Original Article

Melatonin and Epilepsy: Clinical and Laboratory Study

Ahmed Talaat El-Ghoneimy[1], Kamel Hammouda[1], Olfat Gamil Shaker[2], Nermeen Adel[1], Amr Hasan[1]

Departments of Neurology[1], Medical Biochemistry[2], Cairo University; Egypt

ABSTRACT

Background: The pineal gland and melatonin have a major influence on the control of brain electrical activity and were shown to be involved in seizure and sleep mechanisms. **Objective:** To detect possible melatonin alteration in epileptic patients and to examine the relationship between type of epilepsy, seizure control and urinary 6-sulphatoxymelatonin. **Methods:** This study was done on 61 Subjects; 44 patients and 17 controls that were matched regarding age, sex and pubertal stages, Patients' group was divided into 2 subgroups; one group including 26 patients with non-refractory epilepsy and another group including 18 patients with refractory epilepsy, Also patients' group was subdivided into either patients with focal; focal with generalized epilepsy; generalized epilepsy. All subjects were evaluated using Sleep Disturbance Scale for Children questionnaire and a self-administered rating scale for pubertal development and 12 hours urinary 6-hydroxymelatonin sulfate measurement. **Results:** Urinary 6-hydroxymelatonin sulfate level in patients was significantly lower than in the control group (20468.24±19479.38 ng/12 hours urine, 61948.38±38734.98 ng/12 hours urine $p<0.005$). There was no statistically significant difference between focal or focal with secondary generalization epilepsy and generalized epilepsy groups as regards urinary 6 hydroxymelatonin sulfate levels. Raw and T sleep questionnaire scores. There was no statistically significant difference between refractory and non-refractory epilepsy patients as regards urinary 6 hydroxymelatonin sulfate levels, raw and T sleep questionnaire scores. **Conclusion:** Urinary 6 hydroxymelatonin sulfate level was lower in epileptic patients compared to the control group. Type of epilepsy and whether it is refractory or not did not affect melatonin level. **[Egypt J Neurol Psychiat Neurosurg. 2012; 49(4): 387-392]**

Key Words: Melatonin, Epilepsy

INTRODUCTION

Melatonin is a widely occurring neurotransmitter; chemically it is N-acetyl- 5 methoxytryptamine, a derivative of serotonin, which in turn is derived from the amino acid tryptophan[1].

Experimental evidence indicates that the pineal gland and melatonin have a major influence on the control of brain electrical activity and have been shown to be involved in seizure and sleep mechanisms[2].

Melatonin has anticonvulsant properties; it seems to play a role in seizure control and sleep regulation in patients with epilepsy[3].

Melatonin interacts with neurons, the GABA benzodiazepine receptor complex being a likely effector site, the potentiation of this inhibitory neurotransmitter system may explain the anticonvulsant activity of melatonin[4-6]. It also exerts neuroprotective effect due to its antioxidant, anti-excitotoxic, and free radical scavenging properties within the central nervous system[7].

Correspondence to Amr Hasan El Sayed Mohammed, Department of Neurology, Cairo University, Egypt.
Tel.: +201006060809 e-mail: amrneuro77@yahoo.com

The aim of this work is to detect the possible melatonin alteration in epileptic patients and to examine the relationship between type of epilepsy, seizure control and urinary 6-sulphatoxymelatonin [metabolite of melatonin in urine].

SUBJECTS AND METHODS

Subjects

This case-control study was conducted on 61 Egyptian subjects (44 epileptic patients and 17 normal control subjects) recruited from the epilepsy outpatient clinic of Kasr Al-Aini Hospitals in the period from June to December 2010.

The 44 patients (study group or group I) included 18 females and 26 males, their ages ranged from 7 years to 18 years, with mean 15.14 (±2.45) years. The control group (group II) consisted of 17 normal subjects, 6 females and 11 males, their ages ranged from 10 years to 18 years, with mean 13.82 (±2.74) years.

The study group (group I) was divided into 2 subgroups; (group Ia) included patients with focal or

focal with secondary generalization and (group Ib) that included patients with generalized epilepsy according to 1989 report of the Commission of Classification and Terminology of the International League Against Epilepsy (ILAE, 1989).

