Background. The search for biological markers of individual characteristics has produced scanty results. Melatonin (MLT), the main hormonal product of the pineal gland, has been used as a biological marker of neuroticism, introversion-extroversion and morningness-eveningness. Morningness-eveningness indicates preferences associated with morning or evening activities. The goal of this research is to study if serum MLT levels are related to morningness-eveningness preference.

Methods. Twenty-three morning type and twenty-one evening type healthy volunteers took part in the study. Morningness-eveningness was evaluated with the Composite Scale of Morningness. Blood was drawn at 09:00, 12:00 and 00:00 h. MLT levels were measured with an ELISA.

Results. At 09:00 h evening type subjects had significantly higher serum MLT levels than morning type subjects (8.4±3.6 pg/ml vs. 4.6±3.2 pg/ml, p<0.02).

Conclusions. Morning serum MLT may be used as a biological peripheral marker of morningness-eveningness preference. Our results emphasise the convenience of expanding MLT studies until 09:00 h when differences between morning type and evening type subjects may still be found.

Key words: Melatonin, Morningness, Eveningness, Diurnal preference, Circadian typology, Circadian rhythms, Individual differences

Chronotype as modulator of morning serum melatonin levels

El cronotipo como modulador de los niveles séricos diurnos de melatonina

Introducción. La búsqueda de marcadores biológicos que se relacionen con características específicas de las personas no ha producido grandes resultados. Los niveles sanguíneos de la melatonina (MLT), principal producto hormonal de la glándula pineal, han sido utilizados como marcador biológico del neuroticismo, la introversión-extroversión y la matutinidad-vespertinidad. El concepto de matutinidad hace referencia a la preferencia de las personas para realizar actividades por las mañanas, mientras que la vespertinidad hace referencia a la preferencia para realizar actividades por la noche. El objetivo de este trabajo consiste en estudiar si los niveles séricos de MLT se relacionan con la matutinidad o vespertinidad.

Metodología. La muestra está compuesta por 44 voluntarios sanos, de los cuales 23 son del tipo matutino y 21 del tipo vespertino. La matutinidad-vespertinidad fue valorada con la Escala Compuesta de Matutinidad. Se analizaron 3 muestras de sangre, extraídas a las 09:00, 12:00 y 00:00 h. Los niveles de MLT fueron determinados mediante un ELISA.

Resultados. A las 09:00 h, los sujetos vespertinos tenían niveles de MLT significativamente más altos que los sujetos matutinos (8,4±3,6 pg/ml vs 4,6±3,2 pg/ml, p<0,02).

Conclusiones. Los niveles séricos de MLT a las 09:00 h pueden ser usados como un marcador biológico periférico de vespertinidad-matutinidad. Nuestros resultados enfatizan la conveniencia de alargar los estudios de MLT al menos hasta las 09:00 h, cuando aun se pueden encontrar diferencias en los niveles séricos de MLT entre los tipos matutinos y vespertinos.

Palabras clave: Melatonina, Matutinidad, Vespertinidad, Preferencia diurna, Tipología circadiana, Ritmos circadianos, Diferencias individuales

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INTRODUCTION

Chronotype is an individual characteristic that modulates the capacity of the person to be active and alert at certain times of the day. Morning types (MT) are persons who are more alert and active in the morning, while Evening Types (ET) are prone to be more active and alert at night. Morningness and evenness are the extreme poles of a continuum of the chronotype during which persons can be classified according to the time preferences to perform both physical and cognitive activities.2

Melatonin (MLT) is the main pineal gland hormone that transmits information on circadian rhythms as well as seasonal rhythms.3 MLT has been used as a drug with therapeutic properties,4 as well as a biological marker.5

One characteristic of research on MLT as a biological marker is that it has much interindividual variability, regardless of the biological fluid used to measured it. Different variables that affect MLT levels have been described. These variables include a wide range, such as genetic variables,6 environment,7 style of life,8 taking of some medicine9 or drug consumption.10

