The Role of Melatonin in the Pathogenesis of Multiple Sclerosis: A Case-Control Study

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Date of Submission: Feb 18, 2013

Date of Acceptance: Feb 23, 2013

How to cite this article: Ghorbani A, Salari M, Shaygannejad V, Norouzi R. The role of melatonin in the pathogenesis of multiple sclerosis: A case-control study. Int J Prev Med 2013;Suppl 2: S180-4.

Original Article

ABSTRACT

Background: The association between the prevalence of multiple sclerosis (MS) and latitude gradient indicates the importance of environmental factors in MS susceptibility. Sunlight's ultraviolet radiation, its ability to influence melatonin, and an imbalance of melatonin in the central nervous system (CNS) may be involved in this process.

Methods: This case-control study was conducted in Isfahan MS Society (IMSS), Isfahan, Iran. Enrollment was limited to patients with MS referring to the MS clinic of Alzahra and Kashani hospital during January and February 2012.

Results: Thirty-five patients with MS and 35 healthy individuals were included in our study. The melatonin levels were analyzed using enzyme-linked immunosorbent assay (ELISA) kits. There was no significant difference between saliva melatonin level of two groups (patients and healthy individuals) ($P = 0.417$); however, after controlling the effect of age, a significant difference ($P = 0.022$) was found.

Conclusions: In the present study, it is proposed that environmental conditions in Isfahan city might have increased the susceptibility to MS, but more studies in different parts of the world are needed to evaluate this claim.

Keywords: Environmental factors, melatonin, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is a progressive disorder of the central nervous system (CNS) characterized inflammation and demyelination with an unknown cause.[1-3] The relationship between the prevalence of MS and latitude gradient demonstrates the important role of environmental factors in MS susceptibility.[4,5] A previous study had considered sunlight's ultraviolet radiation and how it affects melatonin[1] as well as the imbalance of melatonin in the CNS as the probable factors involved in this process.[4]

Melatonin (*N*-acetyl-5-methoxytryptamine) is a natural hormone secreted by the pineal gland,[6] the suprachiasmatic nucleus receives input from the retina depending on the sunlight level, and it sends feedback to the pineal gland, which regulates melatonin release. Also, it is produced by skin and across the blood–brain barrier.[7] Moreover, melatonin is possibly a ligand

to the retinoic acid-related orphan alpha (ROR). Probably, the paracrine action of local melatonin produced by retina influences the retinal ganglion cells and any other local Th17 cell, which is probably present. While the ROR-alpha receptor can be found in different immune cell lines, the ROR-alpha exists in Th17 cells and may be central to its survival and Th17 secretion.[8,9] Lower levels of sunlight correlate with higher levels of melatonin,[7] when melatonin can be produced and locally released into retinal ganglion cells and Th17 cells, and its circulating level can increase. This would result in higher levels of Th17 cell and higher circulating levels of IL-17. Clinically and symptomatically, this would result in a greater lesion load and volume, because Th17 cells are involved in lesion formation and immune cell recruitment to lesion area.[8,9] On the other hand, several studies have proven that melatonin decreases chronic and acute inflammation.[10,11]

Previous study suggests that melatonin deficiency can play an important role in fatigue,[12] mood disorder,[13] fluctuation of symptoms,[14] and triglyceride level[15] in MS.

However, as indicated above, it is not obvious if the increasing or decreasing level of melatonin plays a role in the pathogenesis of MS, and there is no sufficient data indicating that melatonin level can be different in MS and healthy patients. Although there was a considerable change in the concentration, the percentage of binding of melatonin to plasma proteins did not vary throughout the night. On the other hand, free circulation was totally dependent on salivary melatonin level[16] and, therefore, salivary melatonin level can be a better indicator for melatonin changes in human body; however, previous studies did not measure salivary melatonin level in MS patients.

Therefore, in this case-control study, we investigated the saliva melatonin level in patients with MS and compared them to the control group with regard to sleep quality index (SQI) in each group.

METHODS

Subjects

This case-control study was conducted in Isfahan MS Society (IMSS), Isfahan, Iran. Enrollment was limited to patients with MS referring to the MS clinic of Alzahra and Kashani hospital for routine follow-up between January and February 2012, for preventing day time variation. All patients were from Isfahan and lived in the same city in order to avoid race and climate effects to interfere with the results of this study.

Patients were eligible for inclusion if they were 15-55 years of age with definite MS according to the 2010 revision McDonald criteria and an EDSS <6.

Patients with the following symptoms were excluded: History of endocrine disorder, depression, seizure, and stroke; pregnant patients; and patients who had used a hormone within last 30 days.

All patients filled in the Sleep Quality form that was prepared by sleep medicine Institute of Pittsburgh University and SQI was calculated based on the forms. Based on SQI, sleep is said to be in good quality if the patient gets ≤5 score and poor quality if the score is 6-21.

In this study, 35 patients were included along with another 35 healthy individuals with comparable age, sex, and SQI as the control group.

Saliva sample collection

Light severely affects melatonin secretion, therefore, we collected saliva samples under dim conditions.[17] Subjects were asked to rinse their mouth thoroughly 15 min before sample collection and to collect their saliva in clear sterile plastic tubes at 6.00 PM (passive saliva collection). All saliva samples were centrifuged at 1500 × g for 5 min at room temperature and then kept at 2-8°C until use.

The melatonin levels were analyzed twice using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Direct Saliva Melatonin ELISA; Bühlmann Laboratories, Allschwil, Switzerland), and the average values were used for analyzing the results. The kit sensitivity was 0.5 pg/mL. The intra-and inter-assay coefficients of variation were 12.6% and 22.9%, respectively.

