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Decreased Urinary Level of Melatonin as a Marker of Disease Severity in Patients with Multiple Sclerosis

**Taha Gholipour^{1,2}, Taher Ghazizadeh^{1,2}, Sahand Babapour^{1,2}, Behzad Mansouri³,
Majid Ghaffarpour¹, Bahaadin Siroos¹, Mohammad Hossein Harirchian¹**

¹ Iranian Center of Neurological Research, Tehran University of Medical Sciences, Tehran, Iran

² Interdisciplinary Neuroscience Research Program, Tehran University of Medical Sciences, Tehran, Iran

³ Section of Neurology, Department of Internal Medicine, University of Manitoba, Winnipeg, Canada

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ABSTRACT

Melatonin has both pro-inflammatory and anti-inflammatory properties depending on the stage of inflammation. Despite its therapeutic effect in alleviation of some symptoms of multiple sclerosis; the precise role of melatonin in MS pathogenesis remains a topic of debate. The aim of this study was to measure the urine level of one of melatonin products which is an index of serum melatonin level, in MS patients in the acute phase of relapse and control patients. We also analyzed different clinical and cognitive indices in order to find any correlation with melatonin level.

Twenty eight patients who were diagnosed as relapsing-remitting MS, according to the revised McDonald criteria, along with 10 age- and sex-matched control subjects were recruited in our study.

Here we showed that urine 6-sulphatoxymelatonin levels (aMT6s; the major metabolite of melatonin) were significantly lower in MS patients compared to control group. Interestingly, urine aMT6s levels significantly correlated with MS Functional Composite score, but not Expanded Disability Severity Score.

Based on above findings, there might be new hope in developing a quantitative and objective measure to assess the MS severity especially in neurodegenerative diseases. However, our results should be analyzed cautiously. We didn't evaluate simultaneous level of 25-OH Vitamin D. It has been recently reported that there is a negative correlation between melatonin and vitamin D levels. Further studies are needed to confirm this hypothesis.

Keywords: Cognitive Function; Melatonin; Multiple Sclerosis

Corresponding Author: Mohammad Hossein Harirchian, MD;
Iranian Center of Neurological Research, Imam Khomeini
Hospital, end of Keshavarz Boulevard, 14197, Tehran, Iran. Tel:
(+98 21) 6694 8899, Fax: (+98 21) 6658 1558, E-mail:
harirchm@sina.tums.ac.ir

INTRODUCTION

Multiple sclerosis (MS) is one of the most common neurologic disorders, with a propensity to involve

young adults; making it as a remarkable part of the total neurological diseases burden over society. Insight about the pathophysiology of MS is significantly changed in the last decade.

In general, it is believed that MS is a complex multifactorial disorder so that exposure to an environmental factor in a genetically susceptible person leads to an autoimmune reaction against the myelin antigens in the central nervous system (CNS).¹ The immunopathology of MS is currently believed to involve not only the “autoreactive” T helper cells, but also B cell mediated immunity, as well as neuroimmunomodulatory systems, including glucocorticoids, opioids, prolactin, and N-acetyl-5-methoxy-tryptamine (known as melatonin).²⁻⁷

Melatonin hormone, one of the main regulators of sleep, is secreted from pineal body with a fluctuating pattern which is finely regulated by the biological clock and signaling from the suprachiasmatic nucleus.⁸⁻⁹ Based on its circadian alterations, melatonin is suggested to play a role in the pathogenesis of the diseases that show diurnal and seasonal pattern or are associated with abnormal circadian rhythm of hormones.¹⁰ Recently, it has been elucidated to have neuroprotective, regulatory, and immunomodulatory properties.¹¹⁻¹⁵ The precise mechanism through which the melatonin affects the immune system is still unclear. However, it seems to play as an immune buffer depending on the stage of inflammation, melatonin could have paradoxical pro-inflammatory or anti-inflammatory effects. Its pro-inflammatory effect is secondary to upregulation of major histocompatibility complex-II (MHC-II) expression and increase in Th1-related cytokines through activation of surface melatonin receptor on antigen presenting cells.¹⁶⁻¹⁸ On the other side, melatonin has been shown to have neuroprotective effects because of anti-oxidant

