-tumor necrosis factor [TNF]), and disease duration were recorded. Erythrocyte sedimentation rate (ESR) and the serum C-reactive protein (CRP) level were measured through the Westergren method (mm/h) and nephelometry (mg/dl), respectively.

The AS activity was assessed by the Bath AS disease activity index (BASDAI), AS disease activity score (ASDAS-ESR, ASDAS-CRP) and visual analog scale (VAS). The functional statement was assessed by the Bath AS Functional Index (BASFI). AS quality of life questionnaire (ASQoL) was administered for the assessment of disease-related quality of life. Pittsburgh Sleep Quality Index (PSQI) for sleep assessment and Beck Depression Inventory (BDI) for psychological assessment were administered to all participants.

Outcome Measures

Measurement of disease activity and functional status

The BASDAI^[16] is used to measure disease activity. BASDAI is a self-rated questionnaire that consists of six questions related to the five major symptoms of AS: Fatigue, spinal pain, joint pain/swelling, enthesitis, duration, and severity of morning stiffness. The average score of five major symptoms over the past week is the final BASDAI score (0–10) that a higher score represents more severe disease activity. The reliability and validity of the Turkish version of BASDAI have already been demonstrated.^[17]

VAS is a single-item scale for the degree of disease activity, and 0 cm means "no disease activity" and 10 cm means "very severe activity."

BASFI^[18] is used to assess functional status. The BASFI is a self-administered inventory, which consists 10 items regarding daily activities and ability to perform daily tasks. Each item is scored on a 10-cm horizontal range from 0 (easy) to 10 (impossible). The mean of the 10 items is calculated to obtain the final score with a higher score indicating greater disability. The Turkish version of BASFI has good reliability and validity properties.^[19]

ASDAS^[20] is a scale of self-rated measures and inflammation markers (ESR, CRP) that was developed to assess both the subjective and objective parts of AS disease activity. Items for spinal pain, peripheral joint pain/swelling, and duration of morning stiffness of BASDAI were used in ASDAS-CRP and ASDAS-ESR calculations. In the calculation, CRP is measured in mg/dl and ESR in mm/h. CRP or ESR levels with the scores are calculated in the ASDAS formula. The results are evaluated as follows; above 3.5 is very high disease activity, between 2.1 and 3.5 is high disease activity, between 2.1 and 1.3 is moderate disease activity, and below 1.3 is inactivity disease status.

Measurement of sleep quality assessment

PSQI^[21] is a self-report questionnaire that evaluates sleep quality over 1 month. It consists of nineteen items and measures seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. A global PSQI score corresponding to the total of the individual scores from the seven components is calculated (range=0–21). A total score above 5 is associated with poor sleep quality. The reliability and validity of the Turkish version of this questionnaire have been verified by Agargun et al.^[22]

Measurement of the psychological variables

BDI^[23] is a self-report questionnaire that is widely used to assess the incidence and severity of depressive symptoms in the community. Each answer in BDI is scored on a scale of 0–3. A high total score indicates a high level of depression. The Turkish validity and reliability study was conducted by Hisli.^[24]

Measurement of disease-related quality of life

Health-Related Quality of life (HRQoL) is a multidimensional concept that measures a person's wellbeing, including physical health, psychological state, social functioning, and social relationships.^[25] Chronic pain, which is one of the most important components of AS, causes mental health problems such as distress, loneliness and low quality of life. ^[26] There is a growing interest in the development of disease-specific HRQoL scales designed to be associated with specific health problems. ASQoL^[27] measures the disease-related quality of life. This questionnaire consists of 18 items with binary responses (yes/no). The reliability and validity of the Turkish version of this questionnaire have been verified by Duruöz et al.^[28]



Table 1. Mean scores of the AS patients and controls for the age, gender, and sleep subdimensions

IQR: Interquartile range 25%, 75%; AS: Ankylosing spondylitis; PSQI: Pittsburgh Sleep Quality Index; BDI: Beck depression inventory; SD: Standard deviation.

