Headache
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ISSN 0017-8748
doi: 10.1111/j.1526-4610.2009.01547.x
Published by Wiley Periodicals, Inc.

Research Submission

Urinary 6-Sulphatoxymelatonin Levels Are Depressed in Chronic Migraine and Several Comorbidities

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Objective.—To assess urinary 6-sulphatoxymelatonin levels in a large consecutive series of patients with migraine and several comorbidities (chronic fatigue, fibromyalgia, insomnia, anxiety, and depression) as compared with controls.

Background.—Urine analysis is widely used as a measure of melatonin secretion, as it is correlated with the nocturnal profile of plasma melatonin secretion. Melatonin has critical functions in human physiology and substantial evidence points to its importance in the regulation of circadian rhythms, sleep, and headache disorders.

Methods.—Urine samples were collected into a single plastic container over a 12-hour period from 8:00 PM to 8:00 AM of the next day, and 6-sulphatoxymelatonin was measured by quantitative ELISA. All of the patients were given a detailed questionnaire about headaches and additionally answered the following questionnaires: Chalder fatigue questionnaire, Epworth somnolence questionnaire, State-Trait Anxiety Inventory, and the Beck Depression Inventory.

Results.—A total of 220 subjects were evaluated – 73 (33%) had episodic migraine, 73 (33%) had chronic migraine, and 74 (34%) were enrolled as control subjects. There was a strong correlation between the concentration of 6-sulphatoxymelatonin detected and chronic migraine. Regarding the comorbidities, this study objectively demonstrates an inverse relationship between 6-sulphatoxymelatonin levels and depression, anxiety, and fatigue.

Conclusions.—To our knowledge, this is the first study to evaluate the relationship between the urinary concentration of melatonin and migraine comorbidities. These results support hypothalamic involvement in migraine pathophysiology.

Key words: pathophysiology, hypothalamus, melatonin, migraine, comorbidities

(*Headache* 2010;50:413-419)

The term "comorbidity" refers to the non-casual association between 2 or more morbid conditions in 1 person.[1] In migraine, the study of comorbidities is potentially important for many reasons: (1) the presence of comorbidities has significant impact on the correct diagnosis of migraine due to symptomatological concurrency; (2) their occurrence may either impose therapeutic restrictions or create new treatment opportunities; (3) they may increase the impact of migraine and the search for medical resources to treat them; (4) the study of comorbidities may shed light on the physiopathological mechanisms underlying migraine.[1]

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Funding Source: This study was sponsored by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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Accepted for publication August 13, 2009.

Conflict of Interest: The authors report no conflicts of interest.

The hypothalamus is thought to play an important role in migraine pathogenesis, as well as in the mechanisms involved in the pathogenesis of several comorbid conditions frequently associated with migraine, such as chronic fatigue, sleep disorders, anxiety, depression, and fibromyalgia.[2] It is possible that periodic central disturbances of hypothalamic activity or its labile threshold could account for the observed periodicity of migraine attacks. It also provides a mechanism by which emotional disturbances could be mediated by pathways from the limbic system to the hypothalamus.[3] Almost 60% of patients with migraine report symptoms of elation, irritability, depression, hunger, thirst, or drowsiness during the 24 hours preceding a headache,[4] which also suggests a hypothalamic site of origin.

Melatonin is absent during the day in human beings,[5] and its nocturnal secretion is the main biological event signaling nighttime to the organism.[6] Once melatonin is synthesized in the pineal gland, it is quickly released, generating a blood-melatonin rhythm reminiscent of that seen in the gland itself. Being an amphiphilic molecule, melatonin is capable of entering every cell in the organism; additionally, it readily crosses all morphophysiological barriers, including the blood-brain barrier and the placenta. Melatonin is enzymatically degraded in the liver to 6-hydroxymelatonin and finally excreted in the urine as 6-sulphatoxymelatonin (aMT6s).[6] Urine analysis is widely used as a measure of melatonin secretion, as it is correlated with the nocturnal profile of plasma melatonin secretion.[7] The advantages of urinary metabolite measurement are substantial, particularly the non-invasive nature of fluid collection, the ability to perform very long-term studies, and the feasibility of studies in circumstances where blood sampling is difficult.