properties.^{17,19-20} Oxidative stress plays a cardinal role in neurodegeneration and axonal loss secondary to chronic inflammation in MS. In this way, several studies confirmed neuroprotective effect of melatonin in MS and hypoxia-related oxidative stress. Beside the direct immunomodulatory role of melatonin through lymphocytes, melatonin has been found to be effective in regulation of other immune mediators and cytokines,^{20,21} as well as opioids,²² and the endocrine system (e.g. sex hormones and glucocorticoids)^{12,23}. It is also believed that lymphocytes, like some other tissues, could secrete melatonin which perhaps acts in an autocrine/paracrine manner.²¹

The geographic prevalence of MS in northern hemisphere, beside genetic or other environmental factors, could be contributed to alterations in sera or cellular level of mediators such as melatonin and vitamin D that their metabolism is dependent to sunlight exposure.²⁴ In this dissertation, we compared the urine level of one of melatonin products which is an index of serum melatonin level in MS patients in the acute phase of relapse and control patients. We also measured different clinical and cognitive indices in order to find any correlation with melatonin level.

MATERIALS AND METHODS

Patients

Twenty eight patients who were diagnosed as relapsing-remitting MS, according to the revised McDonald criteria participated in our study.²⁵ Exclusion criteria were any urinary abnormalities (e.g. abnormal urine analysis, incontinence, nocturia, and polyuria), illiteracy, impaired auditory, visual acuity less than 5/10, sleep disorders, medications such as oral contraceptives, estrogen, hydralazine, loop

Table 1. Information collected as patient summary sheets

Personal information	Identifications, Ethnicity, History of migration
Past medical history	medical conditions, Surgery, trauma, or blood transfusion, First symptom(s), History of alternative medicine use, Diagnosis delay, drug used and duration, use of potentially interfering drugs (oral contraceptives, estrogen, hydralazine, loop diuretics, penicillamine, and theophylline)
Current signs and symptom	Checklist of interested neurological signs and symptoms to be filled following a complete neurological history and physical exam
Cognitive assessments	MMSE, EDSS, detailed MSFC, MFIS scores
Laboratory data	Urinalysis +/- urine culture, any abnormal result in routine inpatient tests
Specimen quality	Confirmed accuracy of sampling method, subjective quality of sleep on the night before sampling

MMSE, Mini-Mental Status Exam; EDSS, Expanded Disability Severity Scale; MSFC, Multiple Sclerosis Functional Composite; MFIS, Modified Fatigue Impact Scale

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Table 2. Multiple Sclerosis Functional Composite (MSFC)

Measure	Description	Time limit	
9 hole peg	Minimum time needed to do all replacements with dominant or non-dominant hand (each 2 trials)	5min/trial	arm/hand function
25-foot walk	Minimum time needed to walk 25 feet (2 trials)	3min/trial	leg function/ambulation
PASAT (3s)	Mental addition of each two consequent digits heard every 3 seconds, and saying it	60 stimuli with 3s intervals	Auditory cognitive function

$$\text{MSFC score} = \{Z_{\text{arm, average}} + Z_{\text{leg, average}} + Z_{\text{cognitive}}\} / 3.0$$

diuretics, penicillamine, theophylline, benzodiazepines, narcotic pain medicine, drugs for anxiety, or antiepileptic drugs, which could potentially affect melatonin level or interfere with measurement up to 3 months before admission, and Expanded Disability Severity Scale (EDSS) score more than 8.²⁶ Likewise, affective disorders, migraine headache and cluster cephalgia which have been reported to be associated with decreased level of melatonin, all were excluded. Patient summary sheets were designed in order to precisely summarize potentially important data in each patient (A summary of covered information is translated and presented in table 1). All patients signed informed consent forms which was prepared according to the declaration of Helsinki and approved by the institutional ethic committee.

