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ORIGINAL ARTICLE

Evidence for an association of serum melatonin concentrations with recognition and circadian preferences in patients with schizophrenia

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Abstract

Melatonin, a neuro-differentiation factor, may play a role in the neurodevelopmental origins of schizophrenia. Cognitive impairment and decreased melatonin are reported in schizophrenia; however, the relationship between them remains unclear. We hypothesised that patients with schizophrenia would have lower concentrations of circulating melatonin than healthy controls and that melatonin levels would be associated with cognitive impairment. This study included 47 patients with schizophrenia and 40 healthy controls (HC). Serum melatonin concentrations were measured using the enzyme-linked immunosorbent assay. Positive and Negative Syndrome Scales (PANSS), The Morningness-Eveningness Questionnaire (MEQ), Pittsburgh Sleep Quality Index (PSQI), the Stroop and Oktem verbal memory processes (VMPT) tests were applied. Patients with schizophrenia had lower levels of melatonin compared to the HC group ($p = 0.016$), also after controlling for age, sex, and body mass index (BMI) ($p = 0.024$). In patients with schizophrenia, melatonin concentrations were associated with higher BMI (rho = 0.34, $p = 0.01$) and lower MEQ score (rho = −0.29, $p = 0.035$). The patient sample was split into low and high melatonin categories by using the median melatonin concentration in HC as the cut-off. Patients in the low melatonin group had poorer performance in VMPT-Recognition ($p = 0.026$) and Stroop-Colour Error ($p = 0.032$). Notwithstanding its limitations, the findings of this exploratory study suggest that decreased serum melatonin concentrations observed in schizophrenia might also be associated with cognitive impairment and circadian preferences. Future studies are required to investigate the role of melatonergic pathways in patients with schizophrenia.

Keywords Psychosis · Melatonin · Cognition · Verbal memory · Metabolic disturbance · Circadian preferences

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Introduction

Accumulating evidence suggests that melatonin may play a role in the neurodevelopmental aetiology of schizophrenia. Melatonin acts as a neuro-differentiation factor and stimulates neuron maturation during gestation (Galvan-Arrieta et al. 2017). Melatonin deficiency may be involved in impaired axogenesis reported in schizophrenia, including an aberrant neurotransmitter secretion (Cercos et al. 2017). In this regard, low levels of melatonin may be a trait biomarker of schizophrenia, which maybe reflects an early neurodevelopmental abnormality.

The reduced levels nocturnal (Fanget et al. 1989; Monteleone et al. 1992; Monteleone et al. 1997; Robinson et al. 1991) and early morning (Rao et al. 1990) melatonin in patients with schizophrenia have been reported with the exception of studies showing no difference between groups (Beckmann et al. 1984)

and alteration of sleep-promoting effects with no statistical differences in melatonin concentration (Afonso et al. 2011). Additionally, circadian phase advance of melatonin (Rao et al. 1994), advanced circadian timing of melatonin in rest-cycle (Wirz-Justice et al. 1997), disrupted melatonin patterns (Jiang and Wang 1998), lack of typical diurnal variation in melatonin (Bersani et al. 2003) and extended and not circadian entrained rhythm of melatonin (Wulff et al. 2006) have been reported in patients with schizophrenia. Afonso et al. has found significant lower melatonin production in monozygotic twin-discordant for schizophrenia and proposed that the impaired activity of the pineal gland may be a trait marker of schizophrenia (Afonso et al. 2010). Evidence suggests that melatonin profile is altered in schizophrenia; however, findings are not consistent across studies. The inconsistent findings may be the result of methodological issues and differences across studies, such as relatively small samples, determination of melatonin levels at different time-point and from different biological fluids (Morera-Fumero and Abreu-Gonzalez 2013).