Several studies have addressed the importance of melatonin in the pathogenesis of migraine, and they state in agreement that low levels of melatonin are found in patients who experience migraine,[8] chronic migraine (CM),[2] and migraine attacks regardless of the type of the migraine.[9] These data prompted us to test the hypothesis that melatonin levels are decreased in patients with migraine and its comorbidities (fatigue, sleep disorders, anxiety, depression, and fibromyalgia) by assessing aMT6s levels in a large consecutive series of patients, compared with controls.

METHODS

The study was performed at Albert Einstein Hospital – São Paulo, Brazil. The inclusion criteria were as follows: patients with migraine and control subjects of both sexes, aged 18-65 years, able to understand the consent form and the explanations given by the research team, and with satisfactory diuresis. Migraine was defined according to International Classification of Headache Disorders diagnostic criteria, Second Edition (ICHD-2)[10] and CM was defined according to 2006 appendix criteria.[11] The control subjects did not suffer from migraine or any other primary headache. The exclusion criteria were: chronic diseases, unstable medical condition, secondary headache, continuous usage of any kind of medication, drug addiction, or abusive alcohol use. The patients that remained aroused with light exposure during the night of urine collection were also excluded. All subjects provided written consent for the experimental procedure, which was approved by the local ethics committee.

All of the patients were administered a detailed headache questionnaire and furthermore answered the following questionnaires: Chalder fatigue questionnaire, Epworth somnolence questionnaire, IDATE (State-Trait Anxiety Inventory), and the BDI (Beck Depression Inventory).

The symptoms of fatigue were evaluated based on the Chalder fatigue questionnaire. According to this questionnaire, the sum of several items related to fatigue symptoms are computed for each subject, with the higher values indicative of higher severity of fatigue.[12]

The diagnosis of fibromyalgia was based on clinical criteria determined by the American College of Rheumatology in 1990[13] and the subjects were classified as positive or not for the diagnosis based on the fulfillment of those criteria.

There is not a universally accepted definition for insomnia, and clinical researches use objective criteria that vary widely; however, sleep latency of higher than 30 minutes and sleep efficiency lower than 80% are frequently applied. The most widely accepted definition of insomnia includes the following symptoms: difficulty in maintaining sleep, bad quality of sleep, or an insufficient quantity of sleep. These complaints must be accompanied by an interference with normal diurnal function. To define insomnia in this study, the criteria described by Peres et al[2] were applied. To evaluate diurnal sleepiness, the Epworth somnolence scale[14] was applied to each subject. This scale is based on the probability of 1 subject falling asleep during typical daily situations, with scores higher than 10 associated with a diagnosis of excessive diurnal sleepiness.

To evaluate anxiety, all the subjects were asked to fill out the IDATE-T and IDATE-E scales. There are considered reliable scales with which to assess a person's disposition and state of anxiety, and to measure how those of a relatively stable disposition respond to stressful situations.[15] The higher the values obtained for each subject were, the higher the anxiety levels of that person were considered.

To evaluate major depression, the BDI was applied. The BDI is used as an instrument to quantify behavioral manifestations of depression, with higher scores associated with higher levels of depression.[16]

All of the results from experimental patients were compared with controls. The control subjects did not suffer from migraine or any other primary headache.

Subjects were asked to collect urine in a plastic container over a 12-hour period spanning 8:00 PM to 8:00 AM of the next day. Female patients were advised to collect the samples at least 5 days away from the beginning or the end of menses. All patients were advised not to collect urine samples during migraine attacks.

Sample volumes were recorded and 5-mL aliquots were taken and stored frozen at –20°C until analysis. No special precautions were necessary during sample collection as aMT6s has been found to be extremely stable without preservative for at least 5 days at room temperature and for at least 2 years at –20°C.[17] The concentration of aMT6s was measured by quantitative ELISA (Bühlmann Laboratories, Allschwil, Switzerland). A total of 50 μL of urine sample diluted by 1:200 was assayed directly; where necessary, samples were re-assayed at a dilution of 1:400.

