Disturbed sleep in individuals with idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF) : Melatonin assessment as a biological marker

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Disturbed sleep in individuals with Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF): melatonin assessment as a biological marker

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Abstract

Individuals who suffer from idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF) complain of a variety of adverse health effects. Troubled sleep remains a recurrent and common symptom in IEI-EMF individuals. Melatonin, a circadian hormone, plays a major role in the sleep process. In this study, we compared levels of melatonin between a sensitive group (IEI-EMF, n=30) and a non-sensitive control group (non IEI-EMF, n=25) without exposure to electromagnetic sources. Three questionnaires were used to evaluate the subjective quality and sleep quantity: the Epworth Sleepiness Scale, the Pittsburgh Sleep Quality Index and the Spiegel Sleep Inventory. Melatonin was quantified in saliva and its major metabolite 6-sulfatoxymelatonin (aMT6s) in urine. Melatonin levels were compared by a two-way analysis of variance at various times between the control and IEI-EMF group. Despite significantly different sleep scores between the two groups, with a lower score in the IEI-EMF group ($p<0.001$), no statistical difference was found between the two groups for saliva melatonin ($p>0.05$) and urine aMT6s ($p>0.05$).

Key words: *Idiopathic environmental intolerance, Melatonin, Marker, Urine, Saliva, Electromagnetic fields*

Introduction

During the last decades, the rapid development and extensive use of electric power and wireless communication have increased our exposure to electromagnetic fields (EMF). Potential health effects of EMF are the subject of hot public debate. Moreover, some individuals report adverse effects because of exposure to EMF. This sensitivity is commonly known as idiopathic environmental intolerance of EMF (IEI-EMF) or electromagnetic hypersensitivity (EHS). Among the most common self-reported symptoms of EHS are sleep disorders, headaches, and fatigue [Röösli et al., 2004].

Up until now, numerous double-blind experimental studies have not validated any direct association between the manifestation of these symptoms and EMF exposure [Rubin et al., 2005; Eltiti et al., 2007; Röösli, 2008; Rubin et al., 2009; Kwon et al., 2012] . Individuals claiming EHS did not seem able to detect EMF more accurately than subjects who did not report such hypersensitivity [Eltiti et al., 2007; Röösli, 2008; Rubin et al., 2009] and a generally accepted mechanism of EHS is lacking despite numerous propositions. In addition, there is no known and proven mechanism that triggers EMF-linked symptoms.

Despite lack of association between onset of symptoms and EMF exposure, these symptoms and the suffering of IEI-EMF individuals are real and cannot be ignored.

Recently, we conducted a survey among people self-reporting as suffering from EHS in the French population, and we observed that among reported symptoms, sleep disruption was a recurrent theme and the most common. On the other hand, melatonin (*N*-acetyl-5-methoxytryptamin), a hormone synthesized in the pineal gland with a peak during periods of darkness, has a highly reproducible circadian pattern [Selmaoui and Touitou, 2003] with a

strong endogenous component and is physiologically controlled by light. This neuro-hormone seems to play an important role in initiating and maintaining sleep. It was reported that insomnia, sleep deficiency and shorter sleep time in elderly people are associated with the decline of endogenous melatonin concentrations [Lemoine and Zisapel, 2012]. Melatonin administration is effective in restoring normal sleep and improving quality of life in people over 55 years of age [Lemoine and Zisapel, 2012]. In light of the above, we studied whether complaints of sleep disturbances in the IEI-EMF population could be related to low levels of melatonin concentrations.

The aim of the present study was to compare saliva melatonin and urinary 6-sulfatoxymelatonin (aMT6s) between a self-declared IEI-EMF group and a non-IEI-EMF group. Saliva and urine samples were collected to measure melatonin and its major metabolite, aMT6s, in urine. We also assessed subjective sleep quality. To our knowledge, this is the first time that melatonin has been assessed in IEI-EMF individuals.