Melatonin is a marker of endogenous circadian rhythmicity. Misalignment of the circadian system has profound effects not only on sleep but also on cognition and psychotic-like experiences (Pocivavsek and Rowland 2018). Further, sleep disturbance is a common feature of schizophrenia (Monti et al. 2013) and influences functionality, mood, quality of life and cognition (Krystal et al. 2008; Palmese et al. 2011). Additionally, sleep deprivation in healthy individuals negatively impacts brain function and may result in psychotic-like experiences and impaired cognition (Pocivavsek and Rowland 2018). Additionally, the genetic variability in a number of clock genes has been associated with midbrain dopamine regulation and reward processing that are disturbed in patients with schizophrenia. These findings suggest that sleep–wake disruption in schizophrenia may have a genetic basis and serve as an endophenotype candidate (Manoach et al. 2016). Despite these findings, few studies examined the link between sleep disturbances and impaired cognition in patients with schizophrenia (Manoach et al. 2016; Tek et al. 2014). Only a small sample-sized case-only study showed an association between circadian amplitude and cognitive performance (Bromundt et al. 2011)

In the current study, we hypothesised that patients with schizophrenia would have lower early morning concentrations of circulating melatonin than healthy controls, and that melatonin levels would be associated with cognition scores. In addition, we hypothesized low melatonin level may underlie neurodevelopmental pathoetiology of schizophrenia and not relate with medication status or symptom severity of illness. Therefore, we try to explore the relationship between melatonin levels and clinical severity (PANSS subscales), antipsychotic use and potential cofounder clinical factors (BMI, circadian preferences (MEQ) and subjective sleep parameter (PSQI)). To our knowledge, our study is the first study to investigate whether early morning melatonin concentrations in patients with schizophrenia are lower than those in healthy controls, and if so, whether circulating melatonin concentrations are associated with cognitive performance.

Methods

Study population

Forty-seven patients with schizophrenia and 40 healthy controls (HC) were enrolled to the study (Table 1). Patients were recruited from the outpatient unit of the Department of Psychiatry, Bezmialem Vakıf University. Diagnosis of schizophrenia was ascertained by the medical records and consequently confirmed with the Structured Clinical Interview for DSM-IV-Patient Edition [21]. Clinical stability, defined as no change in medication dosage and no hospitalization in the past six months, was an inclusion criterion. For both groups, exclusion criteria were substance use disorders, learning disabilities, dementia, age < 18 and > 65 years (to prevent confounding of age-related cognitive deficits), use of hormone treatments including oral contraceptives, diagnosis of chronic systemic diseases (e.g., hyper- and hypothyroidism, diabetes mellitus, Cushing syndrome, autonomic nervous system diseases, cardiovascular system diseases), and pregnancy or lactation within the previous 12 months. An experienced psychiatrist interviewed healthy controls for clinical depression and other mental disorders that may influence cognition and melatonin production. Subjects who reported having experienced night shift work or trans meridian travel within the previous 6 months were also excluded. Both groups were matched regarding age, sex, ethnicity, body mass index (BMI), and smoking habits.

This study has been conducted with the approval of the Ethics and Scientific Committees of the Bezmialem Vakıf University with the number of 18/9–13.09.2017. Written informed consent was obtained from all participants.

Instruments

The positive and negative syndrome scale

The Positive and Negative Syndrome Scale (PANSS) is a 30-item clinician-rated scale developed to assess symptom severity in schizophrenia. All patients were applied the PANSS to include three subscales for different types of symptoms: positive symptoms (7 items), negative symptoms (7 items), and general psychopathology (16 items). The items are scored on a 7-point Likert scale ranging from 1 to 7 (absent to extreme severity). Higher scores indicate higher symptoms severity and impairment.

The verbal memory processes test

This test is an analogue of the Rey Auditory Verbal Learning test in Turkish language, and was developed by Oktem–Tanor (Oktem 1992). Oktem the Verbal Memory Processes Test (VMPT) assesses immediate memory, learning or knowledge acquisition process, and retention and recall processes. The VMPT uses a 15-word list that is read to the participant 10 times and provides scores for the following cognitive domains: total learning (the sum of the correct words recalled in all ten trials), highest learning score (the number of most remembered words in a single trial), recognition (the number of correctly recognized words in the list) along with short-term recall, delayed recall, and inconsistency scores that assess an impairment of memory organization.