The study group (group I) was also divided into 2 subgroups; (group Ic) that included patients with non-refractory epilepsy, it included 26 (59.1%) patients and (group Id) that included patients with refractory epilepsy (those who fail to achieve seizure freedom for 12 months or those who fail to respond to first AED after 2 years of onset of epilepsy)[8,9], it included 18 patients (40.9%).

Research Institute (NHTMRI-IRB) approval was taken and a written consent was filled by the patients or their parents.

We excluded from our study:

1. Patients using sedative-hypnotic drugs, psychoactive drugs ,stimulant medications, corticosteroids or other immunosuppressant drugs, also caffeine was prohibited for 24 hours before the testing period.
2. Patients with symptomatic epilepsy, immune disorders, lymphoproliferative disorders.

Methods

1- Thorough **history taking** and neurological examination.

2- Questionnaires:

A. Self-administered rating scale for pubertal development :consisting of five questions which are used to classify our population into either being prepubertal, early pubertal, mid pubertal, late pubertal and post pubertal[10].

B. Sleep was assessed with the Sleep Disturbance Scale for Children (SDSC) questionnaire, Parents were asked to recall the child's sleep during the previous 6 months. The SDSC provides six sleep problems factors grouped from 26 items: disorders of initiating and maintaining sleep (DIMS), sleep breathing disorders (SBDs), disorders of arousal (DA), sleep–wake transition disorders (SWTDs), disorders of excessive somnolence (DOES),and sleep hyperhidrosis (SHY). It also provides a total SDSC score. All sleep factor scores were transformed into age-specific T-scores. T-score of >70 and raw score of >39 were considered pathological. The SDSC in Arabic language was developed by translation and back translation with permission of the original author[11].

3- Urine collection:

Measurement began following an interval of at least 24 hours seizure-free, nocturnal urine was collected over a 12-hour period spanning 8:00 p.m. to 8:00 a.m. of the next day, and as melatonin secretion can be suppressed by exposure to light, parents were asked to report for the night of urine collection if their child was sleeping with the light on and if he or she got up during this night and turned on the light. On the night of urine collection, all subjects (group I and II) went to bed with lights off and none of them was exposed to light until their morning wake – up.

4- Biochemical method:

Determination of melatonin sulfate (synonyms: 6-hydroxymelatonin sulfate, 6-sulfatoxymelatonin) in human urine was measured by quantitative ELISA. Melatonin sulfate in human urine was detected using Enzyme immunoassay kit which was supplied by IBL international GMBP (Hamburg, Germany).

Statistical Analysis

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics: mean, standard deviation, median, minimal and maximum values for quantitative variables and number and percentage for qualitative values. Statistical differences between groups were tested using Chi Square test for qualitative variables, independent sample T test for quantitative normally distributed variables while nonparametric Mann Whitney test and correlations were done to test for linear relations between variables. P-values less than or equal to 0.05 were considered statistically significant.

RESULTS

Both groups, study group (group I) and control group (group II) were matched as regards age, gender, stage of puberty and sleep questionnaire scores.

I) Comparative Data:

A) 6-hydroxymelatonin sulfate:

In the patients group the 6 hydroxymelatonin sulfate ranged from 1044 to 77214 ng/12 hours urine with mean 20468.24±19479.38 SD and in the control group it ranged from 5460 to 127500 ng/12 hours urine with mean 61948.38±38734.98, there was statistically significance difference between the patients and the control groups (p=<0.005).

B) Type of epilepsy:

Comparison between the 2 subgroups (group Ia) that included patients with focal or focal with secondary generalization and (group Ib) that included patients with generalized epilepsy revealed statistically no significant difference as regards age, gender, type of epilepsy, puberty scale, sleep questionnaire scores.

There was also no statistically significant difference in urinary 6-hydroxymelatonin sulfate between the 2 groups (p=0.859) (Tables 1, 2 and 3).