Materials and Methods

Participants

This study is part of the survey we conducted in the French IEI-EMF population. Thirty of 52 people reporting as IEI-EMF in France who responded to our questionnaire were recruited for this study (7 males and 23 females). The IEI-EMF group included participants whose intolerance duration ranged from 1 to 35 years. Twelve participants also reported multiple chemical sensitivity (MCS) and two reported Lyme's disease. Participants reported sensitivity

to multiple EMF sources, especially wireless technology (Wi-Fi), cordless phones, cell phones, cell phone base stations and computers.

Patients were from different regions of France and from various self-help groups and associations (local or national) or were independent. Healthy individuals (n=25 with 21 females and 4 males) were matched for gender, age and body mass Index (BMI). They were recruited through Website advertisements and traditional word of mouth or through blogs or online forums or newsgroups.

Inclusion criteria for the IEI-EMF group were participants who reported at least one symptom that they attributed to one or multiple EMF sources and those who had no acute psychiatric disorders such as acute depressive or paranoid psychosis. Exclusion criteria for both groups was the presence of chronic illness. Participants who had consumed antibiotics, anti-inflammatory agents, and melatonin or any other supplements that might interfere with melatonin assessments were also excluded. In addition, shift workers were not admitted to the study. All study participants gave written, informed consent. Protocol was approved by the ethics committee of the CPP Nord Ouest CHU Amiens, France (CPP, 2014/8).

Study design

Saliva samples were collected with salivettes (Sarstedt, Nümbrecht, Germany). Study subjects collected their two first samples at home to be later delivered to the laboratory for assay. The first sample was collected before bedtime (on day D-1: day before experiment began). The second sample was taken 5 min after they woke up in the morning of experiment day (before coming to laboratory). Participants arrived at the laboratory around 09:00 and spent their waking time in the laboratory. During this period, participants spent

time inside a dimly lit, electrically shielded room serving as a Faraday's cage. This allowed IEI-EMF participants to be in an environment without EMFs. They had a comfortable armchair and were provided with newspapers, magazines, and books. Saliva was then collected every 30 min from 10:00 to 11:30 and from 14:00 to 16:30.

Participants were instructed to abstain from consuming alcohol and coffee for 24 h before and during each experimental session. They were also advised not to brush their teeth or to do physical exercise for at least 1 h before sample collection.

Premenopausal women were studied in the laboratory during the follicular phase of their menstrual cycle. During daytime assessment, participants were advised to take a balanced meal. Total urine was collected overnight from 20:00 to wake-up time as night fraction, and from wake-up time to 16:30 as daytime fraction (Fig. 1).

Saliva melatonin and urinary aMT6s measurements

Salivettes were centrifuged (2 min at 3,000 revolutions per min) aliquoted and stored at -20 °C until analysis. Melatonin concentration in saliva was determined in duplicate with the ELISA immunoassay technique (Salimetrics, State College, PA). Reported minimum detectable concentration of melatonin for the assay was 1.37 pg/mL. Intra-assay and inter-assay coefficients of variation were 1.88% and 10.99%, respectively. Urine samples were stored at -20 °C until analysis. Urinary 6-sulfatoxymelatonin was assessed through a competitive ELISA (Buhlmann Laboratories, Schönenbuch, Switzerland) with intra-assay and inter-assay coefficients of variation ranging from 6.99% and from 12.51%, respectively. The aMT6s concentrations were adjusted for creatinine levels to control for urine volume. Urinary creatinine was measured colorimetrically according to manufacturer's guidelines.

Subjective assessment of sleep quality

Subjects completed all surveys on sleep quality. The Pittsburgh Sleep Quality Index (PSQI) [Buysse et al., 1989] measures self-perceived sleep quality during the past month and contains 10 different questions that relate to normal sleep habits. Subjects were asked to respond according to the majority of days and nights in the past month. A global score greater than five corresponds to a "poor sleeper."

The Epworth Sleepiness Scale (ESS) [Johns, 1991] is a self-administered questionnaire for quantitative subjective measurement of sleepiness. For each activity, the patient rates his or her chances of falling asleep during the activity. Scores range from zero (never dozing in a situation) to three (always dozing). Scores for the eight items are summed. Total ESS scores above 10 suggest chronic sleepiness.