The Stroop test

The Stroop test reflects activities of the frontal lobe and is considered the most selective assessment of inappropriate stimulus inhibition. The Stroop test measures selective attention, interference inhibition, and processing speed, as well as cognitive flexibility and executive function (Kanne et al. 1998). Participants are asked to immediately say the colours of the rectangles at the first stage, and then to immediately say the names of the written colour at the second stage. After the tendencies have been noted, at the final stage, the participants are asked to say the ink colour of the words on the cards instead of reading the names of the written colours. The number of commission errors and time difference between colour and word reading tasks provides the performance measures.

The Morningness-Eveningness questionnaire

The Morningness-Eveningness Questionnaire (MEQ) (Horne and Ostberg 1976), consists of 19 items and evaluates chronotypes on a scale of 16 to 86. MEQ classifies subjects as ‘morning types’ (59–86), ‘intermediate types’ (42–58) and ‘evening types’ (16–41). The psychometric properties of the Turkish version of the MEQ were tested and its validity and reliability were found to be similar to the original version (Punduk et al. 2005).

The Pittsburgh sleep quality index

The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989) was developed to evaluate the subjective sleep quality over the past month. The seven-component scores range from 0 to 21 in total, score of higher than five indicates worse sleep quality.

Enzyme-linked immunosorbent assay (ELISA)

After 12 h of fasting, venous blood samples were collected into tubes (BD Vacutainer®, UK) under low light between 7:00 A.M. and 8:00 A.M. Samples were then separated by centrifugation (10 min at 2500 x g, 4 °C) and stored at −80 °C until subsequent use. The Human Melatonin levels were measured with a Competetive-ELISA using commercial kits (Elabscience; lot no: AK0016JUL05043; PRC) and an ELISA reader (Multiskan FC® Microplate Photometer; Thermo Scientific; United States). Melatonin pre-coated 96-well ELISA plates were used. The standards and samples were added to the micro ELISA plate wells combined with the specific antibody. During the reaction, Human Melatonin in the sample or standard compotes with a fixed amount of melatonin on the solid phase supporter for sites on the Biotinlated Detection Ab specific to Human Melatonin. Excess conjugate and unbound sample or standard were washed from plate, and Avidin conjugated to Horseradish peroxidase was added to each well and incubated. Then a TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of sulphiric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of human melatonin in the samples was then determined by comparing the OD of the samples to the standard curve. The results were expressed in pg/ml. Sensitivity: the minimum detectable dose of Human Melatonin was 9.38 pg/mL. Detection range was 15.63–1000 pg/mL.

Statistical analysis

The characteristics of the study sample were reported using descriptive analyses. Normal distribution was ascertained using the Kolmogorov–Smirnov test; consequently, the Student’s t test or the Mann–Whitney U test were applied to compare continuous variables for normal and non-normal distributions, respectively. To take into account of effects of a priori selected confounds’ (age, sex, BMI), binary logistic regression analysis was performed using the case status as the dependent variable (HC = 0 vs Schizophrenia = 1) and serum melatonin concentration as an independent variable. Spearman correlation analysis were performed for each group separately. For further exploration of the link between cognition and melatonin concentrations, participants in each group were categorized into two subgroups (low melatonin vs high melatonin) using the median level of melatonin in HC conforming to previous study (Balikci et al. 2018); and the cognitive domain scores were compared in schizophrenia and HC groups using one-way analysis of covariance (ANCOVA), also adjusted for the age, sex, and years of education. SPSS for Macintosh version 22.0 was used for statistical analysis. Statistical significance was set at $p < 0.05$.

Results

Table 1 reports demographic features, clinical characteristics, and cognitive scores. The groups differed in terms of marital status, education, and employment.

The group differences in serum melatonin concentrations

The mean serum melatonin concentration was significantly lower in patients with schizophrenia than in HC (Schizophrenia = 134.85 ± 93.93 vs HC = 222.48 ± 22.24; Z = −2.40, $p = 0.016$) (Fig. 1). The logistic regression model controlled for age, sex, and BMI further confirmed that lower serum melatonin concentration was associated with schizophrenia (B = −0.005, SE = 0.002, $p = 0.024$, OR = 0.995).

