

# Reduction of night/day difference in melatonin blood levels as a possible disease-related index in schizophrenia

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## Abstract

**OBJECTIVE:** Aim of this study was to verify if a simple index as night-day plasma MLT level variation is able to confirm the existing data on circadian melatonin alterations in schizophrenia and if a relationship to disease itself instead of the actual clinical state can be suggested.

**SETTING AND DESIGN:** Ten consecutively admitted male schizophrenic inpatients were examined.

**METHODS:** The blood samples for melatonin were collected at 3.00 a.m. and 15.00 p.m. and consequently calculated the values of Delta ( $\Delta$ ) (MLT h.3.00-MLT h.15.00). We divided the sample into two subgroups:  $\Delta < 30$  pg/ml and  $\Delta > 30$  pg/ml taking 30 pg/ml as an arbitrary value, based on literature data, that should indicate a physiologically correct value of  $\Delta$ .

**RESULTS:** The 70% of the sample was under the 30 pg/ml value of  $\Delta$  ( $13.61 \pm 4.0$ ) or was lacking of the characteristic circadian pattern of MLT secretion, whilst the 30% of the sample was over the 30 pg/ml value of  $\Delta$  ( $83.60 \pm 16.34$ ) or was in presence of the characteristic circadian pattern of MLT secretion ( $p = .0001$ ). No correlation was found between  $\Delta$  values and the scale and subscales scores for the assessment of psychopathology.

**MAIN FINDINGS:** The data confirm the lack of the characteristic circadian pattern of MLT secretion in schizophrenics.

**CONCLUSION:** The absence of significant correlation between night / day melatonin level differences and actual psychopathology variables should indicate that the suppression of  $\Delta$  is mostly related to the disease and independent from the clinical state. A neuroleptic-treatment effect cannot be excluded so far.

#### Abbreviations and units

MLT:	melatonin
NAT:	N-acetyltransferase
PC:	pineal calcification
MRI:	magnetic resonance imaging
$\Delta$ :	delta
SAPS:	Scale for the Assessment of Positive Symptoms
SANS:	Scale for the Assessment of Negative Symptoms
PANSS:	Positive and Negative Syndrome Scale
5-HT:	serotonin
PRL:	prolactin
TSH:	thyroid stimulating hormone
GH:	growth hormone

## Introduction

Melatonin (MLT) is a methoxyindole secreted by the pineal gland and rises to maximal concentration during the night. The connection of the pineal with the retina (through which the gland is informed about the light/dark environment condition) and the presence of a gene that activates and disactivates the N-acetyltransferase (NAT), a key enzyme in the melatonin pathway from tryptophan, are the responsible of this rhythmic production. In humans the endogenous rhythm of melatonin secretion is generated by the suprachiasmatic nucleus and follows a distinct circadian pattern, being low during light and increasing in the dark. Therefore, the pineal gland is able to send information concerning outside light/dark cycles to central and peripheral structures, thereby synchronizing endogenous rhythms with the environment in which the organism is living. Light is able to both suppress and entrain MLT production on light schedule. MLT can be considered as the output of the endogenous clock. Since the regulating system follows a central and sympathetic nervous pathway, an abnormality at any level modifies the MLT secretion [1–2].

An involvement of melatonin (MLT) in schizophrenia was suggested by McIsaac on the basis of the structural similarity between MLT and a hallucinatory substance [3]. It has been also hypothesized that MLT catabolism could produce abnormal compounds involved in the pathogenesis of schizophrenia [4]. Recently, data about MLT secretion in schizophrenia are controversial. Several experimental studies indicate reduced nocturnal MLT plasma levels in schizophrenic patients [5–6]. Also in circadian rhythm studies (24 h), the nocturnal MLT peak was absent in schizophrenics [7], showing the lack of the characteristic circadian pattern of MLT secretion [8–9] or a phase advance in drug free schizophrenics [10]. A possible connection between pineal gland and schizophrenia has been suggested also by neuroimaging studies. In a previous Computer Tomography study on pineal calcification (PC) we found no significant differences of calcification size between schizophrenics and healthy controls, even though the incidence of PC was significantly higher in the age subgroup of 21–25 years of schizophrenics [11]. However a MRI study on pineal volume found a significant small volume in schizophrenics than in healthy controls [12].

Aim of this study was to verify if a simple index of MLT circadian rhythm as night-day  $\Delta$  is able to confirm

the existing data in schizophrenia and to investigate if the relationship of possible MLT abnormalities to the schizophrenic disease instead of the actual clinical state can be suggested.

## Material and methods

Ten consecutively admitted male schizophrenic inpatients (2 disorganized, 5 paranoid, 2 indifferentiated, 1 catatonic), as diagnosed by DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) criteria [13], were examined. The mean age of the subjects was  $27.9 \pm 6.4$  years. Six patients were on stabilized neuroleptic treatment, two patients were drug free and two patients were drug naive. All the patients were tested after a stabilization of sleep/wake cycle.