Regarding the use of MLT as a biological marker, two different aspects should be considered. First, MLT secretion rhythms should be studied as this gives us information on different rhythm parameters (initiation time and end of the secretion, maximum peak, etc.).11 To make this type of study, several biological samples must be collected continually and regularly. In the second place, MLT can only be determined at specific times or in short periods of time during the day. This second approach is generally done when attempting to find connections between the MLT levels and some medical conditions.12,13 This second approach is much easier in the research design and, of course, less expensive in the consumption of human resources and time.

There is a lot of information available on the relation between chronotype and circadian rhythms of some biological variables, such as body temperature, cortisol rhythm, blood pressure, heart rate, electrodermal activity and sleep architecture.14-18

Not much information has been published about the relation of circadian rhythm of MLT and the chronotype. It has been found that MT subjects have an advance in the MLT secretion rhythm phase compared to subjects with ET.19 Little information is available on the relations existing between the MLT levels in specific hours of the day and the chronotype.

This research has aimed to study if the serum concentrations of MLT in three specific hours of the day (9 a.m., 12 p.m. and 12 a.m.) are related with the extreme chronotypes (MT vs ET) in healthy subjects.

METHODOLOGY

Subjects

The sample was collected using a mass E-mailing announcement to all the workers in the University of La Laguna who had an E-mail with the extension @ull.es (N=3,500), as well as among persons known to the investigators. In all, 118 persons responded. All the participants signed the informed consent. The Ethics Committee of the University of Laguna approved the study protocol.

Exclusion criteria were: 1) having made a transmeridian trip during the month prior to the study, 2) having sleep problems, 3) having any abnormality in the biochemical or urine analyses made, 4) pregnancy, 5) having a previous history of drug addiction, and finally 6) having a night-time job or job with shifts.

All the subjects participating in the study were asked to abstain from using sunglasses, have drinks with caffeine, tea or alcohol for at least 12 hours prior to the study and during the study. All the participants were also asked to maintain their normal schedules of going to bed and getting up.

Evaluation of the chronotype

Morningness-eveningness was evaluated using the Spanish version of the Composite Scale of Morningness.20 All the participants filled out the composite scale of morningness 4 days prior to the study.

To choose the MT and ET groups, 20% superior and inferior on the scale were chosen as extreme cut offs. Of the initial sample of 118 subjects, 23 were classified as MT and 21 as ET. The day after filling out the scale, all the chosen subjects underwent a general analysis with complete blood test, complete biochemical and urine profile, to rule out to existence of an organic condition.

The study was conducted during one weekend in the month of July. The reason for this was to avoid the seasonal change effect on the MLT levels.21 On the day of the study, the selected study subjects came to the medical school at 8 a.m. while fasting. After, they laid down on a treatment table, remaining in supine position until 9 a.m., when the first blood was drawn. After that they had breakfast. The next two blood drawings were performed at 12 p.m. and 12 a.m. As in the blood drawing at 9 a.m., the subjects had to be lying down one hour prior to it, so as to avoid the postural effect on the MLT levels.22 The nighttime extraction was performed in a room lit with red lamps having 4 luces of intensity. As a means of precaution, to avoid light contamination, at 11 p.m., the eyes of all the subjects were covered with a black eye shield. The nighttime extraction
function was performed with the aid of a lantern with a 20 luces intensity red light that was directly aimed at the forearm of the subject.

After each blood drawing, the blood was placed in vacutainer tubes with no anticoagulant and with separator gel. Clot formation was permitted at room temperature for 15 minutes and then the blood was centrifuged at 2000 rpm for 10 minutes. After, the serum samples were aliquoted in Eppendorf tubes and frozen at -35º C until they were analyzed. MLT was determined by ELISA analysis, using commercial kits from IBL (Immuno Biological Laboratories, Hamburg, Germany). The detection limit of this analysis was 0.3 pg/ml. The intra-analysis and inter-analysis variation coefficients were 8.5% and 11%, respectively. The analyst was blind to whether the samples belonged to the blood drawings of 9 p.m., 12 p.m. or 12 a.m.