The correlations between serum melatonin concentrations and variables

The patients with schizophrenia and HC were analysed separately. There was a positive correlation between serum melatonin levels and BMI only in schizophrenia group (schizophrenia [rho = 0.34, p = 0.01], HC [rho = 0.29, $p = 0.06$]). Serum level of melatonin was negatively correlated with the MEQ scores only in schizophrenia group (rho = −0.29, $p = 0.035$). Table 2 shows the correlation matrix.

The differences between high and low melatonin groups in schizophrenia and healthy controls

The sample was split into low and high melatonin categories by using the median melatonin concentration in HC as the cut-off (Table 3). A statistically significant difference was observed between high and low melatonin groups in schizophrenia with regard to VMPT-Recognition (F = 1.379, df = 41, $p = 0.026$), Stroop-Colour Error (F = 2.01, df = 42, $p = 0.032$), and MEQ scores (F = 1.994, df = 40, $p = 0.003$). In HC, Stroop-Time Difference (F = 1.732, df = 35, $p = 0.008$) was different between high and low melatonin groups. VMPT-Recognition (F = 5969, $p = 0.020$, $N^2 = 0.142$) and MEQ total score (F = 10.562, p = 0.003, $N^2 = 0.227$) remained significant after adjusting cofounders in schizophrenia group, while Stroop-Time Difference (F = 4.498, $p = 0.042$, $N^2 = 0.123$) remained significant in HC.

Table 1 Demographic and clinical features

	Schizophrenia ($n = 47$)	Healthy Controls ($n = 40$)	Test statistic	p
Age (mean ± SD)	42.08 ± 10,69	39.27 ± 11	F = −0.919	0.161
Female (%)	17 (36.2)	19 (47.5)	$X^2(1) = 0.756$	0.384
Marital Status (Single %)	39 (83)	15 (37.5)	$X^2(4) = 20.204$	< 0.001
Education (mean ± SD)	7.67 ± 3.9	11.13 ± 5.19	F = 15.315	0.001
Unemployment (%)	38 (80.8)	9 (22.5)	$X^2(5) = 45.403$	< 0.001
BMI (mean ± SD)	28.16 ± 5.76	26.73 ± 4.64	F = 0.537	0.309
Smoking (Yes %)	23 (48.9)	19 (47.5)	$X^2(2) = 0.777$	0.678
PSQI Total	7.04 ± 3.11	4.81 ± 3,20	F = 0.189	0.001
MEQ Total	52.13 ± 7.54	55.66 ± 7.95	F = 0.116	0.059
VMPT-Immediate memory	4.00 ± 1.63	5.60 ± 1.80	Z = −4.002	< 0.001
VMPT-Total learning score	72.08 ± 21.42	108.40 ± 22.59	F = 0.169	< 0.001
VMPT -The highest learning score	9.71 ± 2.67	13.33 ± 2.15	Z = −5.680	< 0.001
VMPT -Inconsistency	7.73 ± 2.83	5.82 ± 3.98	Z = −2.088	0.037
VMPT-Recognition	6.54 ± 2.11	4.00 ± 2.37	Z = −4.584	< 0.001
Stroop- Colour Error	2.95 ± 5.40	0.51 ± 094	Z = −3.120	0.002
Stroop-Time Differences(sec)	26.06 ± 2.38	14.90 ± 7.64	Z = 7.195	< 0.001
Duration of illness (mean ± SD)	18.36 ± 9.67			
Age of onset (mean ± SD)	23.82 ± 8.26			
CPZe (mean ± SD)	778.61 ± 457			
PANSS-Total (mean ± SD)	66.10 ± 14.12			
PANSS-Global (mean ± SD)	32.63 ± 6.27			
PANSS-Positive (mean ± SD)	15.23 ± 6.44			
PANSS-Negative (mean ± SD)	18.47 ± 6.23			