Refractory and Non-refractory epilepsy:

Comparison between the 2 subgroups (Group Ic) that included non-refractory epilepsy group and (Group Id) that included refractory epilepsy group as regards age, gender, type of epilepsy, type and number of medications, puberty scale scores, sleep questionnaire scores and 6-hydroxymelatonin sulfate levels revealed that there was no statistically significant difference between both groups, but the refractory group had significantly longer duration of epilepsy than the non-refractory group (p=0.029) (Tables 4 and 5).

II. Correlations:

There was a negative correlation between age, puberty scale scores, duration of epilepsy, sleep questionnaire scores (raw and T scores) and 6-hydroxymelatonin sulfate levels but all were not statistically significant (Table 7).

Table 1. Comparative data between patients with focal or focal with secondary generalization epilepsies (Group Ia) and those with generalized epilepsy (Group Ib).

	Group Ia (n = 19)	Group Ib (n = 25)	P-value
Age (mean±SD) in years	15.684 ± 2.29	14.72±2.52	0.199
Gender			
Females	8 (44.4%)	10 (55.6%)	1.00
Males	11 (42.3 %)	15 (57.7 %)	
Sleep questionnaire total raw score	27.21 ± 1.32	27.76 ± 1.54	0.187
Sleep questionnaire total Tscore	39.89 ± 1.85	40.56 ± 2.04	0.183
Duration of epilepsy in years	6.00 ± 4.02	4.08 ± 2.38	0.139
6-hydroxymelatonin sulfate (ng/12 hours urine)	19706.16± 16861.79	21470.97± 22923.64`	0.859

Table 2. Raw sleep questionnaire score of patients with focal or focal with secondary generalization epilepsies (group Ia) and those with generalized epilepsy (group Ib).

	Group Ia (n = 19)	Group Ib (n = 25)	P value
DIMS	7.16 ± 0.50	7.28 ± 0.54	0.289
SBD	3.05 ± 0.23	3.12 ± 0.33	0.447
DA	3.00 ± 0.00	3.00 ± 0.00	1.00
SWTD	6.16 ± 0.37	6.32 ± 0.63	0.448
DOES	5.84 ± 0.9	6.00 ± 1.0	0.615
SHY	2.00 ± 0.00	2.00 ± 0.00	1.00
Total	**27.21±1.32**	**27.76±1.54**	**0.187**

Table 3. T scores of the sleep questionnaire of patients with focal or focal with secondary generalization epilepsies (group Ia) and those with generalized epilepsy (group Ib).

	Group Ia (n = 19)	Group Ib (n = 25)	P value
DIMS	41.47 ± 1.50	41.84 ± 1.62	0.289
SBD	45.37 ± 1.61	45.84 ± 2.32	0.447
DA	47.0 ± 0.00	47.0 ± 0.00	1.00
SWTD	41.63 ± 1.50	42.36 ± 2.74	0.448
DOES	45.32 ± 3.46	45.96 ± 3.88	0.615
SHY	45.0 ± 0.00	45.0 ± 0.00	1.00
Total	**39.89±1.85**	**40.56±2.04**	**0.183**

Table 4. Comparative data of non-refractory (Group Ic) and refractory epileptic patients (Group Id).

	Group Ic (n= 26)	Group Id (n= 18)	P value
Age (mean±SD) in years	14.769±2.78	15.6±1.81	0.236
Gender (no.)			
Males	14 (53.8 %)	12 (46.2%)	0.395
Females	12 (66.7 %)	6 (33.3%)	
Sleep questionnaire total raw score	27.65±1.41	27.33±1.53	0.337
Sleep questionnaire total T score	40.5±1.88	39.94±2.09	0.325
Type of epilepsy			
Focal or Focal with secondary generalization	10 (52.6 %)	9 (47.4 %)	0.447
Generalized	16 (64 %)	9 (36 %)	
Duration: (years)	4.19±3.15	5.94±3.32	0.029*
Medications			
Mono-therapy	23(62.2 %)	14(37.8 %)	0.594
Combined therapy	3(42.9%)	4(57.1 %)	
6-hydroxymelatonin sulfate (ng/12 hours urine)	19389.98±15955.82	22025.72±24104.65	0.599

* Significant at p<0.05.

Table 5. Raw sleep questionnaire scores of non-refractory (Group Ic) and refractory epileptic patients (Group Id).