Data Analysis.—The chi-square test (χ^2) (without Yates correction) was used for categorical data comparisons. Mean differences of continuous measurements were tested by the one-way analysis of variance – ANOVA (F). Whenever the ANOVA showed significant differences, Bonferroni's multiple comparison test was used to verify which groups the differences were found between. The Pearson's product-moment correlation coefficient (r) was used to assess the relationship between 2 continuous variables, and point-biserial correlation coefficients (r_{pb}) were used to assess the relationship between aMT6 and dichotomic categorical variables. A *P* value of less than .05 was considered to indicate statistical significance; all tests were 2-tailed. Ninety-five percent confidence intervals (CI) were calculated for the difference between means and the regression coefficients. All statistical analyses were performed on a personal computer running the statistical package SPSS 11.5.1 for Windows.

RESULTS

A total of 268 subjects were referred to the initial evaluation. From this total, 48 were excluded: 12 patients did not correctly provide their urine samples, 3 refused to take part in the study, 19 did not give reliable headache information, and 17 had at least 1 exclusion criteria. The remaining 220 subjects were in accordance with inclusion criteria. Among them, 73 (33%) had episodic migraine (EM), 73 (33%) had CM, and 74 (34%) were enrolled as control (C) subjects.

There were not statistically significant differences between the 3 groups regarding sex [C = 78% vs EM = 74% vs CM = 88% female, $\chi^2(2) = 4.46$, $P = .107$] or age [C = 38.4 ± 9.0, EM = 37.7 ± 11.6, CM = 39.3 ± 10.8, $F(2, 217) = .44$, $P = .647$].

aMT6 and Migraine.—The levels of aMT6s were significantly different among the 3 groups of subjects [C = 42.6 ± 27.9, EM = 35.2 ± 23.7, CM = 26.0 ± 20.3, $F(2, 217) = 8.64$; $P < .001$]. However, the Bonferroni's post hoc test showed that this difference was significant only between the CM group and controls (95% CI = –26.2 to –6.9, $P < .001$).

The Table summarizes the correlations between aMT6 and the following comorbidities: depression,

Table.—Correlations Between 6-Sulphatoxymelatonin and Comorbidities in All Subjects (Including Controls)

	n	r/r_{pb}	*P*
IDATE-E	170	–0.32*	<.001
IDATE-T	166	–0.36*	<.001
Beck	167	–0.37*	<.001
Epworth	171	–0.14*	.060
Chalder	171	–0.27*	<.001
Insomnia	143	–0.03**	.753
Fibromyalgia	146	–0.08**	.323

*r; **rpb.
IDATE, State-Trait Anxiety Inventory.

anxiety, insomnia, daily somnolence, fatigue, and fibromyalgia.

DISCUSSION

Claustrat et al[8] were the first to demonstrate lower plasma melatonin levels in blood samples from migraine patients compared with controls. Migraine patients without depression also had lower levels than the controls, but migraineurs with superimposed depression exhibited the greatest melatonin deficiency.

Murialdo et al[18] found nocturnal urinary melatonin to be significantly decreased throughout the ovarian cycle of migraine patients without aura as compared with healthy controls. During the luteal phase, when melatonin levels should normally increase, migraine patients showed a less pronounced change than was seen in the controls. Excretion of melatonin was further decreased when patients suffered a migraine attack.

Additionally, Brun et al[19] studied urinary melatonin level changes associated with menses in women suffering from migraine without aura attacks. Melatonin levels throughout the cycle were significantly lower in the migraine patients than in controls. In the control group, melatonin excretion increased significantly from the follicular to the luteal phase, whereas no difference was observed in the migraine group.

To our knowledge, this is the first study to verify diminished levels of aMT6s in a large consecutive series of patients with chronic but not EM. It is possible that a hypothalamic disturbance could lead to both migraine and the diminished secretion of melatonin, as was postulated by Zurak.[20] In addition, lower levels of melatonin could play a role in the pathogenesis of migraine, for instance by enhancing the inhibitory effects of Gamma amino butyric acid (GABA) in the central nervous system (CNS),[21] modulating serotonin receptors,[22] and inhibiting the synthesis of prostaglandin.[23] Thus, the lower levels of melatonin observed in this study may contribute both to the generation of migraine and to the perpetuation of the morbid condition.

It has been suggested that should neuronal hyperexcitability and cortical spreading depression during migraine attacks underlie the pathophysiology of the disorder, then the phenomenon of aura would not represent the initiating event but rather would be initiated by subcortical changes.[24] The suprachiasmatic nucleus is a possible subcortical origin of this change, suggesting that a migraine attack might be an attempt at a drastic resetting of the biological clock in response to a previous decompensation.[20] The strong correlation between CM and aMT6 levels could reflect either a higher frequency of migraine attacks or an increased level of hypothalamic disturbance.