BMI Body-mass Index; *PANSS* Positive and Negative Symptom Scale; *PSQI* Pittsburgh Sleep Quality Index; *MEQ* The Morningness-Eveningness Questionnaire; *VMPT* The Verbal Memory Processes Test; *CPZe* Chlorpromazine Equivalent Dose

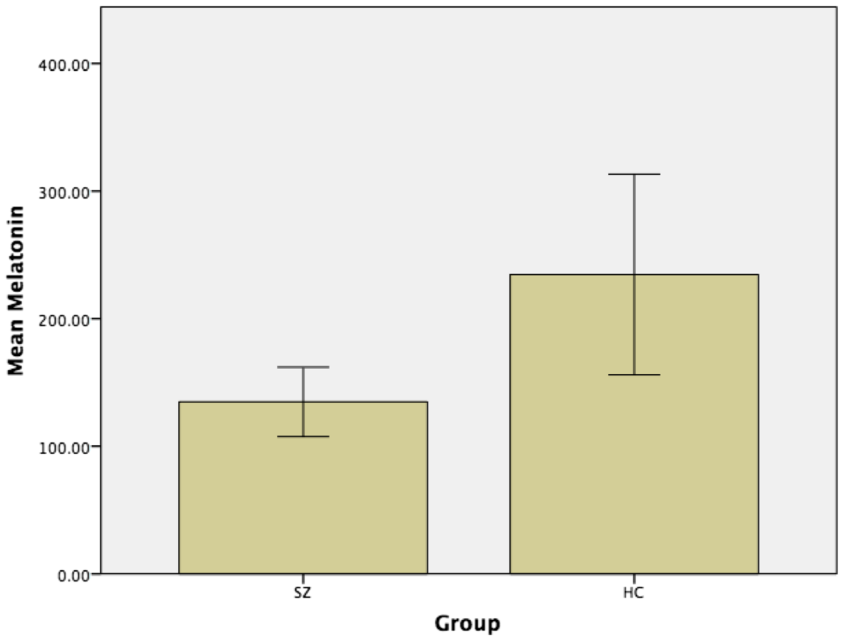

Fig. 1 Serum melatonin concentrations in patients with schizophrenia (SZ) and healthy controls (HC)

Discussion

The major findings of this study were that patients with schizophrenia had lower levels of melatonin compared to the HC group and that melatonin concentrations were associated with lower MEQ score and lower performance on neuropsychological tests of verbal memory in schizophrenia.

Decreased melatonin concentration in schizophrenia

Our findings suggest that patients with schizophrenia have lower circulating concentrations of melatonin. Further analysis revealed that the difference between groups remained significant after controlling for age, sex, and BMI. This finding fits well with previous findings that point out a "low melatonin syndrome" in schizophrenia. The lower and blunted nocturnal melatonin secretion in schizophrenia has been supported by substantial evidence showing that melatonin levels are lower in both drug-naive and treated patients with schizophrenia than in HC (Suresh Kumar et al. 2007) with the exception of one study (Rao et al. 1994). Melatonin production from serotonin is regulated by two important enzymes, the arylalkylamine N-acetyltransferase and the hydroxyindole-O-methyltransferase (HIOMT). A post-mortem study investigating the pineal enzyme activity of serotonin N-acetyltransferase and HIOMT in the brain autopsy of 11 patients with schizophrenia and 67 individuals without a diagnosis of psychotic disorders observed an elevated HIOMT activity by about 25% and speculated that a lack of substrate or abnormally low activity of an enzyme preceding HIOMT in the biosynthesis of melatonin might be involved in the "low melatonin syndrome" observed in schizophrenia. (Smith et al. 1981). Additionally, the disrupted circadian rhythm and the melatonergic system have been associated with the pineal gland abnormalities (Liebrich et al. 2014). Patients with schizophrenia had significantly reduced pineal gland volumes compared to both healthy controls (Bersani et al. 2002) and patients with mood disorders (including unipolar depression and bipolar disorder) (Findikli et al. 2015). Moreover, pineal gland volume reductions appear to be a trait marker and likely the result of delays in the neurodevelopmental process, as neither duration of illness nor treatment differences have been associated with pineal volumetric changes (Gupta 2010). Pineal gland calcification, which may influence the volume of the gland and secretion of melatonin, has been associated with early onset and prefrontal cortical atrophy in schizophrenia as well (Sandyk and Kay 1991).

