A Novel Study of Predictive Utility of Serum Melatonin in Diagnosis of Systemic Sclerosis: A Case-Control Study

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ABSTRACT:

BACKGROUND:

Melatonin is a pineal gland hormone with complex roles in the pathogenesis of autoimmune disorders. Immune abnormalities and disturbed endocrine secretion have been reported in patients with systemic sclerosis (SSc). A possible role of melatonin in pathogenesis of SSc patients might be present

OBJECTIVE:

To assess the predictive utility of serum melatonin in diagnosis of SSc patients.

METHODS:

A case-control study conducted at Rheumatology Unit of Baghdad Teaching Hospital-Medical City from November 2015 to April 2016 on 40 SSc patients diagnosed according to the 1980 criteria for classification of systemic sclerosis or 2013 American College of Rheumatology/European League Against Rheumatism for the classification of systemic sclerosis criteria and compared with 40 healthy controls matched in age, sex, and body mass index. Patients with overlapping other inflammatory arthritis or connective tissue disease or autoimmune diseases were excluded. Serum melatonin was measured in both groups by enzyme-linked immunosorbent assay (ELISA).

RESULTS:

Mean level of serum melatonin concentration was significantly lower in patients compared with controls (Mean ± SEM of patients was 602.9 ± 30.69 vs 782.1 ± 43.66 pg/ml for controls, p=0.001). The optimum cut-off value of serum melatonin that differentiate between patients and controls was ≤ 810.8 pg/ml (AUC=0.72, p=0.0005). Validity of serum melatonin at the optimum cutoff value showed highest level of accuracy (75%) with sensitivity 87.5% and specificity 62.5% and if the test was positive and the clinical suspicion of prestest probability was 50% then we have 70% confidence that the patient has systemic sclerosis [Positive predictive value (PPV) at pretest 50%=70%]. However, this confidence will increase to 95.5% if the clinical suspicion of pretest probability was 90% (PPV at pretest probability 90%=95.5%). Also, if the test was negative then we can exclude the disease with 97.8% with confidence [Negative predictive value (NPV) =97.8%].

CONCLUSION:

Serum melatonin was a valid measure to diagnose SSc.

KEYWORDS: serum melatonin, pineal gland, systemic sclerosis (SSc).

INTRODUCTION:

Systemic sclerosis (SSc) is a systemic connective tissue disease with a high morbidity and mortality characterised by fibrosis of the skin and internal organs and vasculopathy [1,2]. The current knowledge regarding the pathogenesis of SSc indicates that the pathological process arises from the complex interrelation between three main components: vascular dysfunction, innate and adaptive immunity dysregulation, and excess activation of fibroblasts and related cells, which culminates in the development of fibrosis. [3,4].

Melatonin is the major secretory hormone produced by the pineal gland during the night and has multifunctional effects. These effects are a consequence of antioxidant, antiinflammatory, and immunomodulatory properties of melatonin [5,6]. Recent studies have confirmed that melatonin plays an important role in the immune system [6]. Melatonin receptors are expressed on the membrane of CD4 T cells, CD8 T cells, and B cells [7,8]. It has been reported that the proliferation of T cells increases in mice treated with melatonin [9]. Melatonin treatment has also been reported to enhance the production of natural killer (NK) cells and monocytes in the bone marrow of mice [10], and can induce cytokine production in human peripheral blood mononuclear cells via the nuclear melatonin

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receptor [11]. By contrast, other studies have demonstrated that the expression of interleukin (IL)-2 and interferon (IFN)-γ is decreased and the expression of T helper (Th)2 cell cytokines, such as IL-4 and IL-10, is upregulated in mice treated with melatonin [12, 13]. The effects of melatonin have been investigated in several animal models and evaluated in patients with clinical autoimmune disease (14-16). However, in systemic sclerosis rarely reported and diagnostic utility in SSc has not been demonstrated. The aim of this study was to assess the predictive validity of serum melatonin in diagnosis of SSc.

