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Diagnostic utility of serum melatonin levels in systemic lupus erythematosus: a case-control study

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SUMMARY

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune inflammatory disease and early diagnosis is of clinical and therapeutic importance. Melatonin is an endogenous endolamine hormone that plays an important role in the immune system due to its anti-inflammatory action. This study was designed to assess serum melatonin levels in SLE patients and to evaluate the possible correlation between serum melatonin and patients' baseline characteristics.

A case-control study was performed on 50 SLE patients (48 females and 2 males), diagnosed according to the revised 1997 ACR Criteria, and 25 healthy controls (24 females and 1 male), matched by age and sex. Daily serum melatonin levels were investigated in all participants using human melatonin enzyme linked immunosorbent assay (ELISA) kit (MYBIOSOURCE (MBS), United States).

Serum melatonin concentration was significantly lower in patients with SLE compared to healthy controls (19.17±6.86 pg/mL *vs* 23.26±6.71 pg/mL, p=0.017). Serum melatonin concentration ≤18.51 pg/mL was the optimum cut off value to differentiate between SLE patients and healthy controls with an accuracy of 69.3%, a sensitivity of 66%, and a specificity of 76%. The positive predictive value (PPV) at pretest 50% was 73.3% and PPV at pretest 90% was 96.1%; the negative predictive value (NPV) at 10% was 95.3%. Patients' characteristics were not significantly correlated with serum melatonin concentrations using multiple logistic regression analysis. Serum melatonin was a valid measure to differentiate between SLE patients and healthy controls with good accuracy, sensitivity and specificity and PPV and NPV. There was no significant correlation between serum melatonin concentrations and patients' baseline characteristics.

***Key words**: Serum melatonin; systemic lupus erythematosus; autoimmune diseases.*

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease, characterized by a multitude of autoantibodies, complement activation and immune-complex deposition, which causes tissue and organ damage (1). The etiology and pathogenetic mechanisms of SLE are still unclear; no single cause for SLE has been identified (2). It is possible that the autoimmune disorder results from the combination of predisposing genetic factors and the disturbed status of stress response mechanisms, including the sympathetic nervous system and various hormones (3). Melatonin, produced by the pineal gland during the night, is the major secretory hormone and a key player in the neuroendocrine-immune pathway (4, 5). Melatonin can stimulate cytokine production, phagocytosis and natural killer cell activity. *In vitro* studies and animal experiments have provided evidence for a modulatory effect of melatonin in the immune response: melatonin receptors are expressed on the membrane of CD4 T cells, CD8 T cells, and B cells (6, 7), and melatonin treatment in mice showed increasing proliferation of T cells (8). In addition, it can antagonize the increasing levels of IgM anti-ssDNA and histone autoantibodies, and could also decrease IL-6 and IL-13 production, and increase IL-2 production in the pristane-induced lupus mice (9).

Melatonin concentrations have been in-

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vestigated in different autoimmune and allergic diseases (10, 11). However, the function of melatonin in humans remains unclear. It is believed that melatonin could induce cytokine production by human peripheral blood mononuclear cells via the nuclear melatonin receptor (12). Another study reported seasonal changes in melatonin concentrations in SLE patients, the daily melatonin plasma concentrations in SLE patients being higher in December than in June (13). A subsequent study showed lower daily melatonin concentrations in SLE patients compared to healthy women (14). A recent study showed that melatonin increases the number of T regulatory (Treg) cells expressing FOXP3 and offset BAFF overexpression in SLE patients' cells (15). These findings open a new field of research in SLE that may lead to the use of melatonin as treatment or cotreatment for SLE. However, studies on the plasma melatonin levels and its clinical associations in SLE patients are still very limited.

The aim of this study was to assess the diagnostic utility of measuring melatonin concentrations in human SLE, and its correlations with clinical and laboratory features.

PATIENTS AND METHODS

Study design

This case control study was conducted at the Rheumatology Unit of Baghdad Teaching Hospital/Medical City from January 2015 to the end of March 2015. Informed written consent was obtained from all participants, and this study was approved by the Ethics Committee of Baghdad University, College of Medicine-Medical Department.

Participants

A total of 50 Iraqi SLE patients diagnosed by a rheumatologist according to the American College of Rheumatology (ACR) 1997 revised classification criteria (16) were included in this study and compared with 25 healthy controls matched by age and sex. Patients were excluded if they had features of other overlapping inflammatory arthritides or connective tissue diseases.

Clinical and laboratory evaluation

Using interviews and questionnaires, we assessed age, sex, body mass index (BMI), disease duration, and disease activity measured by the systemic lupus erythematosus disease activity index (SLEDAI) and medications taken. Complete blood count (CBC), general urine examination (GUE), complement (C3 and C4), and anti-ds-DNA antibodies were recorded.