Clinical and Cognitive Measures

In addition to neurological history and physical examination, disability was assessed using the standardized scoring system EDSS before any anti-inflammatory intervention. Patients with EDSS greater than 8 were excluded because of their possible inability to complete motor tasks.

All the patients were tested with Persian standardized version of Mini-mental status exam (MMSE).²⁷⁻²⁸ Each patient's overall score out of 30 was considered as the outcome.

Multiple Sclerosis Functional Composite (MSFC) was used to assess the overall disability impact of the disease. We used a combination of different tests including (a) 3s paced auditory serial addition task (PASAT) as a cognitive assessment, (b) timed 9 hole peg test for both hands, and (c) timed 25-foot walk test. This combination of tests has been previously examined by the authors and was shown to not differ from those of widely used tests in the English version.

Using standard norms, each score resulted in a Z-score. The MSFC overall score yielded from averaging these scores. A detailed description of the tests is presented in table 2.

A pen and paper assessment using Persian standardized version of Modified Fatigue Impact Scale (MFIS) for neurologic patient, filled by patients before intervention was used to assess fatigue in patients.²⁹⁻³⁰

Urine 6-Sulphatoxymelatonin Levels

Enzyme-linked immunosorbent assay (ELISA) kits (IBL, Hamburg, Germany) were used to measure the urine 6-sulphatoxymelatonin (aMT6s; aka melatonin sulfate) levels. In brief, according to suggested method of sampling by the kit manufacturer, the patient was asked to empty his/her bladder at 10:00 PM. First morning micturation (i.e. overnight urine sample) before any corticosteroid therapy was collected and aliquoted in sterile polyethylene tubes, then stored at -21°C. Specimen quality was confirmed on the sampling morning by asking the patient the sampling details and subjective quality of sleep. Patients were withdrawn from the study in case of any concurrent abnormal urinalysis report, or any missed overnight sample. Control group included 7 newly hospitalized patients at the same hospital for non-neurological causes. Patients in this group were also carefully interviewed and examined. If there was any doubt about neurological, psychiatric or other medical complications, control subjects were screened with more sophisticated examinations. It is assumed that the stress from hospitalization equally affects both groups and the different serum melatonin reflects the effect of the disease. Ten microliter (µL) of the samples was used according to the protocol. We used semi-automatic ELISA-reader to read for optic density of the plates. Six provided concentration standards were used to

interpolate the cubic spline curve, so the concentration of melatonin for samples could be calculated using millimetric paper.

Statistical Analysis

Data were tested for normality and equality of variances using Kolmogorov-Smirnov and Equal Variance tests, respectively. Considering confirmed normality but unequal variances, Mann-Whitney Rank Sum Test was used to compare patients with control group. Data are presented as median and interquartile range (IQ). The significance level was defined as $p < 0.05$.

Correlations between different numerical outcomes were tested using Pearson Product Moment Correlation test. Again, the significance level was defined as $p < 0.05$.

Table 3. Demographic properties of patients

N	28	10
Age, mean (SD)	34.6 (8.7)	30.2 (7.4)
Sex (M/F)	6/22	2/5
Age of onset, mean (SD)	28.8(9.0)	--
EDSS, median (IQ)	3.5 (2.75-5.5)	--
MSFC, median (IQ)	-1.32 (-1.59:-0.75)	--
MFIS, median (IQ)	16 (6.5-44)	--

M, male; F, female; SD, standard deviation; EDSS, Expanded Disability Severity Scale; MSFC, Multiple Sclerosis Functional Composite (mean Z-score); MFIS, Modified Fatigue Impact Scale; IQ, interquartile range; N/A, not applicable.