Statistics

Statistical analyses were done using SPSS version 20 (SPSS, Chicago, IL, USA).

The Mann-Whitney U test and paired sample *t*-test was used for the statistical analysis. A P value <0.05 was considered statistically significant.

RESULTS

Thirty-five patients with MS and 35 healthy individuals were included during the study period.

Table 1 shows the demographic characteristics of cases and controls as well as the clinical characteristics of patients. Both groups had 6 males and 29 females, with an average age of 32.74 (SD = 9.23) years; the average age of controls was 31.14 (SD = 1.31) years. All patients had relapsing remitting type of MS, the first attack for 40% of them was optic neuritis, for 22.85% motor presentation, for 14.28% sensory, for 14.28% diplopia, and for 8.57% ataxia.

There was no significant difference between saliva melatonin level of two groups (patients and healthy individual) (P = 0.417), but when we controlled the effect of age, we found significant difference between them (P = 0.022).

Table 1: Demographic and clinical characteristics

Variable	Patients	Controls
Age	32.74±9.23 (year)	31.14±1.31 (year)
Men/ women	6/29	6/29
Disease duration	5.11±4.50 (year)	
First attack (%)	ON=14 (40) Motor=8 (22.8) Diplopia=5 (14.28) Sensory=5 (14.28) Ataxia=3 (8.57)	
EDSS	1.68±1.27	
Treatment (%)	*Interferon beta 1-a (Cinnovex)=17 (48.57) *Interferon beta 1-a (Recigen)=3 (8.57) *Interferon beta 1-a (Rebif)=2 (5.71) *Interferon beta 1-b Betaferon=3 (8.57) *Mitoxantrone=3 (8.57) *No treatment=7 (20)	
SQI	7.66±4.57	6.02±2.39
Good/ poor sleep quality	14/21	14/21
Melatonin level	1.70±1.63 (pg/ml)	2.34±2.73 (pg/ml)

EDSS=Expanded disability status scale, SQI=Sleep quality index

Also, there was no difference between melatonin level between the two groups with poor and good quality sleep (P = 0.349).

Although mean melatonin level was different in males (2.58 ± 4.12) and females (1.90 ± 1.67), no statistically significant difference was observed between them (P = 0.345).

Finally, there was no difference between melatonin levels in patients with different clinical characteristics such as disease duration, first attack, treatment type, and EDSS.

DISCUSSION

According to the results of this case-control study, saliva melatonin level was significantly low among patients with MS after controlling the effect of age. However, the level of melatonin was not affected by the duration of disease, treatment, EDSS, and sex. Also, we did not find any relation between melatonin levels and sleep quality.

Results suggested that melatonin can have a role in neurogenesis,[18,19] immunomodulation,[18,19] improving immune defense,[20] eliminating free radicals,[21-24] intervening in lipid metabolism,[25,26] and neuroprotection.[4]

As mentioned before, due to the lack of sunlight in the high altitudes, melatonin level raises and triggers immune system; but, this conflicts the previously found result that melatonin can be neuroprotective and antinflamatory. Therefore more investigations are needed to clear the exact role of melatonin in inflammatory processes such as MS.

In a recently published case-control, Melamud *et al*., reported significantly decreased levels of 6-sulphatoxy-melatonin (6-SMT) and disrupted circadian regulation of its secretion, which were increased with IFN-β treatment, caused by fatigue. Sleep quality in the MS group was significantly lower than in controls.[12] However, to the best of our knowledge, there is no report from case-control study that evaluates saliva melatonin level based on SQI.

Sandyk *et al*.,[27] suggest that a specific association between nocturnal melatonin secretion and duration of clinical symptoms of MS and suggest that the activity of the pineal gland may decline with progression of the disease. However, we did not find this association.

In the pineal gland of the CNS, 5-HT is the metabolic precursor for melatonin, and it is catalyzed by the melatonin synthetic enzyme serotonin N-acetyltransferase to N-acetlserotonin and then metabolized by hydroxyindole o-methyltransferase to produce the melatonin. Supra abundant synthesis of melatonin in individuals living in high altitude areas result in deficiency in 5-HT, which originally has a limited metabolic source;[4] therefore, we suggest that 5-HT acts as a neuroprotective and antinflamatory agent and that melatonin plays a role in MS pathogenesis.

Another hypothesis for describing this controversial finding is that, in the initial phase, melatonin raises and triggers immune system, but when disease progresses due to pineal gland dysfunction, melatonin secretion decreases. But, based on our results, regarding the low level of melatonin, in our population-normal level was >10 pg/ml, but the average melatonin level in normal population was 2.34 ± 2.73 pg/ml. Also, as Isfahan is a medium to high risk area for MS,[28] we suggest that dysregulation in diurnal variation of melatonin might increase the susceptibility for MS. Although the level of saliva melatonin was low, melatonin may rise to very high levels during the day and cause immune reaction, on the other hand, maintaining adequate level of melatonin may protect the body against inflammatory process and act as a neuroprotective agent; in MS patients, this maintenance level is low. Thus, oral use of melatonin supplement may provide adequate level of melatonin and protect again periodic rise in its level.

Consequently, we suggest that environmental conditions in Isfahan city may increase susceptibility to MS.

ACKNOWLEDGMENTS

We greatly appreciate the staff of MS clinic and Isfahan University of Medical Science.

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Source of Support: Isfahan University of Medical Science,
Conflict of Interest: None declared.