The Spiegel Sleep Inventory is a self-administered mini-questionnaire that inquires about the previous night via six questions on sleep initiation, quality, and length; nocturnal awakenings; dreams; and feeling refreshed in the morning. A score below 15 suggests pathologic sleep, whereas a score above 20 is considered good sleep [Spiegel, 1981].

Statistical analysis

Comparisons between control and IEI-EMF groups were performed with U Mann Whitney for sleep evaluation of PSQI, Spiegel and Epworth scores; two-way analysis of variance (ANOVA) for testing time and groups effect considering saliva melatonin or urinary aMT6s as variables. For characteristics and sleep scores comparisons, the student t-test and chi square test were used.

Statistical analysis was performed with the help of SPSS statistical software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY) and plotted with the Graphpad Prism (Prism, version 5, San Diego, CA). Values were considered to be significantly different when $p < 0.05$.

Results

The characteristics of the two groups are presented in Table 1. Groups did not differ statistically in age, gender and smoker proportions. The IEI-EMF group reported significantly different scores from the control group, showing a lower quantity ($p < 0.001$) and quality ($p<0.001$) of sleep. The ESS was similar for both groups ($p=0.183$). Additionally, the IEI-EMF group had a significantly higher proportion of participants with Spiegel score under the cutoff value of 15 ($p<0.01$).

Figure 2a represents data of 6-sulfatoxymelatonin concentrations in urine (aMT6s) and Figure 2b those of aMT6s adjusted by urinary creatinine concentrations. Two-way ANOVA performed for both sets of data did not reveal any differences between aMT6s levels measured in the IEI-EMF group and those in the control ($p>0.05$). However, time effect was found to be very significant ($p<0.0001$) for aMT6s levels between daytime and nighttime fractions.

Figure 3 represents data of saliva melatonin assessed at different times of day: bedtime, wake-up time, then subsequently every 30 min between 10:00 and 11:30 and between 14:00 and 16:30. Here again, no significant differences ($p>0.05$) were shown between saliva melatonin concentrations in the IEI-EMF group and those obtained in the control. Participants noted their bedtime and wake-up times (Table 2).

Discussion

The present work was intended to determine whether melatonin levels are affected in patients suffering from IEI-EMF. In fact, IEI-EMF patients complained about insomnia or difficulties in sleeping and headache disorders. On the other hand, lower nocturnal melatonin levels were reported to be associated with worsened daytime sleepiness, sleep deficiency, and shorter sleep time in older men [Lemoine and Zisapel, 2012]. Moreover, a strong association has also been reported between melatonin secretion and headache disorders. Indeed, some studies suggest that some people who have migraine might be deficient in melatonin production [Bruera et al., 2008; Masruha et al., 2010].

Our results showed that urinary aMT6s levels were not affected in IEI-EMF patients compared with non-IEI-EMF individuals. Despite poor sleep quality reported by IEI-EMF patients (PSQI index) and the high proportion of IEI-EMF presenting a pathologic Spiegel score, their day/night levels of urinary melatonin were comparable to those of non-IEI-EMF individuals. In addition, day/night variations of melatonin levels were in accordance with what is observed in normal circadian rhythm, with high levels during the night and low levels during the day. This suggests that circadian rhythm in IEI-EMF patients is not affected.

In addition, saliva melatonin was assessed in a sample collected just before bedtime and analyzing melatonin in this sample could be an indicator for melatonin onset. Indeed, the onset of melatonin secretion is associated with an increase in sleep propensity in both sighted and blind individuals [Lewy et al., 1980; Lockley et al., 1997; Wyatt et al., 2006] and it was reported that exogenous administration of melatonin especially during the day can

facilitate sleep [Dollins et al., 1994; Cajochen et al., 1996; Zhdanova et al., 1996; Hughes and Badia, 1997; Stone et al., 2000; Rajaratnam et al., 2004; Wyatt et al., 2006; Arendt et al., 2008].

In our present work, saliva melatonin concentrations at bedtime did not differ between the IEI-EMF group and the control. This means that melatonin levels before bedtime were similar in both groups. Moreover, saliva melatonin concentrations collected immediately after wake-up time and in subsequent samples collected every 30 min from 10.00 to 16.30 did not reveal any changes in daytime pattern of melatonin secretion. These results suggest therefore that the circadian rhythm of melatonin secretion is most likely not phase-shifted in IEI-EMF patients.