PATIENTS AND METHODS:

Study design

This case control study was conducted at Rheumatology Unit of Baghdad Teaching Hospital-Medical City from November 2015 to April 2016. Informed consent was obtained from each participant included in this study according to the declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Baghdad University, College of Medicine, Medical Department.

Participants

Eligible patients included in the study were: any gender >18 years with features of systemic sclerosis diagnosed by a rheumatologist according to the criteria developed by the 1980 criteria for classification of systemic sclerosis or 2013 American College of Rheumatology/European League Against Rheumatism for the classification of systemic sclerosis[17,18]. Patients were excluded if they had overlapped features of other inflammatory arthritis or connective tissue disease or autoimmune diseases. Another group healthy individuals matched in age and gender were taken as a control group.

Clinical and laboratory evaluation

Using interviews and questionnaires: age, gender, and body mass index (BMI) were recorded Complete blood count (CBC), urinalysis, fasting blood sugar (FBS) , renal function test (blood urea, serum creatinine) , antinuclear antibody (ANA), anti-scleroderma 70 (AntiScl70), Anticentromer antibody , Anti-Sjögren's-syndrome-related antigen A (Anti-Ro) and Anti-Sjögren's-syndrome-related antigen B (Anti-La antibody), Thyroid function tests(T3 T4 and TSH) when appropriate were measured

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

Statistical Analysis

Statistical analysis was done using SPSS software version 23 IMB. Kolmogrove-Smirnove test was used to test the normality of distribution of variables. Continuous variables with normal distribution were reported as Mean ± SD. Difference between normally distributed continuous variables was done using independent T-test (Student Test). The optimum cutoff value of serum melatonin was calculated using receiver operating characteristics curve (ROC) with its validity parameters (sensitivity, specificity, accuracy, PPV, and NPV. P value < 0.05 was considered statistically significant.

RESULTS:

A total of 40 female SSc patients and 40 healthy female controls were involved in the study. The mean age of patients and controls were statistically not significantly different (34.53 ±4.47 vs 34.83±3.48 years , p=0.36).The mean BMI of patients and controls was also statistically not significantly different as 17.24 ± 1.66 vs 17.6 ± 1.69 kg/m², p=0.97) as shown in Table 1.

The mean level of serum melatonin concentration was significantly lower in patients compared with controls (Mean ± SEM of patients was 602.9 ± 30.69 vs 782.1 ± 43.66 pg/ml for controls, p=0.001) as shown in Figure 1.

The validity of serum melatonin as a test to differentiate between SSc patients and controls was measured by ROC test. The optimum cut off value was ≤ 810.8 pg/ml with AUC=0.72 and statistically was significant (p=0.0005) as shown in Figure 2.

In Addition, the validity parameters of serum melatonin at optimum cutoff value(≤ 810.8 pg/ml) showed highest level of accuracy(75%) with sensitivity 87.5 % and specificity 62.5%. and if the test was positive and the clinical suspicion of the prestest probability was 50% then we have 70% confidence that the patient has SSc (PPV at pretest

probability 50%=70%). However, this confidence will increase to 95.5% if the clinical suspicion of the pretest probability was 90% (PPV at pretest probability 90%=95.5%) as shown in Table 2.

Finally, if serum melatonin was higher than the optimum cut off value (>810.8) this means the test was negative, then we can exclude the disease with 97.8% with confidence.

Table 1: Demographic distribution of systemic sclerosis patients and controls.

Variables	Patients	Controls	P
Age	35.53±4.47	34.83±3.48	0.36
Female n(%)	40(40%)	40(40%)	0.82
BMI	17.24 ± 1.66	17.6 ± 1.69	0.97
BMI, body mass index. P<0.05 is not significant			

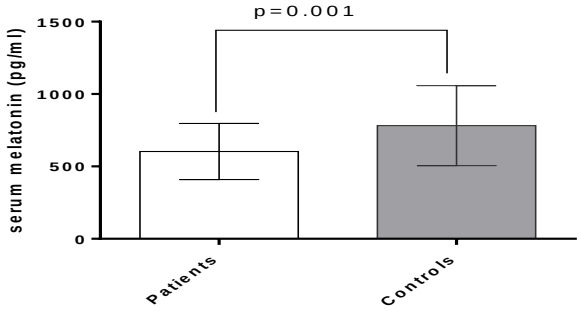

Figure1: Serum melatonin level in patients and controls.