Five milliliters of venous blood were drawn from a peripheral vein of each patient and control using disposable needles and syringes. The blood samples were then allowed to clot at room temperature in plain tubes for 30-45 minutes. Sera were obtained by centrifugation of these tubes at (3000 rpm) for 10 minutes and kept frozen in plain plastic tubes at deep freeze temperature (-60°C). Daily melatonin concentrations were investigated through human Melatonin (MT) Enzyme Linked Immunosorbent Assay (ELISA) kit, from Mybiosource (MBS), United States. Reliability of measurements was assessed by intra-assay calibration. There was no statistical significant intra-assay variation, which fact suggests that the results are reproducible.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 20 IMB. The Anderson-Darling normality test was used to test the distribution of variables by a Minitab version 17 software. Continuous variables were reported as mean±SD when normally distributed and median interquartile range (IQR) when not normally distributed. Categorical variables were presented as numbers and percentages. The difference between normally distributed continuous variables was assessed using the independent T-test (Student Test) and Mann-Whitney test for not normally distributed continuous variables. The difference between categorical variables was measured using the Chi square Test or the Fisher Exact test. The cut-off value of serum melatonin was calculated using the Receiver Operating Curve (ROC) with its validity parameters (sensitivity, accuracy and specificity). The multiple logistic regression analysis test

Table I - Baseline characteristics of patients and controls.

Variable		Patients (n=50)	Controls (n=25)	p value
Age (years)		31.88±9.16	27.56±7.33	0.044
Gender	Female n=72	48 (96%)	24 (96%)	1.00
	Male=3	2 (4%)	1 (4%)	
BMI (kg/m^2)		25.47±4.13	25.31 ± 3.96	0.872
Disease duration (months)		27 (12-63)*		
SLEDAI	Mild-moderate	10 (20%)		
	Severe	40 (80%)		
	Median (IQR)	25 (14-42.25)*		
ANA	Positive	47 (94%)		
Anti-ds-DNA	Positive	28 (56%)		
Prednisolone	N(%)	41 (82%)		
Hydroxychloroquine	N(%)	35 (70%)		
Azathioprine	N(%)	15 (30%)		
Mycophenolate mofetil	N(%)	5 (10%)		
Methotrexate	N(%)	3 (6%)		
Chloroquine	N(%)	1 (2%)		
Aspirin	N(%)	1 (2%)		

BMI, body mass index; SLEDAI, systemic lupus erythematosus disease activity index; ANA, antinuclear antibody; Ant-ds-DNA, anti double-stranded deoxyribonucleic acid antibody. *Median (Interquartile range).

was used to evaluate the effect of baseline patient characteristics on serum melatonin. P value ≤0.05 was considered statistically significant.

RESULTS

A total of 75 individuals were enrolled in this study. Of these, 50 were SLE patients and 25 healthy controls. Gender and mean BMI showed no statistically significant difference between the groups (p>0.05). The mean age of the patients was slightly higher than that of the controls (p=0.044). Sociodemographic variables were similar between both groups. Other baseline characteristics of patients and controls are shown in Table I.

Serum melatonin level was significantly lower in patients with SLE compared to healthy controls (19.17 pg/mL±6.86 pg/mL *vs* 23.26 pg/mL±6.71 pg/mL, p=0.017) (Figure 1). The optimum cut off value of serum melatonin as a test to differentiate between SLE patients and healthy controls was tested by the ROC method. We found that the area under the curve (AUC) at value 0.71 was statistically significant (p=0.002) and had high accuracy. Serum melatonin concentration ≤18.51 pg/mL was the optimum cut off value to differentiate SLE patients from

Table II - Multiple linear regression analysis showing the effect of baseline patients' characteristics on serum melatonin (p=0.971; R_2=0.14).

Baseline characteristics	Partial regression coefficient	p value
Age	0.036	0.829
Sex	–0.013	0.938
BMI	0.041	0 .806
Disease duration	–0.028	0 .870
SLEDAI Score	–0.117	0 .483
ANA	0.017	0.918
Anti-ds-DNA	0.114	0.496
Azathioprine	0.094	0 .576
Methotrexate	–0.008	0.960
Mycophenolate mofetil	0.282	0 .086
Prednisolone	–0.106	0.525
Hydroxychloroquine	0.025	0.880
Chloroquine	–0.099	0.554
Aspirin	0.036	0.829

healthy controls with accuracy 69.3%, sensitivity 66%, and specificity 76%; positive predictive value (PPV) at pretest 50% was 73.3% and PPV at pretest 90% was 96.1%, whereas negative predictive value (NPV) at 10 % was 95.3% (Figure 2).

The baseline characteristics of patients: age, sex, BMI, disease duration, disease activity measured by SLEDAI, ANA, Anti-ds-DNA, use of azathioprine, MTX, mycophenolate mofetil, prednisolone, chloroquine, hydroxychloroquine, and aspirin showed no statistically significant effect on serum melatonin using multiple linear regression analysis as shown in Table II.

DISCUSSION AND CONCLUSIONS

This case-control study assessed the diagnostic utility of serum melatonin in SLE patients and the correlation of melatonin concentrations with the baseline characteristics of the patients. It revealed that serum melatonin was significantly lower in SLE patients compared to healthy controls and there was no significant correlation between baseline characteristics of the patients and serum melatonin.