However, the best way to evaluate the phase shift of melatonin circadian rhythm would be a temporal sampling over 24 h or at least during the whole night to include melatonin peak secretion. Nevertheless, our protocol combining day/night variation of melatonin levels in urine and saliva melatonin analyzed in samples collected at specific moments of the day, such as before bedtime and immediately after wake-up time, remains quite informative about the circadian rhythm of melatonin phase shifting.

Although chronotype profile was not investigated by an appropriate questionnaire, our results indicate an earlier waking time in the IEI-EMF group despite comparability to the control group in terms of age and gender. The bedtimes (or wake-up times) differed between the two groups by about 1 h. This difference in timing of the sleep/wake schedule cannot be described in terms of chronotype. Indeed, evening chronotypes typically have time of sleep delayed 2 to 3 h longer than morning chronotypes [Lack et al., 2009]. In terms of circadian markers, our study did not find any significant differences between IEI-EMF and

control groups in the timing of saliva melatonin levels analyzed at bedtime, wake-up time and in subsequent samples. It should be noted that the chronotype depends also on environmental [Roenneberg et al., 2003] and genetic [Toh et al., 2001] factors.

The main limitation of this study is the variable time of sampling in participants' homes. Although we used a largely acceptable form of sampling for saliva collection and despite participants being instructed to collect samples in a given time range, variations in time of collection at home were unavoidable. However, sampling was performed so as not to disrupt participant habits and to reduce stress. Home collections concern only a small proportion of study samples.

Our hypothesis in studying melatonin in IEI-EMF individuals is based on the fact that its secretion is strongly inhibited by light [Lewy et al., 1980], which is the visible portion of EMFs. Therefore it is speculated that melatonin could likely be sensitive to another part of the electromagnetic spectrum. With regard to extremely low-frequency magnetic fields (1 to 300 Hz), data from the literature on the effect of EMFs on melatonin secretion are contradictory in animal studies [Touitou and Selmaoui, 2012]. On the other hand, the circadian pattern of the hormone can be phase-shifted (advanced or delayed) by light according to the time of exposure [Touitou Y et al., 1993], and this phenomenon might occur with exposure to EMFs and could in part explain the poor sleep quality in IEI-EMF patients.

Data showed no differences between melatonin concentrations measured in the IEI-EMF group and those measured in the control. In light of these results, it is suggested that melatonin was not affected in our group of IEI-EMF patients and cannot be related to their sleep disturbance. Likewise, it is not excluded that perception of sleep instead of sleep can be affected. While sleep quality measured by self-report questionnaire is suitable, it can be

argued that time taken to fall asleep and/or number of sleeping hours could be more precisely assessed with physiological measures.

Origins of this sleep disruption or eventual troubled perception of sleep should also be investigated more precisely.

Conclusions

The present work was conducted to determine whether IEI-EMF patients have low levels of melatonin that might explain their poor sleep.

However, these results must be interpreted with caution. Absence of a correlation between sleep complaints and melatonin levels does not mean that IEI-EMF patients are not really suffering from sleep disturbance. In view of this, polysomnographic recordings are an effective way to evaluate sleep disturbances objectively. In addition, the origin of these problems must be sought as well as EMF's role in occurrence of symptoms.

Additionally, some IEI-EMF patients have reported to us that symptoms such as headache, itching, etc. occur several hours after exposure and might appear at bedtime. Headache pain and so on could likely be the cause of sleep disturbance. Indeed, some studies have shown that more than 50% of patients with diverse sources of chronic pain complain significantly of sleep disturbance [Pilowsky et al., 1985; Atkinson et al., 1988 ; Smith et al., 2000].

Overall, there are very few data available on IEI-EMF's biomarkers [Dahmen et al., 2009; Ghezel-Ahmadi et al., 2010]. Further studies are needed to look for other biomarkers that may help in diagnosing IEI-EMF.