Statistical Analysis

Statistical Package for the Social Sciences 20 version was used to evaluate the data. The descriptive statistics were presented in median values and interquartile ranges (IQR; 25–75%) for the quantitative variables; and frequencies and percentages for the categorical variables. The Chi-square test was used to determine possible differences between groups in terms of categorical variables. Shapiro-Wilk test of normality indicated that the scale scores were not normally distributed in many instances. Therefore, non-parametric tests were used: The Mann-Whitney U test for comparing the continuous variables among two groups was used. And correlations between the PSQI, BDI, ASQoL, and the clinical parameters of AS variables were investigated with Spearman's correlation. A multiple linear regression model was used with a stepwise technique to investigate potentially predictive factors for the worse PSQI and BDI scores. Any predicted variables that were highly correlated (-.7, and +.7) with the dependent variable were not included in the regression equation. In this context, regression analysis was not performed for ASQoL because most of the variables indicating AS disease activity were highly correlated with ASQoL. Regression analyzes were performed for PSQI and BDI with age, gender, disease duration, ASDAS-ESR, ASDAS-CRP, BASDAI, BASFI, and VAS variables. p<0.05 was considered statistically significant.

Results

A total of 100 AS patients and 100 healthy controls were included in the study. Gender ratios were the same in both groups, 27 females and 73 males. As the mean age was 41.96±9.11 in the AS patients, it was 42.11±9.05 in the control group. There was no significant difference between AS patients and control groups in terms of age (p=0.849) and gender (p=1.000) (Table 1). The duration of the disease was 13.2±9.1 years in the patients. A total of 84 (84%) patients were on regular medication, and the remaining 16 (16%) patients were on drug-free follow-up. Of the patients using drugs, 14 were receiving NSAIDs, 27 were receiving DMARD group drugs, and 43 were receiving anti-TNF treatment. The AS patients had higher scores in the BDI (0.042), subjective sleep quality (p=0.014), sleep latency (p<0.001), sleep disturbance (p<0.001), and total PSQI scores (p=0.001) (Table 1).

The median (IQR) global PSQI score was 6 (4-8), with 57 % of the AS patients classified as poor sleepers (PSQI global score >5). ASDAS-ESR (p<0.001), ASDAS-CRP (p<0.001), BASDAI (p<0.001), BASFI (p<0.001), BDI (p<0.001), ASQoL (p<0.001), and VAS (p<0.001) scores in AS patients with poor sleep quality were found to be statistically significantly higher than AS patients without poor sleep quality (PSQI global score \leq 5) (Table 2).

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Variables	Total median (IQR) (n=100)	PSQI≤5 median (IQR) (n=43)	PSQI >5 median (IQR) (n=57)	р
ASDAS-ESR	2.3 (1.72–2.97)	1.8 (1.3–2.3)	2.7 (2.2–3.4)	<0.001
ASDAS-CRP	2.4 (1.9–3.1)	1.9 (1.6–2.4)	2.9 (2.3–3.7)	<0.001
BASDAI	4 (2.45–5.95)	2.4 (1.6–3.9)	5.4 (3.8–6.3)	<0.001
BASFI	2.7 (1.2–4.7)	1.2 (0–8)	4.4 (2.1–6.3)	<0.001
BDI	8.5 (3.25–15.75)	4 (2–6)	13 (8–19)	<0.001
ASQoL	5.5 (1–12)	1 (0–3)	10 (6–15)	<0.001
VAS	4 (3–6)	3 (2–4)	6 (4–7)	<0.001

IQR: Interquartile range 25%, 75%; AS: Ankylosing spondylitis; BASDAI: Bath AS Disease Activity Index; BASFI: Bath AS Functional Index; ASDAS-ESR: AS disease activity score-erythrocyte sedimentation rate; ASDAS-CRP: AS Disease Activity Score-C-reactive protein; ASQoL: AS quality of life questionnaire; PSQI: Pittsburgh Sleep Quality Index; BDI: Beck depression inventory; VAS: Visual analog scale.

	Coefficient of correlations between PSQI, BDI, ASQoL, and measurements of disease variables						
	Total-PSQI	BDI	ASQoL				
Total-PSQI	-						
BDI	0.555*	-					
ASQoL	0.598*	0.714*	-				
BASDAI	0.536*	0.551*	0.798*				
BASFI	0.485*	0.608*	0.820*				
ASDAS-ESR	0.494*	0.519*	0.685*				
ASDAS-CRP	0.476*	0.482*	0.709*				
VAS	0.455*	0.579*	0.686*				

*: P<0.001; IQR: Interquartile range 25%, 75%; AS: Ankylosing spondylitis; BASDAI: Bath AS Disease Activity Index; BASFI: Bath AS Functional Index; ASDAS-ESR: AS disease activity score-erythrocyte sedimentation rate; ASDAS-CRP: AS disease activity score-C-reactive protein; ASQoL: AS quality of life questionnaire; PSQI: Pittsburgh Sleep Quality Index; BDI: Beck depression inventory; VAS: Visual analog scale.