The blood samples for melatonin measurement were collected at 3.00 a.m. and 15.00 p.m. Radioimmunoassay melatonin determination in plasma was performed using available commercial kit (Stockgrand Ltd., Guildford, U.K.), without extraction of the sample [14]. Briefly, 500  $\mu$ l of each sample or standard were added to assay tubes, in duplicate. Then, 200  $\mu$ l of a specific antiserum were combined with the previous (except total counts and non-specific binding tubes), vortexed and incubated at room temperature for 30 min. The sheep antiserum used in the assay (G/S/704-8483), raised against N-acetyl-5-methoxytryptophan, was sufficiently specific for clinical application, without pre-assay sample preparation. The relative specificity was 100% for melatonin, less than 1% against N-acetyltryptamine, 6-hydroxymelatonin, N-acetyltryptophan, and less than 0.07% for other indoles. The lower limit of sensitivity of the standard curve was 2.5 pg/tube. The coefficients of variation intra- and inter-assay were 6.8% and 10.2%, respectively. After incubation, all the tubes received 100  $\mu$ l  $^3$ H-melatonin, then vortexed and incubated for 18 hours at 4°C. The separation between the antibody-bound melatonin and the free fraction was obtained by incubation for 15 min at 4°C in ice, with 500  $\mu$ l dextran-coated charcoal, stirred continually on ice 30 min before and during addition. After quick vortex, in order to reduce intra-assay variation, the tubes were centrifuged at 1500 g for 15 min at 4°C. The supernatant was removed (700  $\mu$ l) into vials containing 4ml of a toluene-based scintillation fluid. Vials were shaken for 1 hour, and the radioactivity counted in all tubes. Melatonin concentration was determined from the standard curve and the results expressed in pg/ml.

The difference between night and day MLT (MLT h.3.00–MLT h.15.00 =  $\Delta$ ) concentrations was then calculated. According to the value obtained patients were divided into two subgroups:  $\Delta < 30$  pg/ml and  $\Delta > 30$  pg/ml, taking 30 pg/ml as an arbitrary value, based on literature data, that should indicate a physiologically correct value of  $\Delta$ .

Psychopathological assessment of patients was conducted by two independent senior psychiatrists blind to other protocol data (interrater reliability I.R. = .97) using the Scale for the Assessment of Positive Symp-

toms (SAPS), the Scale for the Assessment of Negative Symptoms (SANS) [15] and the Positive and Negative Syndrome Scale (PANSS) [16].

Student's *t*-test was used to assess statistical differences between groups. The Pearson's coefficient was used to investigate statistical correlations between  $\Delta$  with psychopathological scales (subscales scores included) in the overall sample.

## Results

The 70% of the sample was under the 30 pg/ml value of  $\Delta$  (mean  $\pm$  sd: 13.61  $\pm$  4.0) therefore lacking of the characteristic circadian pattern of MLT secretion, whilst the 30% of the sample was over the 30 pg/ml value of  $\Delta$  (mean  $\pm$  sd: 83.60  $\pm$  16.34) or was in presence of the characteristic circadian pattern of MLT secretion. Student's *t*-test showed that this difference was statistically significant ( $p=.0001$ ).

No correlation was found between  $\Delta$  values and the psychopathology scale and subscales scores.

## Discussion

The use of a simple index such as night/day  $\Delta$  leads to confirm the lack of the characteristic circadian pattern of MLT secretion in schizophrenics with respect to normal adult population. The absence of significant correlation between night / day melatonin level differences and actual psychopathology variables should indicate that the reduction of  $\Delta$  could be mostly related to the disease and independent from the clinical state. The sample size in our study is too small to get indications about differences in clinical forms of the illness, previously suggested by Monteleone *et al.* in 1992 [17] regarding the circadian rhythm absence of plasma melatonin in paranoid schizophrenics.

Due to the wide potential clinical heterogeneity of the illness, several factors could influence the pattern of MLT secretion in the patients, but the substantial homogeneity of the results and their statistical significance even in a not large group of subjects are suggestive of a disease-related finding. If MLT secretion is altered in schizophrenics independently from symptom features, some more direct relationship to pathogenetic aspects of the illness can be hypothesized. These could mainly regard neurotransmitter system involvement and hormonal / behavioural biorhythm modifications.

The secretion of melatonin, as the endocrine output of the suprachiasmatic circadian clock, is regulated by several neurochemical systems (mostly noradrenergic, serotonergic and cholinergic), but a dopamine influence has been hypothesized as well. In the striatum and limbic areas MLT was found to reduce dopaminergic function in the rat [18]. This could be mediated by 5-HT, known to have a role in regulating dopaminergic activity in the nigro-striatal pathway. It can be speculated that in schizophrenia the reduction of MLT  $\Delta$  can be due either to a disturbance of a dopaminergic modulating action to some extent related to the

complex implication of dopamine in the pathogenesis of the schizophrenic syndromes, or, conversely, to the decreased inhibitory action of reduced MLT levels which may contribute to the dopamine dysregulation, more directly related to the clinical symptoms of the disease.

Data on circadian patterns modifications in schizophrenia are poor so far. Even if few results suggest some potential neuroendocrine alterations (PRL, TSH, GH) [19], most of the findings are still based on clinical observation. The sleep / wake cycle is often disrupted, and also associated to changes in general activity patterns, but to date the role of general aspects of psychopathology cannot be considered in a separate manner, with respect to that of potential primary hypothalamic or pituitary alterations. As a matter of fact, the patients in our sample presented a stabilized sleep pattern in the period preceding the study, therefore reducing the weight of this factor on the results, even though a long lasting influence of previous circadian alterations acting on MLT rhythm cannot be definitely excluded.

A role of neuroleptic treatment in MLT modification can not be excluded even if some reports suggest that antipsychotic drugs do not induce any change in melatonin rhythmicity (7–9–10). Actually, the small sample size of this study does not allow to evaluate the real ability of the treatment to influence the results, therefore opening an interesting issue to be deepened in further studies.

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