	Group Ic (n= 26)	Group Id (n= 18)	P value
DIMS	7.19 ± 0.49	7.28 ± 0.57	0.569
SBD	3.08 ± 0.27	3.11 ± 0.32	0.701
DA	3.00 ± 0.00	3.00 ± 0.00	1.00
SWTD	6.346 ± 0.628	6.11 ± 0.32	0.185
DOES	6.0 ± 0.98	5.83 ± 0.02	0.621
SHY	2.0 ± 0.00	2.00 ± 0.00	1.00
Total	**27.65±1.41**	**27.33±1.53**	**0.337**

Table 6. T scores of the sleep questionnaire of non-refractory (group Ic) and refractory epileptic patients (group Id).

	Group Ic (n= 26)	Group Id (n= 18)	P value
DIMS	41.58 ± 1.88	39.94 ± 2.09	0.569
SBD	45.54 ± 1.47	45.78 ± 2.26	0.701
DA	47 ± 0.00	47 ± 0.00	1.00
SWTD	42.46 ± 2.73	41.44 ± 1.294	0.185
DOES	45.92 ± 3.72	45.33 ± 3.694	0.621
SHY	45.00 ± 0.00	45.00 ± 0.00	1.00
Total	**40.5 ± 1.88**	**39.94 ± 2.09**	**0.325**

Table 7. Correlations between urinary 6 hydroxymelatonin sulfate and age, duration of epilepsy, sleep questionnaire scores and puberty scale scores.

	6 hydroxymelatoninsulfate	
	R	P value
Age	-0.002	0.989
Duration of epilepsy(in years)	-0.203	0.185
Total raw sleep questionnaire score	-0.139	0.368
Total T sleep questionnaire score	-0.135	0.381
Puberty scale	-0.071	0.649

DISCUSSION

In this study we aimed to detect the possible melatonin alteration in epileptic patients and to examine the relationship between type of epilepsy, seizure control and urinary 6 -sulphatoxymelatonin [metabolite of melatonin in urine] level.

Melatonin can be measured using multiple plasma sampling but in children it is more reasonable to be measured in the urine, where the stable metabolite of melatonin, 6 sulfatoxymelatonin, is easily measured and reflects plasma melatonin levels.[12]

Our study showed that the urinary 6-hydroxymelatonin sulfate level in patients was

significantly lower than in the control group (20468.24±19479.38, 61948.38±38734.98 p< 0.005).

Supporting these results, the study done by Elkhayat et al.[14], who found that melatonin levels in epileptic patients were significantly lower than those of the healthy controls.

Bazil et al.[13], reported that patients taking anticonvulsants have lower baseline levels of melatonin compared to healthy controls. This might explain lower levels of melatonin in our patients as all patients included in our study were on antiepileptic medications.

Also seizures could alter the feedback regulatory mechanisms coordinated by the pineal gland thus low level of melatonin reflects a disorder in neuroendocrine regulation among patients with epilepsy[13].

Moreover, patients and control groups in our study matched as regards age, sex, pubertal stage and sleep questionnaire scores thus excluding the possibility that sleep disturbances in the epileptic group accounted for their lower melatonin levels.

On the other hand, a study by Ardura et al.[15], conducted on 40 children with ages ranging from 15 days to 11 years, divided into 4 groups epilepsy, febrile seizures, and 2 control groups, salivary melatonin levels was collected in 6 samples every 4 hours all through the day, showed no significant difference between the epileptic patients and their control group, however a significant difference was found between patients with febrile convulsions and their control group.

The different methodology, age range of patients may be responsible for these results.

There was no statistically significant difference between refractory and non-refractory epilepsy groups as regards urinary 6 hydroxymelatonin sulfate levels. supporting these results the study done by Elkhayat et al.[14].

In our study there was also no statistically significant difference between sleep questionnaire scores (either raw or T scores) in both patients with non-refractory epilepsy and patients with refractory epilepsy.

This is congruent with Elkhayat et al.[14] study, who found no statistical significant difference between patients with controlled and uncontrolled epilepsy regarding Children's Sleep Habits Questionnaire and Epworth sleepiness scale.

The patients group was also subdivided into 2 groups, group including patients with focal or focal with generalized epilepsy and another group including patients with generalized epilepsy.

