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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Serum Melatonin Levels Related with Night Pain and High Disease Activity in Seronegative Spondyloarthritis Patients

Seronegatif Spondiloartriti Hastalarında Gece Ağrısı ve Artmış Hastalık Aktivitesi İle Serum Melatonin Düzeyleri İlişkisi

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ABSTRACT

Aim: Circadian rhythms play a major role in regulation of human physiological functions. Many signs and symptoms of inflammatory arthritis, especially in rheumatoid arthritis (RA), show intra- and inter-day variations. We aimed to measure serum levels of melatonin (MLT), interferon gamma (IFNγ), interleukin-10 (IL-10) and cortisol and to investigate relation with clinical parameters in patients with seronegative spondyloarthritis (SPA).

Methods: The study was designed prospectively. 17 patients with SPA (9 patients with Anklyosing Sponylitis, 8 patients with psoriatic arthritis), 20

patients with RA and 20 healthy controls were included to study. Serum MLT, IFNγ, IL-10 and cortisol measurement were made in patients and healthy controls at 8 p.m., 3 a.m. and 8 a.m.

Results: We found that MLT values of SPA patients were statistically lower (p=0,017) than healthy groups at 3 a.m.. Level of cortisol in RA patients at 8 a.m. were significantly lower than SPA patient and healthy control groups (p=0.047 and p=0.006; respectively). Cortisol levels in SPA patients which is measured at 8 a.m. was significantly higher (p=0.001) than other measurement in day. There was also significant positive correlation between Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and MLT levels measured at 8:00 p.m. in patients with SPA (r=0.688; p=0.040).

Conclusion: Serum MLT levels well correlated with disease activity in SPA patients. Back pain which is seen midnight can be related to decrease in MLT secretion. Our findings suggests MLT have a role in pathogenesis of SPA but, it is need more detailed studies for explaining of this role of MLT in pathogenesis of SPA.

Key Words: Circadian rhythm, Melatonin, Rheumatoid arthritis, Spondylo-arthropathies, Hypothalamopituitary adrenal axis

ÖZET

Amaç: Sirkadiyen ritim, insan fizyolojik fonksiyonlarının düzenlenmesinde önemli bir rol oynar. Özellikle romatoid artritte (RA) inflamatuar artritin bir çok belirti ve semptomu gün içi ve günler arası farklılıklar gösterir. Seronegatif spondiloartritli (SPA) hastalarda serum melatonin (MLT), interferon-gamma (IFNγ), interlökin-10 (IL-10) ve kortizol düzeylerini ölçmeyi ve klinik parametrelerle ilişkisini araştırmayı amaçladık.

Yöntemler: Çalışma prospektif olarak tasarlandı. SPA'lı 17 hasta (Anklyozan Sponylitli 9 hasta, 8 psoriatik artritli hasta), RA'lı 20 hasta ve 20 sağlıklı kontrol çalışmaya dahil edildi. Serum MLT, IFNγ, IL-10 ve kortizol ölçümü hasta ve sağlıklı kontrollerde 20:00, 03:00 ve 08:00 saatlerinde yapıldı.

Bulgular: SPA hastalarının MLT değerlerinin sabah 3'te sağlıklı gruplara göre istatistiksel olarak daha düşük (p=0,017), RA hastalarında sabah 8'deki kortizol düzeyinin SPA hasta ve sağlıklı kontrol gruplarına göre anlamlı olarak düşük olduğunu bulduk (p = 0,047 ve p = 0.006; sırasıyla). SPA hastalarında sabah 8'de ölçülen kortizol düzeyleri diğer gün ölçümlerine göre anlamlı derecede yüksekti (p = 0,001). SPA hastalarında Ankilozan Spondilit Hastalık Aktivite İndeksi (ASHAI) ve saat 20:00'de ölçülen MLT seviyeleri arasında da anlamlı pozitif korelasyon vardı(r = 0.688; p = 0.040).

Sonuç: Serum MLT düzeyleri SPA hastalarında hastalık aktivitesi ile iyi korelasyon gösteriyordu. Gece yarısı görülen sırt ağrısı MLT salgısının azalması ile ilişkili olabilir. Bulgularımız, MLT'nin SPA patogenezinde rolü olduğunu, ancak MLT'nin SPA patogenezindeki bu rolünü açıklamak için daha detaylı çalışmalara ihtiyaç olduğunu göstermektedir.