Cognition and melatonin

We found that lower melatonin level in schizophrenia was associated with poorer performance on verbal memory recognition task after adjusting confounding factors (age, sex, and education). Our findings are in line with a previous study showing that better cognitive functioning is associated with standard circadian properties and poor performance on the Stroop colour-word interference test, while semantic verbal tasks performance in patients with schizophrenia is associated with the low- amplitude melatonin profiles (Bromundt et al. 2011). Additionally, a recent study comparing circadian profiles in young people with attenuated mood syndromes and patients with established mood disorders found that reduced melatonin secretion and shorter phase angles are associated with poorer performance of verbal memory and lower

Table 2 Correlations between serum level of melatonin and variables in schizophrenia and healthy controls

	BMI	MEQ	PSQI	PANSS-G	PANSS-P	PANSS-N	CPZe
Schizophrenia	0.345*	−0.289*	0.121	0.116	−0.104	−0.093	−0.228
Healthy controls	0.287	−0.211	−0.114				

BMI Body-mass index; *PANSS* Positive and Negative Symptom Scale; *PSQI* Pittsburgh Sleep Quality Index; *MEQ* The Morningness-Eveningness Questionnaire; *CPZe* chlorpromazine equivalent dose

*Spearman Rho, correlation is significant at $P = 0.05$

subjective sleepiness in young people with attenuated mood syndromes (Naismith et al. 2012). Patients with schizophrenia have memory impairments in verbal and nonverbal recognition (Aleman et al. 1999; Tripathi et al. 2018) that are associated with deficits in the prefrontal cortex and hippocampus (Ragland et al. 2015). Melatonin may have a potential link with memory and performance, the actions of melatonin may vary with circadian phase, task specificity, or receptor signalling. Furthermore, the function of melatonin on cognition may vary between MT1 and MT2 signalling as an MT2 receptor deletion results in impairments in long-term hippocampal memory (Larson et al. 2006). Pharmacological studies indicate that acute administration of melatonin inhibits long-term potentiation and affects hippocampal neuronal plasticity (Wang et al. 2013), whereas melatonin receptor antagonists phase-specifically enhance night-time memory in diurnal animals (Rawashdeh et al. 2007).

Moreover, the increased level in kynurenic acid (KYNA), which is potentiated by the cAMP/PKA pathway, in the frontal cortex is suggested to cause cognitive deficits and hypofrontality in schizophrenia (Miller et al. 2006). Melatonin is a significant inhibitor of the cAMP/ PKA pathway and therefore would be expected to inhibit the KYNA induced cognitive deficits in schizophrenia (Anderson and Maes 2012). Additionally, cortisol significantly induces Tryptophan 2,3-dioxygenase (TDO) and therefore KYNA, contributing to cortex inhibition and melatonin has been also shown to have a modulatory stress effect on cognitive processing in a stress paradigm via inhibition of cortisol's induction of TDO (Anderson and Maes 2012; Rimmele et al. 2009)

BMI and melatonin

We found a negative correlation between BMI and serum melatonin concentrations in schizophrenia but not in HC. Body weight, BMI, waist-hip ratio, and waist circumference have all been shown to be significantly higher in patients with schizophrenia even when compared to lifestyle-matched controls (Ryan et al. 2004). This suggests that while possibly intensified by external factors such as medication, socio-economic variables, eating habits, and exercise levels, endogenous mechanisms are likely involved in dramatic weight gain observed in schizophrenia (Robillard et al. 2012). Melatonin is relevant in the regulation of several biological functions, and decreased activity of melatonin is linked with the metabolic syndrome (Anderson and Maes 2012; Kamath and Rather 2018). In this regard, it should be noted that people with schizophrenia have lower melatonin levels (Robinson et al. 1991) and higher cortisol levels at night (Thakore et al. 2002). Few preclinical studies suggest that atypical antipsychotics may lower melatonin concentrations and thereby magnify the risk of adverse metabolic effects (Kamath and Rather 2018). However, these findings have not been confirmed in human clinical trials. A comprehensive meta-analysis of randomized-controlled clinical trials of melatonin administration for atypical antipsychotic-induced metabolic adverse effects in patients with mental disorders yielded a slight decrease in BMI in melatonin treatment group but this difference was not significant in the random-effects analysis model (Kamath and Rather 2018).