In the current study, there was no statistically significant difference regarding the level of 6 hydroxymelatonin sulfate between the 2 subgroups, (group Ia) that included patients with focal or focal with secondary generalization and (group Ib) that included patients with generalized epilepsy.

Our results were supported by Elkhayat et al.[14]. On the other hand, a study by Paprocka et al.[15] found that patients with focal epilepsy had lower melatonin levels than patients with generalized epilepsy.

In the current study, there was also no statistically significant difference in sleep questionnaire raw scores and T scores either total or subscales scores between patients of (group Ia) and those of (group Ib) (p=0.183).

These results were supported by findings of Elkhayat et al.[14].

Moreover, Gupta et al.[7], studied 31 epileptic patients aged 3-12 years. Parental questionnaire, sleep behavior questionnaire were taken either by patients or their parents and it was found that type of seizures did not correlate significantly with total sleep questionnaire scores.

On the other hand another study by Shouse et al.[17], found that seizure type influence the occurrence of sleep abnormalities and that patients with generalized epilepsy have sleep alterations more than patients with simple or complex seizures.

Also a study by Hoeppner et al.[18], conducted on three groups of adult epileptic subjects with simple partial, complex partial and generalized seizures and normal control subjects. All subjects completed a brief self-report sleep questionnaire. The simple partial and complex partial groups indicated significantly more sleep disorder symptoms than the generalized epilepsy group.

In the current study there was negative correlation between age, puberty scale scores and 6-hydroxymelatonin sulfate level but it was not statistically significant (r -0.002 p=0.989) and (r -0.071, p=0.649) respectively.

Supporting these results; a study done by Mahlberg et al.[19], on 75 healthy subjects aging from 20 to 84 years. levels of 6 hydroxymelatonin sulfate was measured in urine collected in 5 collection periods: first night time period, morning period, daytime period, evening period, second night time period, found that the morning and evening samples were negatively correlated to age.

Questions regarding the anticonvulsive activity of melatonin have not yet been fully answered and the lower levels of melatonin in epileptic patients need further studying to be able to conclude whether it is a cause or a result of epilepsy.

Conclusions

* Urinary 6 hydroxymelatonin sulfate level was lower in epileptic patients compared to the control group.
* Type of epilepsy and whether it is refractory or not did not affect melatonin level.

[Disclosure: Authors report no conflict of interest]