Anahtar Kelimeler: Melatonin, Spondiloartropatiler, Sirkadiyen ritim, Romatoid Artrit, Hipotalamus Hipofiz Adrenal Aksı

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INTRODUCTION

Melatonin (MLT) is a neurohormone secreted by the pineal gland and recently involved in the pathogenesis of some inflammatory diseases, particularly rheumatoid arthritis (RA). MLT secretion is sensitive to light and its serum levels have diurnal variations. It also exerts immunomodulatory effects via its receptors in the immune system.[1]

Joint stiffness, which is more noticeable in the morning, is one of the main characteristics of RA. Although these patients had lower serum cortisol levels, their melatonin levels were suggested to be significantly higher in early morning and evening hours compared to healthy controls (2-4). This finding suggests that circadian characteristics of RA, such as morning stiffness, might be related to circadian secretion of melatonin.[2,4] On the other hand, in patients with RA, proinflammatory cytokines also show parallel changes.[4,5] However, there are not enough studies performed other inflammatory arthritis in this regard.

In this study, we aimed to measure the levels of serum melatonin, IFNγ, a Th-1 derived cytokine, IL-10, a Th-2 derived cytokine and serum cortisol in patients with RA, SPA which are other forms of inflammatory arthritis, in accordance with diurnal variations and compare these findings with clinical parameters.

METHODS

Patients: This study was included 20 patients diagnosed with SPA according to European Spondyloarthropathy Study Group criteria[6], 10 of which with ankylosing spondylitis (AS)and 10 with Psoriatic arthritis (PSA), 20 patients with RA according to ACR/EULAR criteria[7], who were admitted to and followed in our rheumatology division, Internal Medicine Department and 20 healthy controls (HCs) consisting of hospital staff and patient caregivers who have no disease. The patients were recruited by their order of admission to the hospital.

Patients with diseases, such as malignancy, diabetes mellitus, hypertension, decreased vision, chronic obstructive pulmonary disease and those who smoke and use alcohol were excluded from the study. All patients and controls were documented for age, gender and general demographic characteristics and underwent physical examinations.

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was used to determine the disease activity of patients with SPA. The disease activity score assessing 28 joints (Disease

Activity Score 28: DAS28) was used to determine the disease activity of patients with RA. ESH, CRP levels for each patient and RF level for patients with RA were evaluated. The sample were taken randomly over 3 months.

Inform concepts were taken from all participants. The study was approved by local ethics committee (12.05.2008–43).

Biochemical analyses:

Blood collected from each participant at 8:00 p.m., at 3:00 am and at 8:00 am, three times a day according to the procedure was centrifuged at 3000 rpm for 20 minutes at 4°C and stored at -70° C after the obtained plasma and serum samples were separated into 1.5 cc serum storage tubes with clear cap. Analyses were performed by ELISA methods using Hamburg melatonin kit (Catalog Number; RE54021, IBL, HAMBURG, GERMANY) for serum melatonin level IBL; Biosource invitrogen IFN-γ kit (Catalog Number; KHC4021, Invitrogen Corporation Carlsbad, California, USA) for serum IFN-γ level; Assay Max Human Interleukin-10 (IL-10) kit (Catalog Number: EI3010-1, ASSAYPRO South Drive Saint Charles, MOUSA) for serum IL-10 level. Serum cortisol level was analyzed in Abbott C8000 auto analyzer using Abbott kit (Catalog no: 7D62-20, Chicago, IL, USA).

Statistical Analysis

For quantitative data, we compared means of two groups using student-t test for normally distributed variables and Mann-Whitney U test for variables that were not normally distributed. One-way analysis of variance (ANOVA) or Kruskall Wallis tests were used to compare means of multiple groups. Chi-square test was employed to compare qualitative data. Correlation analysis was performed to quantify the association between data. The significance limit was accepted at $P < 0.05$.

RESULTS

General characteristics of the groups: Three of the patients with SPA did not participate in the study for various reasons. Out of the remaining patients, 17 were in the SPA group (9 of which were with AS, 8 with PSA), 20 with RA and 20 were HCs. Clinical and some demographic data of the patients are summarized in Table 1.

Comparison of the biochemical analysis in patients with SPA, RA and of healthy controls: The cortisol levels measured at 8:00 am were found different between groups (F=4.445, p=0.016). LSD analysis showed that the cortisol levels measured at 8:00 am were significantly lower in patients with RA than in patients with SPA and HCs (p=0.047 and p=0.006 respectively). MLT values measured at 3:00 am in patients with SPA were found statistically significantly lower than HCs group (p=0.017). If we take account only Ankylosing Spondylitis cases, the MLT level at 3:00 am was lower than that of healthy controls, again (P: 0.02, chi-square: 12.13).