**Statistical analysis**

The statistical analysis was performed with version 15 of the SPSS. Quantitative variables were compared by chronotype using the Student’s T test. Qualitative variable distribution was analyzed with the Chi Square test. Significance level was established at 0.05 and all the statistical tests were two-tail. Quantitative data are presented as mean ± standard deviation.

**RESULTS**

Table 1 shows the summary of the sociodemographic and psychometric characteristics of both groups. The samples are homogeneous in age, gender, weight and height. As was to be expected, MT subjects scored significantly higher on the morningness scale than the ET subjects. It has been published that hormone contraceptives affect MLT level. In our sample, no woman was taking hormone contraceptives.

Figure 1 represents the results of the comparison of the MLT levels in each chronotype and at each study hour. As can be concluded from the observation of this figure, ET subjects had significantly higher serum levels of MLT at 9 a.m. than the MT subjects.

MLT levels at 12 a.m. were significantly higher than MLT levels at 9 a.m. and 12 p.m., both in the MT subjects (12 a.m.: 35.8±24.2 pg./ml vs. 9 a.m.: 4.6±3.2 pg./ml, 12 p.m.: 3.0±1.6 pg./ml, p<0.001) and in the ET subjects (12 a.m.: 40.0±24.1 pg./ml vs. 9 a.m.: 8.4±3.6 pg./ml, 12 p.m.: 3.2±1.7 pg./ml, p<0.001).

**DISCUSSION**

This is the first work that has found that serum levels of MLT at 9 a.m. may be useful to differentiate MT subjects from ET subjects. This higher level of MLT at 9 a.m. in the ET subjects compared to those of the MT subjects may help to explain why the ETs are more clumsy and sleepy in the morning.

In our review of the literature, we have only found one work that has compared MLT levels at specific times of the day with the chronotype. Gibertini et al. found that MLT concentrations did not discriminate between MT and ET subjects. Our results at 12 a.m. are comparable to those of Gibertini et al. MLT levels were not differentiated between the MT and ET subjects. The rest of the time measurements were homogeneous in age, gender, weight and height.
could be compared since Gibertini et al. measured MLT every hour from 12 a.m. to 7 a.m., while we did so at 12 a.m., 9 a.m. and 12 p.m.

Most of the investigations on MLT and chronotypes focus on the study of MLT secretion rhythms. Research done in short periods of time or simply at specific times of the day are comparatively less. Two of the main markers used to determine MLT circadian rhythms are initiation and end of MLT secretion. The level of 10 pg./ml of MLT is considered as the threshold of initiation and termination of the MLT secretion. However, given the improvement in both sensitivity and specificity of the new analytic tests, it is recommended that in the future we reconsider the values of this figure. In fact, in our samples, the MLT serum level is inferior to 10 pg./ml, but in spite of this, the MLT levels in our study are differentiated between MT and ET subjects.

Both ET and MT subjects have nighttime levels (12 a.m.) that are significantly higher than the daytime ones (9 a.m. and 12 p.m.). These results confirm that both groups showed differences between the day and night time serum levels of MLT, the nighttime levels of MLT being significantly higher than the daytime ones. This result, that of higher nighttime levels of MLT then daytime ones, is in already known fact and has been reported by other investigators.

In conclusion, the increase of the MLT levels in the morning in the ET subjects helps to explain why the ET subjects are more clumsy and sleepy in the morning. Our results stress the importance of extending the MLT studies at least until 9 a.m., when it would still be possible to find differences between the ET subjects and the MT subjects. MLT serum concentrations can be used as a marker of morningness-eveningness.

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CONFLICT OF INTEREST

None.

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