REFERENCES

1. Sanchez-Barcelo E. Melatonin and mammary cancer: a short review. Endocr Relat Cancer 2003; 10:153–9.
2. Yalyn O, Arman F, Erdogan F, Kula M. A comparison of the circadian rhythms and the levels of melatonin in patients with diurnal and nocturnal complex partial seizures. Epilepsy Behav 2006; 8(3):542–6.
3. Gordon N. The therapeutics of melatonin: a paediatric perspective. Brain Dev. 2000; 22:213-217.
4. Gomar MD, Del Aguila CM, Fernandez B, Castillo JL, Acuña Castrovijo D. Influence of the behaviorally active peptides ACTH1-10 and ACTH4-10 on the melatonin modulation of [3H] flunitrazepam receptor binding in rat cerebral cortex. Neuroendocrinology. 1994; 60:252-60.
5 Acuña-Castroviejo D, Escames G, Macías M, Muñóz Hoyos A, Molina Carballo A, Arauzo M, et al. Cell protective role of melatonin in the brain. J Pineal Res. 1995, 19, 57–63.
6. Stockmeier, CA, Blask DE. Pinealectomy-induced convulsions: current status as a model of epilepsy. In Advances in pineal research. Reiter, RJ, Karasek M, eds. John Libbey, London, 1986; 197-206.
7. Gupta M, Gupta YK, Aneja S, Kohli K. Effects of add-on melatonin administration on antioxidant enzymes in children with epilepsy taking carbamazepine monotherapy: a randomized, double-blind, placebo controlled trial. Epilepsia 2004;45:1636-9.
8. Dlugos DJ, Samuel MD, Strom BL, Farrar JT. Response to first drug trial predicts outcome in childhood temporal lobe epilepsy. Neurology. 2001; 57: 2259-64.
9. Kwan P, Sander JW. The natural history of epilepsy: an epidemiological view. J Neurol Neurosurg Psychiatry. 2004; 75:1376–81.
10. Carskadon MA, Acebo C. A self adminsterd rating scale for pubertal development journal of adolescent Health 1993; 14:190-5.
11. Bruni O., Ottaviano S., Guidetti V., Romoli M, Innocenzi M. et al., the sleep disturbance scale for children (SDSC) construction and validation of an instrument to evaluate sleep disturbances in childhood and adolescence, J Sleep Res.1996:5;251-61.
12. Hancock E, O'Callaghan F, English J, John P, Osborne JP. Melatonin Excretion in Normal Children and in Tuberous Sclerosis Complex With Sleep Disorder Responsive to Melatonin. J Child Neurol. 2005; 20:21-5.
13. Bazil CW, Short D, Crispin D, Zheng W. Patients with intractable epilepsy have low melatonin, which increases following seizures. Neurology. 2000; 55(11):1746–8.
14. Elkhayat HA, Hassanein SM, Tomoum HY, Abd-Elhamid IA, Asaad T, Elwakkad AS. Melatonin and sleep-related problems in children with intractable epilepsy. Pediatr Neurol. 2010; 42:249-54.
15. Ardura J, Jesus Andres J, Jose R. Garmendia and Francisco Ardura Melatonin in Epilepsy and Febrile Seizures J Child Neurol. 2010; 25: 888.
16. Paprocka MJ, Dec R Jamroz E, Marzal E. Melatonin and child hood refractory epilepsy. Med Sci. 2010; 16(9):389-96.
17. Shouse MN, da Silva AM, Sammaritano M. Circadian rhythm, sleep, and epilepsy. J Clin Neurophysiol. 1996; 13: 32–50.
18. Hoeppner JB, Garron DC, Cartwright RD. Self-reported sleep disorder symptoms in epilepsy. Epilepsia. 1984; 25: 434–7.
19. Mahlberg R, Tilmann A, Salewski L, Kunz D. Normative data on the daily profile of urinary 6 sulfatoxymelatonin in healthy subjects between the ages of 20 and 84. Psychoneuroendocrinology, 2006; 31:634-41.

الملخص العربي

الميلاتونين ومرض الصرع، دراسة إكلينيكية ومعملية

الميلاتونين مركب يفرز أساسا من الغدة الصنوبرية وقد أظهرت الدراسات وجود علاقة بين مستوى الميلاتونين والنوم ووظائف الغدة الصنوبرية وللميلاتونين دور في التحكم في النشاط الكهربائى للمخ. والهدف من هذا البحث هو دراسة تغيرات الميلاتونين فى مرضى الصرع وما إذا كانت هناك علاقة بين نوع الصرع واستجابته للعلاج وبين مستوى الميلاتونين فى البول (٦ هيدروكسى كبريتات الميلاتونين فى البول).

وقد أجريت هذه الدراسة على عدد 61 شخص منهم 44 مريض بالصرع و 17 شخص مجموعة ضابطة، ولم يكن هناك فرق ذو دلالة إحصائية فى السن أو الجنس بين المجموعتين. وقد خضعت جميع الحالات لفحص إكلينيكي ومقياس اضطرابات النوم وأيضا مقياس لدرجة البلوغ وتم قياس مستوى (٦ هيدروكسى كبريتات الميلاتونين فى البول) فى البول الذى تم تجميعه من الساعة ٨ مساءا إلى الساعة ٨ صباحا لجميع الحالات والمجموعة الضابطة .. وقد أظهرت النتائج فى هذا البحث أن نسب (٦ هيدروكسى كبريتات الميلاتونين فى البول) فى مرضى الصرع أقل منها فى المجموعة الضابطة وكان هذا الاختلاف ذو دلالة إحصائية. ولكن لا توجد فروق ذات دلالة إحصائية في نسب (6 هيدروكسى كبريتات الميلاتونين فى البول) فى حالات الصرع مع اختلاف نوع الصرع، وأيضا لا توجد فروق بينهم فى مقياس اضطرابات النوم.