There was a strong inverse correlation between aMT6s levels and depression across all subjects, with those who had higher concentrations of aMT6s presenting lower levels of depression. This finding corroborates studies that demonstrated lower levels of melatonin in patients with depression,[25] and supports the hypothesis that depression is a syndrome that is associated with low melatonin secretion.[26,27]

Studies addressing the role of melatonin are important to understanding the pathophysiological processes of both depression and migraine. Seasonal depression is thought to be caused by alterations in the secretion of melatonin since it is influenced by light/dark cycle variations during the year[28] and the administration of melatonin and its analogous have been shown to be beneficial in both animal models of depression and patients with this condition.[29,30] As melatonin is considered an indirect marker of adrenergic function, its possible relation to depression becomes clear when one considers that the adrenergic system is involved in the pathogenesis of depression.

6-sulphatoxymelatonin levels were also strongly related to anxiety levels across all subjects. According to our results, the subjects presenting higher scores on the IDATE-T and IDATE-E scales (meaning higher levels of anxiety) had lower levels of aMT6s. To date, this is also the first study to demonstrate a relationship between anxiety and melatonin in humans. It is currently known that melatonin receptors are present in the cerebral nuclei that take part in behavioral control. Animal models of anxiety have been used to evaluate the effect of melatonin administration. In one of these studies, it was shown that the administration of melatonin reduced neophobic behavior, and that the compound S22153 (a melatonin antagonist) blocked this effect.[31] It is thought that melatonin exerts a role in the pathogenesis of primary anxiety disorders as well as in associated diseases, such as the migraine comorbidities.

A relationship between aMT6s levels and fatigue was also revealed when the results obtained using the Chalder fatigue scale were analyzed. Our results showed that subjects with a higher urinary concentration of aMT6s reported lower levels of fatigue. This corroborates the findings of other studies that also showed diminished levels of melatonin in patients who presented high levels of fatigue, although the previous findings were not as clear as those shown in our results.[2]

6-sulphatoxymelatonin levels did not differ statistically among patients with or without the diagnosis of insomnia, a finding that is not in accordance with previous literature data. Low levels of melatonin are usually found in patients with insomnia, particularly elderly patients or those presenting circadian rhythm disorders, with several reports demonstrating beneficial effects of melatonin in the treatment of insomnia.[32] Among patients with CM, it has previously been shown that in those who also present the diagnosis of insomnia, the levels of melatonin are significantly lower than in those who do not. This may indicate that the insomnia in those patients may be caused by a chronobiological disorder, marked by an abnormal secretion of melatonin.[2]

Finally, our study showed that the levels of aMT6s did not differ between patients with or without the diagnosis of fibromyalgia. Abnormal levels of melatonin in patients with the diagnosis of fibromyalgia has been reported in the literature, with the fibromyalgic patients exhibiting lower levels of melatonin when compared with controls.[33] However, there was a failure in an attempt to replicate these results.[34]

Nevertheless, there are possible explanations linking a rheumatological disease with a pineal gland dysfunction. Fibromyalgic patients present severe sleep disturbances, and melatonin is known to be important in the regulation of sleep. Thus it is possible that the sleep problems among patients with fibromyalgia are related to altered melatonin secretion.[35] Another possible explanation for the lower melatonin levels in these patients is the fact that serotonin, a precursor of melatonin, is synthesized from tryptofan, which the fibromyalgic population is deficient in the absorption of.[35]

Treatment of headache disorders with melatonin is promising, particularly in cluster headaches, hypnic headaches, indomethacin-responsive headaches, and migraine. Insomnia in headache patients is the most likely associated condition in migraine to respond to melatonin therapy and decreased melatonin levels may predict response to melatonin treatment. Other chronobiotic agents, such as melatonin receptor agonists, light therapy, and magnetic fields can also be tested.[6]

CONCLUSION

To our knowledge, this is the first study thoroughly addressing the association between melatonin levels and several migraine comorbidities. This is also the first report to demonstrate objectively that there is an inverse relationship between aMT6s levels with CM, depression, anxiety, and fatigue levels. These results support hypothalamic involvement in migraine pathophysiology.

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