When MLT, IL-10, IFNγ and cortisol levels of RA cases were compared at 8:00 p.m., 3:00 a.m. and 8:00 a.m., no difference was found in IFNγ and IL-10 levels measured at different time points (p >0.05 for all). There was, however, a difference between cortisol levels at different time points (F=3.874; p=0.027), In LSD analysis, the cortisol measured at 8:00 a.m. was significantly higher than that measured at 8:00 p.m. and 3:00 a.m. in RA patients. (p=0.049 and p=0.010, respectively) (Table 1).

There was a significant negative correlation between the IFNγ/IL-10 ratio measured at 8:00 am and the cortisol level measured at 03.00 a.m. in patients with SPA (r=-0.548, p=023). There was also a significant positive correlation between BASDAI and MLT levels measured at 8:00 pm in patients with SPA (r=0.688; p=0.040). Considering only cases of Ankylosing Spondylitis, this correlation was still ongoing (p:0.040; pearson 0.688). There was no statistically significant difference between IFNγ /IL-10 ratios at all time points in RA, SPA and HCs (p >0.05 for all). There was no correlation of IFNγ/IL-10 ratios measured at each three time points with DAS28 in RA patients and with BASDAI in SPA patients.

17 of the patients with RA were receiving corticosteroids equivalent to a mean of 6.75 ± 4.05(4-16) mg methyl prednisolone. MLT, IFNγ, IL-10 and cortisol levels of patients not receiving corticosteroid therapy were not different from those on corticosteroids (p >0.05).

Laboratory data of the patients and HC group are summarized in Table 1. The course of serum MLT, cortisol, IFN-γ and IL-10 values measured at each three time points in SPA, RA and HC groups are graphically illustrated in Figures 1, 2, 3 and 4 respectively.

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Table1. Demographic and Laboratory Results of the Patients with SPA, RA and Healthy Control Groups

Variable	SPA N=17	RA N=20	HC N=20	P
Age, years	45,7±14,2 (21-73)	54,8±12,185 (29-73)	37,8±6,0 (29–49)	**NI**
Gender(F/M)	10/7(%59/41)	12/8(%60/40)	9/11 (%45/55)	**NI**
Disease Duration, months	129,5±135,2(6-480)	135,6±132,1(12-420)	-	
MLT–20, pcg/ml	2.8±19.3 (18.4–38.3)	2.1±10.6 (9.7-49.6)	3.9±23.5 (3.5-71.6)	**0.01**
MLT–03, pcg/ml	2.4±16.5 (16.0-33.0)	2.0±8.6 (11.3–37.7)	3.6±18.7(6.0–73.8)	**0.003**
MLT-08, pcg/ml	2.0±11.4 (14.2-26.0)	1.9±9.7 (11.5–46.8)	2.8±18.1(6.9–65.5)	**>0.05**
Cortisol-20, µg/dl	4.1±2.5 (2.8-5.4)	4.8±4.0 (0.4-18.0)	5.0±2.8 (1.6–11.9)	**>0.05**
Cortisol-03, µg/dl	5.9±4.6 (3.5–8.3)	5.8±5.2 (0.3–18.6)	7.4±4.9 (1.0–14.9)	**>0.05**
Cortisol-08, µg/dl	13.6±7.6 (9.5–17.7)	9.3±6.2(0.9–19.2)	15.1±5.3 (2.7–26.4)	**0.016**
IFN-γ-20, pcg/ml	30.7±15.5 (22.7–38.7)	40.6±14.8(19.6-83.8)	53.6±88.8 (16.9–423.5)	**>0.05**
IFN –γ-03, pcg/ml	36.8±31.8 (20.5–53.2)	42.6±14.3 (25.6–77.4)	40.9±36.8 (19.3–188.6)	**>0.05**
IFN –γ-08, pcg/ml	27.9±8.0 (23.8–32.1)	44.1±16.3(26.8-101.2)	49.0±57.9 (18.4–281.0)	**>0.05**
IL-10-20, ng/ml	1.0±1.0 (0.5-1.5)	1.0±1.0(0.3-4.2)	0.8±1.3 (0.2–6.4)	**>0.05**
IL-10-03, ng/ml	0.4±0.2 (0.3-0.6)	1.1±1.3 (0.2-4.7)	1.7±4.9 (0.2–22.5)	**>0.05**
IL-10-08, ng/ml	**0.4±0.2 (0.3–0.5)**	**1.1±1.6 (0.3–6.8)**	**0.8±1.4 (0.2–6.0)**	**>0.05**