Circadian preferences in patients with schizophrenia MEQ: Phase-advance

Abundant evidence indicates a link between circadian preferences (chronotypes) and circadian rhythms of various biological measures, such as body temperature, melatonin, cortisol, blood pressure, heart rate, and sleep architecture. (Morera-Fumero et al. 2013). However, few studies have investigated the possible relation between the circadian rhythm of melatonin and chronotypes. Higher MEQ scores (prone morningness) have an advance in the melatonin secretion rhythm phase compared to persons with lower MEQ score (prone eveningness) in the general population (Morera-Fumero et al. 2013). We showed that the MEQ scores and melatonin concentrations were negatively associated (phase advance) in patients with schizophrenia. In a previous study, a phase advance was reported in medication-free patients with schizophrenia, and a similar phase advance persisted in the neuroleptic-treated patients (Rao et al. 1994; Wulff et al. 2012). Although it is somewhat difficult to interpret these findings in the absence of a complete melatonin profile, these findings may suggest that the circadian pacemaker is advanced/ phase-shifted relative to the timing of the sleep-wake cycle.

Table 3 The comparison of low and high melatonin groups within schizophrenia and healthy controls

	Schizophrenia								Healthy Controls							
	Low melatonin $n = 34$	High melatonin $n = 13$	Unadjusted			Adjusted			Low melatonin $n = 20$	High melatonin n = 20	Unadjusted			Adjusted		
			F	df	*P*	F	p	N^2			F	df	*P*	F	p	N^2
VMPT-Immediate memory	3.87 ± 1.49	4.27 ± 1.95	0.248	41	0.408											
VMPT-Total learning score	70.21 ± 22.83	78.63 ± 18.74	1.220	41	0.278				103.61 ± 24.77	111.80 ± 21.34	0.691	39	0.268			
VMPT-The highest learning score	9.50 ± 2.77	10.63 ± 2.46	0.602	41	0.235				12.94 ± 2.51	13.57 ± 1.85	3.563	38	0.380			
VMPT-Inconsistency	7.46 ± 2.98	8.18 ± 2.31	2.013	41	0.476				6.17 ± 3.86	5.71 ± 4.17	1.122	37	0.728			
VMPT-Recognition	7.20 ± 2.16	5.66 ± 1.23	1.379	41	0.026*	5969	**0.020**[a]*	.142	3.95 ± 2.08	3.85 ± 2.60	0.410	38	0.894			
Stroop-Color Error	3.21 ± 5.07	1.14 ± 1.09	6.170	42	0.032*	1.655	0.206[a]	.040	0.47 ± 1.02	0.52 ± 0.87	0.000	38	0.868			
Stroop-Time Differences	26.35 ± 18.25	27.51 ± 11.07	0.877	41	0.839				18.41 ± 8.17	11.91 ± 5.84	1.732	35	0.008**	4.498	**0.042**[a]*	0.123
MEQ total	54.21 ± 6.29	46.50 ± 8.27	1.994	40	0.003**	10.562	**0.003**[b]**	.227	54.63 ± 9.52	57.65 ± 6.40	2.531	27	0.419			
PSQI total	6.62 ± 2.62	8.07 ± 4.03	2.987	42	0.159				5.40 ± 3.18	4.09 ± 3.04	0.000	35	0.222			

VMPT The Verbal Memory Processes Test; *PSQI* Pittsburgh Sleep Quality Index; *MEQ* The Morningness-Eveningness Questionnaire