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Figure and table legends

Figure 1. Description of protocol: saliva and urine collection

Urine collection was fractioned (1): night fraction: from 20:00 (D-1) to wake-up on D; (2): daytime fraction: from participant's arrival to 16:30 on D.

Saliva collection: on day D-1: before sleeping; on day D: 5 min after wake-up, and every 30 min from 10:00 to 11:30 and from 14:00 to 16:30.

D-1: day before experiment; D: day of experiment

Figure 2. Day/night variation of 6-sulfatoxymelatonin concentrations in urine.

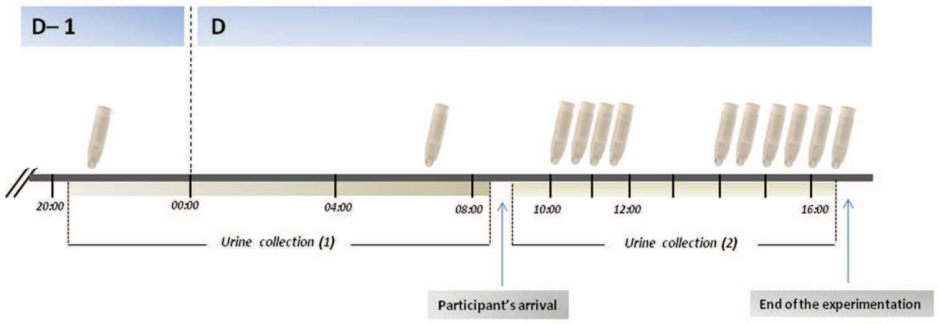
a) aMT6s concentrations, no significant difference between control (white bars) and IEI-EMF group (black bars).

b) Creatinine-adjusted 6-sulfatoxymelatonin, no significant difference between control (white bars) and IEI-EMF group (black bars). Data represent the mean ± S.E.M. of concentrations in control (n=25) and IEI-EMF (n=30) group.

Figure 3. Saliva melatonin concentrations (pg/mL) in samples collected at bedtime, waking time and every 30 min from 10:00 to 11:30 and from 14:00 to 16:30. No significant difference between control (white bars) and IEI-EMF (black bars) group. Data represent mean ± S.E.M. Control (n=25) and IEI-EMF (n=30).

Table 1. Participants' characteristic and sleep scores. Information presented as mean ± SD or total number n (percentage).

Table 2. Intervals, mean and median of bedtimes and wake-up time of participants collected during and before day of experimentation (D-1).



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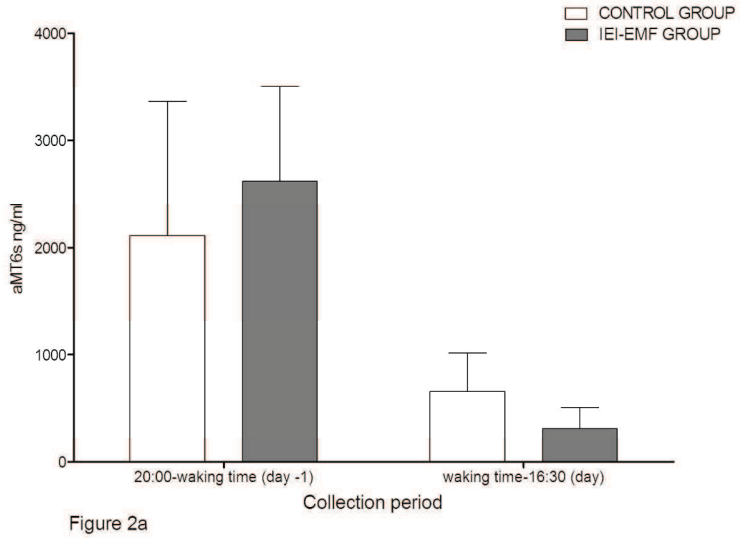