According to Spearman's correlation analysis, poor sleep quality (higher total PSQI) was strong positively correlated with higher depressive symptoms, poor quality of life, and higher BASDAI, BASFI, ASDAS-ESR, ASDAS-CRP, and VAS scores (for all p<0.001). Higher depressive symptoms were strongly positively correlated with poor quality of life (p<0.001). Moreover, higher depressive symptoms and poor quality of life were strong positively correlated with higher BAS-DAI, BASFI, ASDAS-ESR, ASDAS-CRP, and VAS scores (for all p<0.001). Correlations between PSQI, BDI, and ASQOL and disease variables are shown in Table 3.

As a result of regression analysis, it was determined that higher PSQI scores in AS patients were associated with higher BASDAI scores (p<0.001). However,

higher subjective sleep quality scores with higher BASDAI (p=0.041) scores and higher VAS scores (p=0.047); higher sleep latency scores with higher BASFI scores (p<0.001); higher sleep duration scores with low age (p=0.010) and higher ASDAS-ESR scores (p<0.001); higher sleep disturbance scores with higher BASDAI scores (p=0.041); and higher Daytime dysfunction scores were found to be associated with higher BASDAI scores (p=0.041) (Table 4). Higher depressive symptoms were found to be associated with higher BASFI scores (p<0.001) and higher VAS scores (p=0.002) (Table 5).

Discussion

In this study, our findings suggested that patients with AS were significantly more affected by sleep problems than healthy individuals. According to our findings, 57% of AS patients had sleep problems (PSQI total score >5). The prevalence of sleep problems in patients with AS has been reported as 64.5%, 54%, and 54.8% by three different studies, respective-ly.^[29–31] This ratio appears 32.1% in the general population.^[32] In patients with AS, total PSQI and three of the PSQI components scores were found to be significantly worse compared to normal individuals (Table 1). The "subjective sleep quality," "sleep latency," and "sleep disturbances" were significantly impaired.

In the current study, analyses were performed between patients who have good and poor sleep quality; poor-sleep patients had significantly higher scores of BASDAI, BASFI, BDI, ASQoL, VAS, ESR, and CRP compared with good sleepers. These factors may explain a good correlation between poor sleep

			-				
	UC					95% CI	
	В	SE	ß	t	р	Lower bound	Upper bound
Total PSQI ¹							
BASDAI	0.758	0.121	0.536	6.279	p<0.001	0.518	0.997
Subjective sleep quality ²							
BASDAI	0.096	0.046	0.277	2.076	0.041	0.004	0.189
VAS	0.097	0.048	0.269	2.016	0.047	0.001	0.192
Sleep latency ³							
BASFI	0.174	0.035	0.450	4.984	p<0.001	0.105	0.243
Sleep duration ^₄							
Age	-0.021	0.008	-0.251	-2.635	0.010	-0.038	-0.005
ASDAS-ESR	0.207	0.084	0.235	2.467	0.015	0.040	0.373
Sleep disturbance⁵							
BASDAI	0.120	0.029	0.384	4.119	p<0.001	0.062	0.177
Daytime dysfunction ⁶							
BASDAI	0.168	0.040	0.394	4.245	p<0.001	0.090	0.247

Table 4. Final multivariate regression models in AS patients, PSQI domains as dependent variable

UC: Unstandardized coefficients; B: Unstandardized coefficients; SE: Standard error of the estimate; β : Adjusted coefficients; CI: Confidence interval; AS: Ankylosing spondylitis; BASDAI: Bath AS Disease Activity Index; BASFI: Bath AS Functional Index; ASDAS-ESR: AS disease activity score-erythrocyte sedimentation rate; PSQI: Pittsburgh Sleep Quality Index; VAS: Visual Analog Scale, 1: n=100, R²=0.287, F(1, 98)=39.43, p<0.001; 2: n=100, R²=0.261, F(2, 97)=16.91, p<0.001; 3: n=100, R²=0.202, F(1, 98)=24.83, p<0.001; 4: n=100, R²=0.125, F(2, 97)=7.35, p<0.001; 5: n=100, R²=0.148, F(1, 98)=16.96, p<0.001; 6: n=100, R²=0.155, F(1, 98)=18.02, p<0.001.