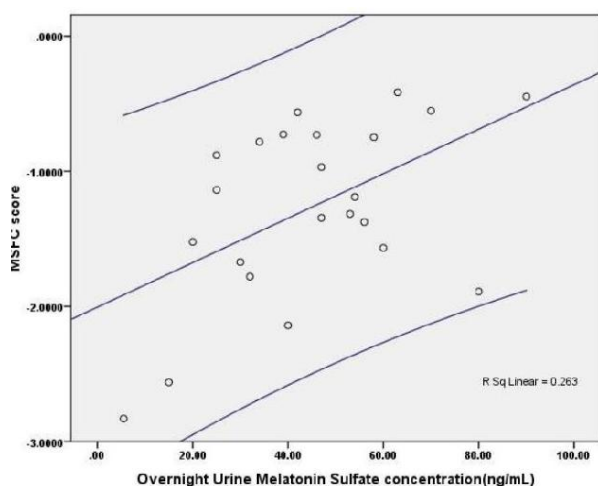


Figure1. Estimated regression line obtained by means of a linear regression model linking MSFC to Urine level of aMT6s levels (R sq linear = 0.263).

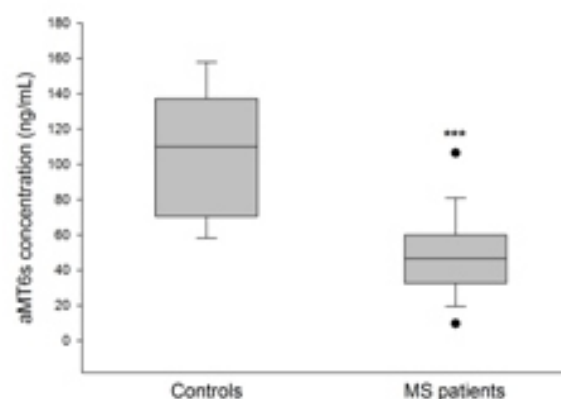


Figure2: Measured concentration of 6-sulfatoxymelatonin (aMT6s) in controls (N=10) and multiple sclerosis (MS) patients (N=28). Boxes and the index lines represent interquartile range and median, respectively. Black dots represent outliers. * $p=0.001$.**

RESULTS

The demographic properties of the sample are presented in table 3. There was no significant difference between MS and control groups for age, sex, and education, according to Mann-Whitney test ($p > 0.05$).

Using the method described above, urine aMT6s levels were measured in patients and controls and results were compared. Patients had median aMT6s levels of 46.5 ng/mL (IQ 33-60 ng/mL), comparing to controls with a median of 110 ng/mL (IQ 70.75-136.25 ng/mL; figure 1). Mann-Whitney rank sum test revealed significant lower levels in MS patients ($U = 18.50$; $p = 0.001$).

Within the patient group, correlations between aMT6s levels and clinical and cognitive data were tested. As revealed by Pearson's correlation coefficient, aMT6s levels were found to significantly correlate with MSFC ($r = 0.607$, $p < 0.001$) [figure 1 & 2] but not with EDSS and MMSE (both $p > 0.05$).

As shown by Pearson's correlation coefficient, MFIS scores inversely correlated with MSFC as well ($r = -0.992$, $p < 0.01$). In other words, patients reporting higher fatigue had lower cognitive performance. No correlation was found for MFIS and EDSS or MMSE (both $p > 0.05$).