Figure 2a

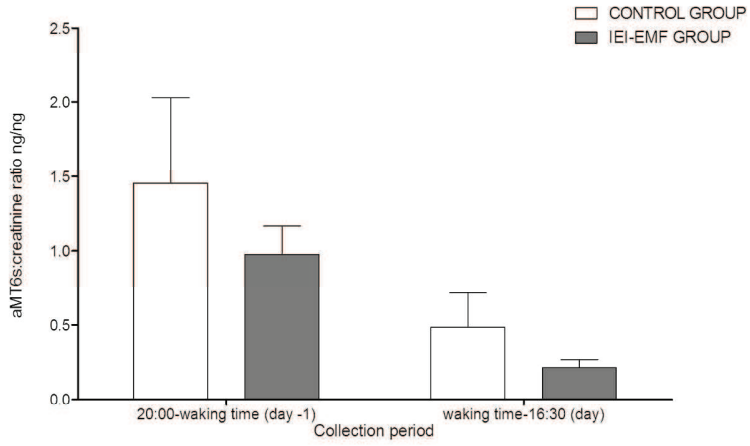

Figure 2b

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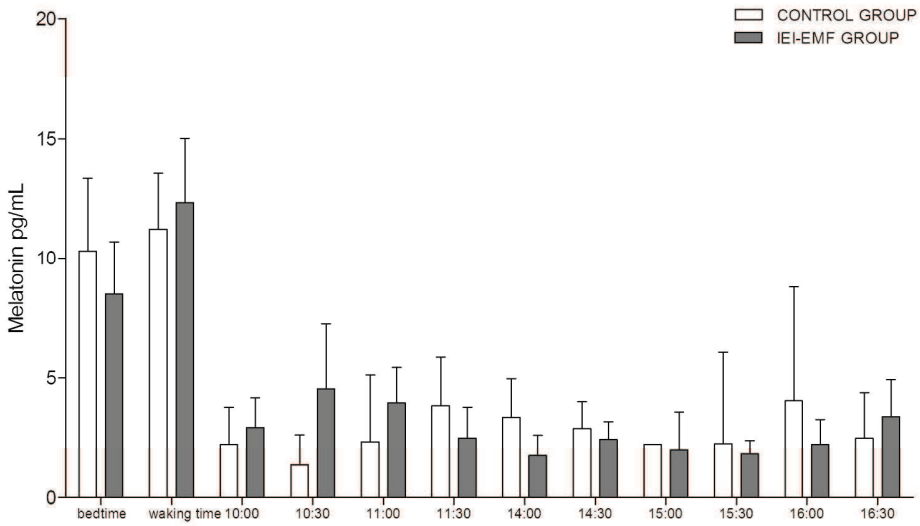

196x112mm (300 x 300 DPI)

Table 1. Participants' characteristic and sleep scores. Data presented as mean ± S.D. or total number n (percentage)

Variable	Control group (n=25)	IEI-EMF group (n=30)	p-value
Age, years	46 ± 10	47 ± 9	0.999
IEI-EMF duration, years	-	8.43 ± 7.3	-
Female gender, n (%)	21 (84)	23 (77)	0.498
BMI, kg/m²	22.80 ± 2.60	22.35 ± 3.33	0.268
Smoker n (%)	6 (24)	4 (13)	0.307
Spiegel score	21 ± 6	16 ± 6	<0.001
Spiegel score <15, n (%)	8 (27)	21 (70)	<0.01
ESS	7 ± 4	9 ± 4	0.183
ESS >10, n (%)	7 (28)	12 (40)	0.404
PSQI index	3 ± 2	8 ± 3	<0.001
PSQI >5, n (%)	1 (4)	5 (20)	0.134

Abbreviations: IEI-EMF: Idiopathic environmental Intolerance attributed to electromagnetic fields; BMI: body mass Index, ESS: Epworth Sleep Score PSQI: Pittsburgh Sleep Quality Index

Respective cutoffs for sleep scores were: Spiegel score under 15 ; PSQI above 5 and ESS above 10

Table 2. Intervals, mean and median of bedtimes and wake-up time of participants collected during and before the day of experimentation (D-1)

	Control group (n=25)	IEI-EMF group (n=30)
Bedtime		
Mean	**23:30**	**22:48**
Width	**21:30 – 01:00**	**21:30 – 01:00**
Median	**23:30**	**22:30**
Waking time		
Mean	**07:29**	**06:23**
Width	**06:45 - 08:20**	**04:00 - 08:40**
Median	**07:30**	**06:45**