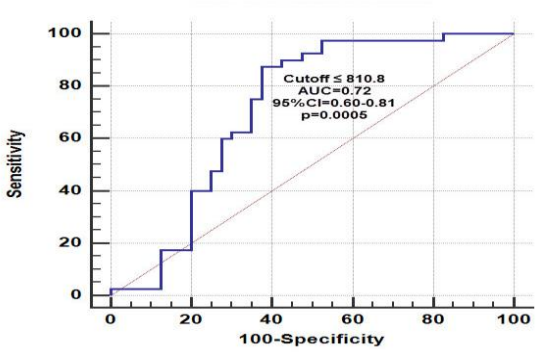

Figure 2: Validity of serum melatonin as a test to differentiate between systemic sclerosis patients and controls using ROC test.

Table 2: Validity parameters of serum melatonin to differentiate between systemic sclerosis patients and healthy controlsز

Optimum Cut off value positive if	Sensitivity	Specificity	Accuracy	PPV at pretest 50%	PPV at pretest 90%	NPV at pretest 10%
Serum melatonin ≤ 810.8	87.5 %	62.5 %	75 %	70 %	95.5 %	97.8 %

PPV, positive predictive value, NPV, negative predictive value

DISCUSSION:

This case control study assessed the predictive utility of serum melatonin in diagnosis of patients with SSc. It showed that serum melatonin was significantly lower in patients with SSc compared with healthy controls and was a valid marker to differentiate between SSc patients and controls with high sensitivity, accuracy, and very high PPV and NPV but with lower specificity. These results are statistically significant and clinically relevant because it will help in early diagnosis and prognosis of SSc with subsequently a possible therapy to prevent disease complication.

The mechanism of impaired melatonin secretion remains unknown. It may be speculated that a low melatonin level is involved in development of immune abnormalities, neuroendocrine dysfunctions and/or impaired free radical elimination. All these phenomena may contribute to development of fibrosis [19].

According to the current evaluation of the literature, there was only one pilot study published by kotluska et al [19] evaluated the circadian profile of serum melatonin levels in six patients with SSc and compared it with six age-matched healthy women and reported that the general nature of the circadian serum melatonin rhythm is maintained in the SSc patients although secretion of melatonin is depressed. However, in that study the sample of patients was little and diagnostic validity of serum melatonin was not assessed.

Other reports suggested low levels of serum melatonin in other autoimmune connective tissue diseases and diurnal variations. Robeva et al [20] investigated the role of daily serum melatonin concentrations in the development of systemic lupus erythematosus patients and demonstrated that daily melatonin levels decreased in women with systemic lupus erythematosus and correlated inversely to the activity of the autoimmune disease. Keskin etal [21]investigated the levels of serum melatonin in Sjögren patients and melatonin's diurnal rhythm and found that diurnal rhythm of the melatonin changed in Sjögren patients. These findings can be due to the effects of melatonin on the inflammatory responses.

The main limitation of the current study are: first, only daily melatonin level was assessed as in other studies [22,23]. Therefore, no information about the possible disturbances in the circadian melatonin rhythm was obtained. Second, the lack of longitudinal data allowing the follow up of the individual melatonin changes during the SSc progression. Finally, the interrelation with SSc severity and patients' quality of life was not evaluated. Nevertheless, our results showed up to the best of our knowledge for the first time the predictive utility of serum melatonin in diagnosis and differentiation of SSc patients from healthy controls with strong inclusion and exclusion criteria. This may suggest a probrable use of melation in SSc diagnosis, follow up, and treatment.

CONCLUSION:

Serum melatonin was a valid measure in diagnosis and prediction of SSc. Further studies are needed to clarify the importance of the pineal and extrapineal melatonin secretion in patients with SSc as well as the interrelations between the hormone and the autoimmunity.

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