DISCUSSION

The importance of melatonin in the MS

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pathogenesis is a topic of debate. Some studies elucidated that melatonin receptor antagonist could ameliorate experimental allergic encephalomyelitis (EAE).³¹ Of interest, a similar modulatory effect was reported by melatonin itself.³² Jankovic et al. showed that rats with early pinealectomy are prone to EAE, while those with late (after 6 weeks age) pinealectomy are protected against EAE induction.³³⁻³⁴ This effect emphasizes on a dual effect of melatonin on thymus in early life, where self tolerance is learnt by T-cells, whereas late life when melatonin is more frequently believed to increase autoimmunity. Previous animal studies demonstrated that thymic tissue, as well as spleen and other lymphoid organs, could be labeled with [125I] iodomelatonin, indicating possible presence of melatonin receptor in lymphoid immune system.³⁵⁻³⁶ More interestingly, the binding site density in thymus membrane shows a diurnal pattern, with maximum density levels at the lowest melatonin serum level (i.e. middle of light period).³⁶ One can assume that a viral agent, a genetic factor or even a traumatic event in MS patients, can affect the pineal gland in childhood similar to the pinealectomy in rats.³³ The exciting evidence to support this hypothesis is that the risk of developing MS after immigration from areas with high prevalence of MS to low prevalence areas remains high only if emigration happens before a median age of 15. Beyond that age, the incidence rate of developing MS gets close to incidence rate at the new region. However moving to high prevalence area after this age does not change the risk of developing MS.^{5,24,38-39} This hypothesis is corroborated by the fact that exposure to a viral agent such as EBV in childhood, probably by affecting pineal body, predisposes the patient to multiple sclerosis in adulthood.³² Likewise, above thesis is compatible with our observations showing lower level of urine aMT6s, as a marker of serum melatonin and thereby pineal gland function, in MS patients when samples taken within relapse period prior to any anti-inflammatory intervention.⁴⁰

Previous studies found that there might be a stronger correlation between brain atrophy and the MS Functional Composite (MSFC) compared to EDSS.⁴¹ Here we found a significant correlation between melatonin level and MSFC score but not between melatonin level and EDSS. Since EDSS represents the severity of the functional disability over the lower extremities, the correlation of aMT6s with MSFC suggests a significant association with a disability index, considering that it

represents patients' cognitive abilities.

In some studies, fatigue is presented as the most common and disabling symptom of the MS patients.⁴²⁻⁴³ It has a major impact on quality of life and performance in MS patients.⁴⁴ Modified Fatigue Impact Scale is developed and recommended by the panel of the Multiple Sclerosis Council for Clinical Practice Guidelines (1988) for clinical and research purposes. MFIS is already translated and normalized in Persian (the most common language spoken in Iran), and its validity and reliability has been confirmed in our research center as well as independent institutes.²⁹ Fatigue was a common symptom in our MS patients. Of note, the MFIS was correlated with MSFC and melatonin level but not EDSS in our study. This is congruent with the current common view that fatigue, as a common MS presentation, alleviated with melatonin administration.⁴³⁻⁴⁴ We also noticed that some of our MS patients who primarily had elevated mood in MMSE screening test, were found to suffer from severe fatigue.

We demonstrated that aMT6s urine levels, as a reliable measure for melatonin production, is significantly lower in MS patients during an acute relapse compared to control subjects. This might help to better understand the pathophysiology of MS and to some extent explain some epidemiologic variations of the MS disease. Of note, our results should be analyzed cautiously. We didn't evaluate simultaneous level of 25-OH Vitamin D. Vitamin D and melatonin interaction is a topic of debate so that recently has been reported that there is a negative correlation between melatonin and vitamin D levels in INF- β treated patients.⁴⁵ Supplementation with 25-OH Vitamin D, as a message of light, was associated with decreased nighttime secretion of melatonin. So, there is not clear that decreased level of melatonin is a primary event which play a role in neurodegenerative inflammation or a secondary event in a setting of inflammation or vitamin D administration. Nonetheless, confirming the neuroprotective effect of melatonin in MS patients by several clinical trials is suggestive for this hypothesis that melatonin dysregulation may be contributed in MS pathogenesis, at least in neurodegenerative stage and axonal loss due to oxidative events. Since the aMT6s urine level correlates with patients cognitive functionality (i.e. MSFC scores), there might be new hope in developing a quantitative and objective measure to assess the MS severity especially in neurodegenerative stage disease.

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