Interestingly, this is the first study showing that serum melatonin was a statically significant valid measure with fair diagnostic performance accuracy and a good predictive value to differentiate between SLE patients and healthy controls at optimum cut off value 18.51 pg/mL.

Only one previous study, conducted by Robeva et al. (17), investigated the role of daily serum melatonin concentrations in the development of SLE and concluded that daily melatonin levels were decreased in women with SLE. However the diagnostic validity of serum melatonin to differentiate between patients and healthy controls was not measured.

Another observation of note in the current study was that there was no significant correlation between patients' baseline characteristics and serum melatonin. This may suggest that the reduced serum level of melatonin is mostly related to SLE rather than to other confounders. In particular, age, which was slightly different in patients and controls, did not affect serum melatonin.

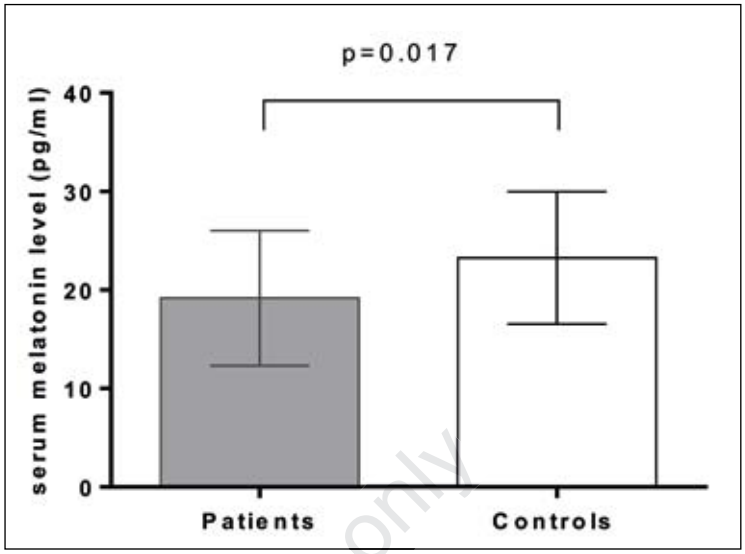

Figure 1 - Mean serum melatonin concentrations in patients and controls.

A previous study by Haga et al. (18) observed a difference in plasma melatonin concentrations between winter and summer

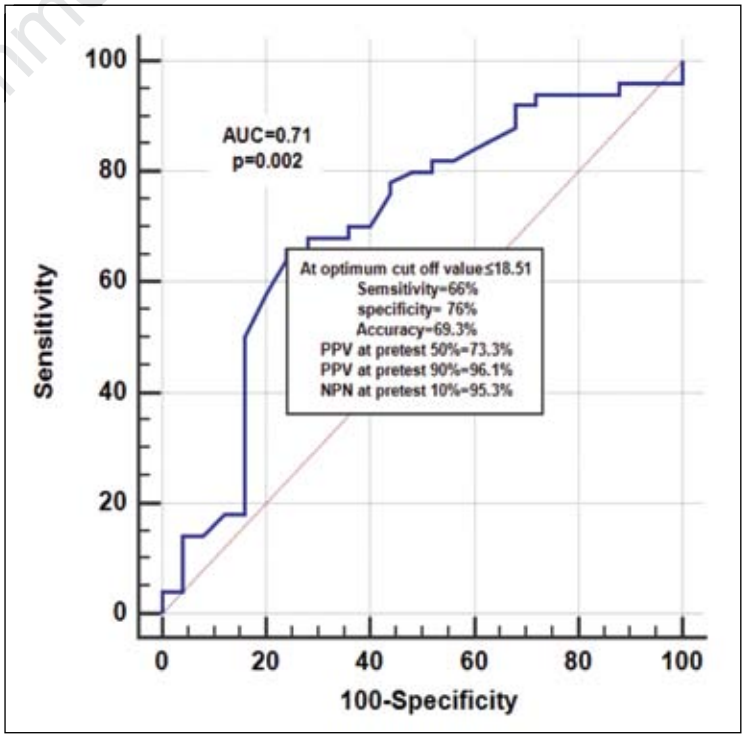

Figure 2 - ROC curve of serum melatonin showing the optimum cut off value that differentiates between SLE patients and healthy controls.

months. Serum melatonin concentration did not correlate with disease activity. Another study (17) reported that the decrease in the daily melatonin level in SLE patients was not a consequence of chronic steroid treatment, because a significant correlation between melatonin levels and prednisolone doses was lacking.

In the present study, only daily melatonin concentrations were determined, Therefore, no information about the possible disturbances in the circadian melatonin rhythm was obtained, which we consider the main limitation of the study. Another important limitation is the lack of longitudinal follow up data of the individual melatonin changes during the SLE course.

In conclusion, serum melatonin level was a simple and valid diagnostic measure for SLE patients with high sensitivity, specificity and accuracy. This may help to differentiate between SLE patients and healthy controls. Further studies with a larger number of patients, longer follow-up time, and comparison with controls affected by other conditions are needed. Studies on the effect of seasonal variations on melatonin level in SLE patients to clarify the importance of pineal and extrapineal melatonin secretion on autoimmunity would be warranted.

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