MLT:Melatonin, IFN-γ:Interferon-gamma, IL:Interleukin, RA:Rheumatoid arthritis; SPA: Seronegativespondiloartropathy; HC: Healthy control; NI: Not investigated

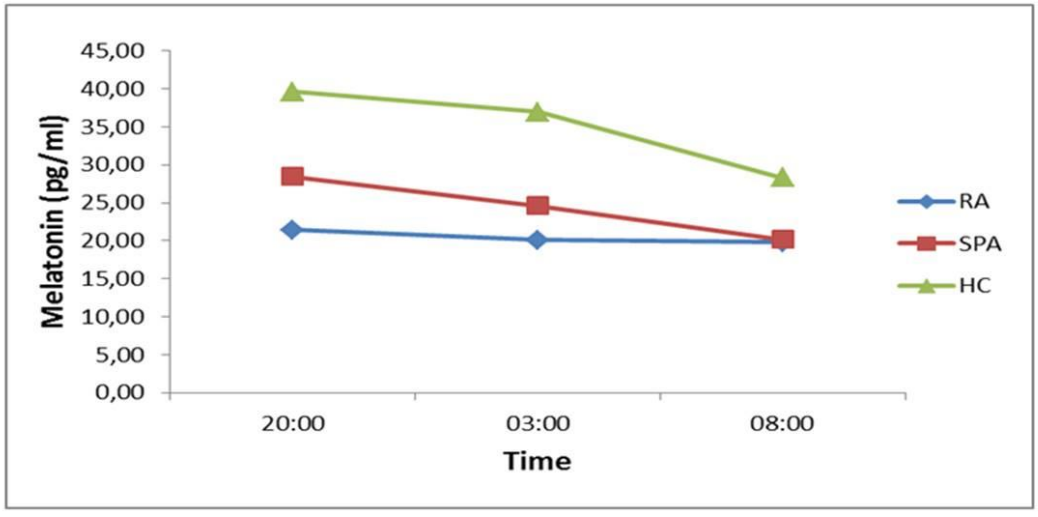

Figure 1.1. The course of serum melatonin levels at various times.

RA: Rheumatoid Arthritis, SPA: spondyloarthropaties, HC= Healty Control

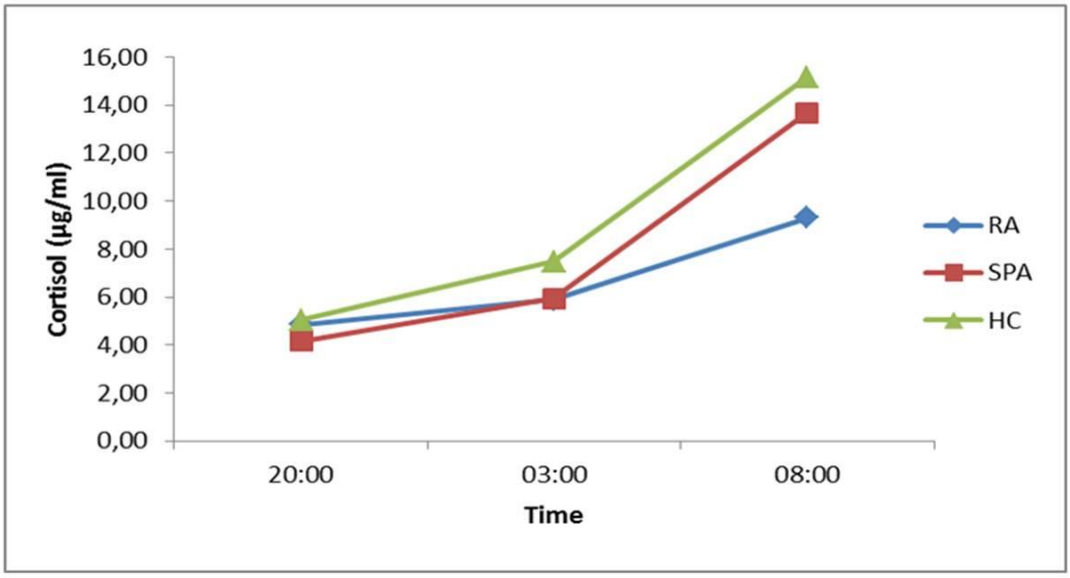

Figure 1.2. The course of serum cortisol levels at various times. RA: Rheumatoid Arthritis, SPA: spondyloarthropaties, HC= Healty Control