	U	UC				95% CI	
	В	SE	ß	t	p-value	Lower bound	Upper bound
BDI ¹							
BASFI	1.542	0.379	0.404	4.068	p<0.001	0.790	2.294
VAS	1.419	0.438	0.322	3.240	0.002	0.550	2.288

Table 5. Final multivariate regression models in AS patients, BDI as dependent variable

UC: Unstandardized coefficients; B: Unstandardized coefficients; SE: Standard error of the estimate; β : Adjusted coefficients; CI: Confidence interval; AS: Ankylosing spondylitis; BASFI: Bath AS Functional Index, BDI: Beck depression inventory; VAS: Visual analog scale; 1: n=100, R²=0.432, F(2, 97)=36.84, p<0.001.

quality and all clinical parameters (pain, disease activity, inflammatory status, functionality, and disease-related quality of life) in patients with AS. In addition, depression was also strongly associated with all clinical parameters of AS. The previous studies reported that disease activity, pain, stiffness, and loss in functionality in AS contributed to fatigue, depression, and poor sleep quality.^[1,33] A reciprocal relationship was observed between poor quality of life and depression in this study.

Pain and stiffness in the early stages of AS are thought to be caused by inflammation and related to disease activity and physical disability.^[34,35] Several studies have found that pain is an important factor associated with sleep disturbance in AS patients.^[36,37] Sleep problems are often reported by AS patients who wake up due to inflammatory pain.^[31] In our study, a positive correlation was found between inflammatory markers CRP and ESR, and sleep disorders. This finding was also reported by Li et al.^[38]

Consistent with the literature, this research found that BASDAI was independently associated with sleep problems.^[38] The multiple regression analysis indicated that disease activity was independently associated with sleep disturbance, subjective sleep quality, daytime dysfunction, and poor sleep quality. BASDAI focuses on four major symptoms including fatigue, axial and peripheral pain, enthesitis, and morning stiffness. Sleep disorders in rheumatism diseases are positively associated with pain and fatigue.^[39] It has been reported that pain is the most associated factor with fatigue.^[40] In a study of patients with AS, 41% of patients complaining of fatigue experienced more than three episodes of awakening per night.^[41] Possible explanations for the increased sleep disturbance and daytime dysfunction, and reduced subjective sleep quality might be the nocturnal increases in inflammation, pain, and stiffness. Many of the patients with AS have difficulty in performing their daily life activities and daytime dysfunction appears. Daytime dysfunction can occur with extreme tiredness during the day and recurrent sleep interruptions at night. In the continuation of this situation, it is inevitable that the quality of life will decrease and sleep disorders will deepen. Our results indicated that the patients with higher BASFI scores had increased sleep latency according to PSQI. This finding was consistent with the study of Li et al.^[42] who suggested that sleep latency was significantly associated with BASFI.

It is well known from epidemiological studies that depressive symptoms are more common in AS patients. It has been demonstrated that the risk of depression is 80% higher in AS patients compared to the general population.^[14] In this study, it was found that decreased functional status and pain were strongly associated with depression in AS patients. The reason for this finding may be that AS patients have difficulty in adjusting to daily life due to their physical limitations and consequently loss of functionality. This finding is consistent with the study suggesting that functional impairment has a robust and strong association with depressive symptoms.^[43] The relationship between pain and depression in AS may be explained by the fact that patients stop participating in many daily activities for fear of worsening pain and become more isolated. In a study by Baysal et al.,[44] the relationship between chronic pain and depression in patients with AS was clearly demonstrated.

A number of limitations need to be noted regarding the present study. The principal limitation of this study was the cross-sectional design. Prospective studies are needed to reveal the relationship between clinical parameters of AS and sleep quality and depression. Second, we evaluated the use of sleep quality with a questionnaire. On the other hand, polysomnographic evaluation could have given more accurate results.

The findings clearly indicate that sleep disorders and depressive symptoms in patients with AS are more common compared to the healthy individuals. Overall, this study strengthens the idea that there is a strong positive correlation between depression symptoms, poor sleep, poor quality of life, and AS. The current data highlight the importance of sleep problems and mental health, which are often overlooked during the routine examinations of patients with AS. Further research is required to better understand the underlying causes of sleep problems and depression in patients with AS. More information on sleep problems and mental health related to the AS would help us to establish a greater degree of accuracy on this matter.

Ethics Committee Approval: The İstanbul Trainig and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 21.12.2018, number: 1588).

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