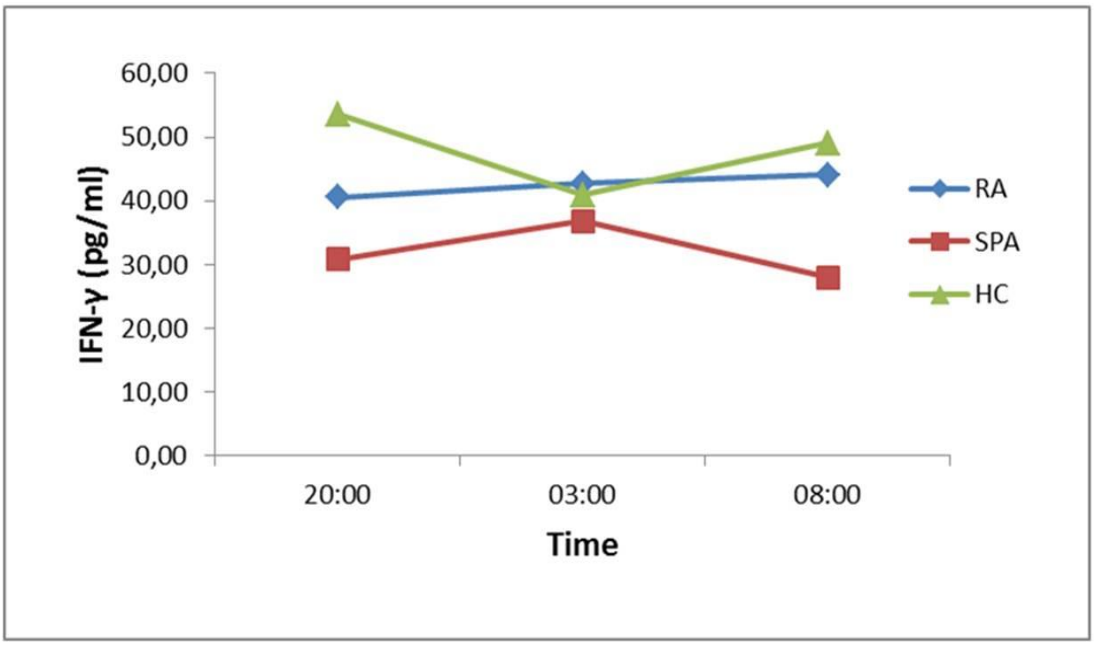

Figure 1.3. The course of serum INF gamma levels at various times. RA: Rheumatoid Arthritis, SPA: spondyloarthropaties, HC:Healty Control

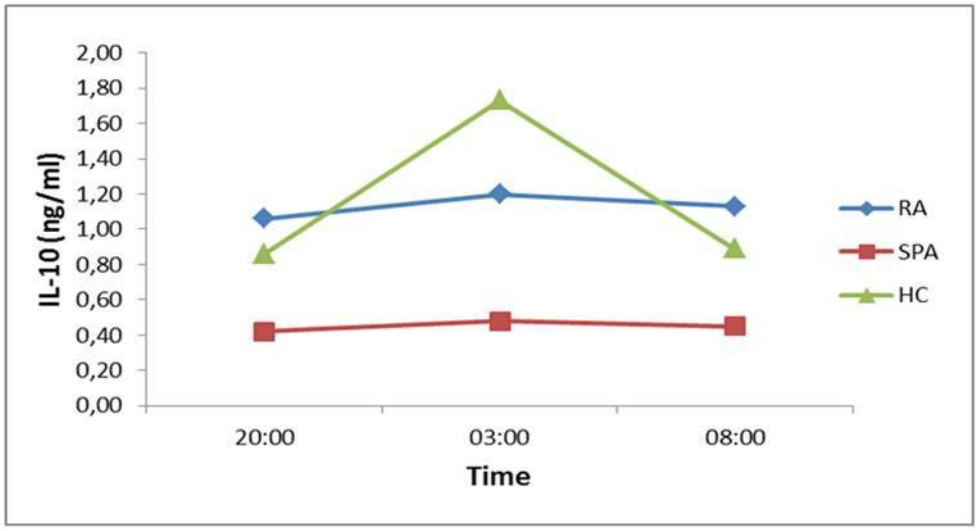

Figure 1.4: The course of serum IL-10 a levels at various times. RA: Rheumatoid Arthritis, SPA: Spondyloarthropaties, HC: Healty Control

DISCUSSION

Circadian rhythms play a major role in the regulation of human physiological functions. This study investigated the clinical relationship between circadian rhythm and inflammatory cytokines in patients with SPA and RA. Many signs and symptoms of arthritis, especially in RA, show intra- and inter- day variations. Morning stiffness around hand joints is the main determinant characteristics of RA and back pain that occurs after midnight and more noticeable in the morning is the main determinant characteristics of SPA.[8,9] There is many studies which investigated the circadian rhythm in RA[2-5,10], few studies were found in the literature regarding MLT values in patients with SPA (11). Senel et al reported that MLT levels correlated disease activity in AS patients.[12] Diurnal rhythm of MLT has not been investigated in these studies. In our study, MLT values of SPA patients at 3:00 a.m. were found statistically significantly lower than HC group (p=0.017). MLT measured at 8:00 p.m. in SPA patients only was significantly higher than MLT measured at other times of day (p=0.049). There was a significant relationship between BASDAI and MLT levels measured at 8:00 p.m., but no such relationship was found with MLT values at other times of day. These findings suggest that MLT might be involved in the pathogenesis of SPA and that low

melatonin levels may play a role in low back pain that is more noticeable at midnight. In healthy people, MLT peaks at 3:00 a.m. and cortisol peaks after 05:00 a.m. in parallel to the Th1 and Th2 derived cytokines peak at similar hours.[13] Our result suggests, increased pain at midnight pain may be related to MLT reduction in SPA patients. In studies targeting T cell, decreased Th1 response was detected in HLA B27-positive patients with SPA. When the T cells were treated with Chlamydia antigens, there was not adequate increase in IFN-γ and TNF-alpha secretion.[14] Low MLT may also induce inflammation by leading to inadequate Th1 activation and Th1/Th2 imbalance, or a deficiency in Th1 activity may result in low MLT levels. In our study, IFNγ levels were lower in patients with SPA when compared to RA patients and HC, but this difference was not statistically significant.

A correlation was reported between IL-10 levels and morning stiffness in AS patients.[15] However, no correlation was found between BASDAI and IL-10 levels in our study. In our study, there was a significant correlation between IL-10 and cortisol measured at 8:00 a.m. in SPA patients (r=0.558; p=0.025). This may be explained by the parallel increase of cortisol and Th2-derived cytokine levels. There was a significant negative correlation (r=-0.471, p=0.036) between MLT and cortisol levels measured at 8:00am in patients with RA, which is consistent with the literatüre.[16]

MLT achieves its peak level at about 03:00 a.m. and cortisol at about 9:00 a.m. in HCs.[13,17] In a study conducted by Cutolo et al, found that MLT and TNF-alpha levels were significantly higher at night in RA patients than in healthy people. More frequent measurement has applied in this study.[16] In our study, measurements were done at 8:00 p.m., 3:00 a.m. and 8:00 a.m., and the MLT levels were not found higher in patients with RA. Statistically significantly lower levels of MLT were observed at 8:00 p.m. and 3:00 a.m. in RA cases compared to HCSs (p=0.003; p=0.001, respectively). Lack of measurement performed at night and early in the morning at more frequent intervals and low number of patients are major limitations of our study. Also, in our study, blood was collected randomly over a period of up to 20 months. The fact that the blood from patients and controls was not collected at a homogeneous time frame can be another factor explaining why our results are not consistent with the literature. There is many limitations of our study. They are; low number of patients; we did not determine serum vitamin D; some patients with RA recieve corticosteroids and samples were not taken within a certain period of time.

CONCLUSION

Serum MLT levels well correlated with disease activity in SPA patients during evening hours. Back pain which is seen midnight can be related to decrease in MLT secretion. Our findings suggest MLT have a role in pathogenesis of SPA. But, it is need more detailed studies for explaining of this role of MLT in the pathogenesis of SPA.

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***Conflict of Interests*:** The authors declare that there is no conflict of interests.

The study was approved by local ethics committee (12.05.2008–43).

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