[a] One-way analysis of covariance Adjusted for age, sex, years of education

[b] One-way analysis of covariance Adjusted for age, sex

Clinical status and melatonin

We found no correlation between melatonin concentrations and clinical characteristics including positive and negative symptoms. However, these findings are to a degree contradicting the idea that negative symptoms (e.g., lack of interest, negative affect, and social withdrawal) mark weak zeitgebers, such as social cues and daylight exposure, and therefore point out a desynchronised circadian activity rhythm in schizophrenia. Clinical trials of melatonin and agomelatine also suggest that melatonin receptor modulation may be helpful in reducing psychiatric symptoms. In a randomized double-blind placebo-controlled study of add-on melatonin, olanzapine plus melatonin 3 mg/day group showed greater reduction in PANSS scores than the placebo group (Modabbernia et al. 2014). Similarly, in an open-label preliminary study testing the efficacy of agomelatine (melatonin receptor agonist) combination with clozapine in 20 outpatients with schizophrenia showed that agomelatine augmentation significantly improved PANSS negative, general psychopathology, total score and overall clinical symptoms (measured by the Brief Psychiatric Rating Scale) (Bruno et al. 2014; De Berardis et al. 2015). To understand the possible relationship with melatonin level and psychopathology, future studies may benefit from differentiating subtypes of schizophrenia.

Antipsychotic use and melatonin

In our study, all patients were on antipsychotics treatment, and we found no correlation between melatonin concentrations and chlorpromazine equivalent dose. In the literature, in medication-free patients with schizophrenia, melatonin concentrations remained stable after treatment with typical antipsychotics (Monteleone et al. 1997; Robinson et al. 1991). Additionally, olanzapine treatment did not influence the melatonin circadian rhythm of a group of previously medication-free patients with schizophrenia (Mann et al. 2006). Quetiapine, another atypical antipsychotic, likewise did not affect melatonin secretion in healthy participants (Morera-Fumero and Abreu-Gonzalez 2013). Melatonin concentrations in the CSF were not different in medicated, drug-free patients, and healthy controls; (Beckmann et al. 1984) while patients on chlorpromazine had increased melatonin concentrations. (Smith et al. 1977).

Limitations of the study

Our findings from the current exploratory research require further confirmation in future studies. We investigated total melatonin levels from the serum collected in the early morning. Our findings may imply that the patient group is advanced/phase-shifted relative to the timing of the circadian system, but it is difficult to interpret these findings in the

absence of full melatonin profile. However, there are concerns that the clinical setting is a challenging environment and continuous collection of biological samples in patients with schizophrenia would be a difficult task to perform. Therefore, total melatonin measurement have been proposed as a pragmatic alternative to continuous collection (Morera-Fumero and Abreu-Gonzalez 2013). Measuring melatonin in serum yields a better resolution and higher sensitivity compared to urine or saliva measurements. Also, serum measurement is particularly helpful for detecting low melatonin concentrations. Although a variety of methods for sampling and analysing melatonin has been defined, there is no consensus on when and how these various methods should be used. In our study, to avoid heterogeneity due to sampling, we collected blood samples under same light and time conditions for all participants. Early morning melatonin concentration is associated with the termination of melatonin synthesis (Synoff), which is also likely to present a unique and essential aspect of melatonin physiology, but there is no consensus on the gold standard for measurement (Benloucif et al. 2008). Synoff represents the changeover from maximum nocturnal melatonin production to the morning start descend (synthesis off), and ensures a measure of the return to daytime melatonin levels. Results should be interpreted cautiously because the melatonin metabolism or excretion can influence morning decline by alterations. Also, we used self-reported measurements of sleep-wake disruption. Actigraphy may provide us with a more granular and objective assessment of sleep and circadian patterns.

Conclusion

Notwithstanding its limitations, our findings from this exploratory study suggest that decreased serum melatonin concentrations observed in schizophrenia might also be associated with cognitive impairment. Further confirmatory studies are warranted to understand the potential link between alterations in levels of melatonin (phase advance and low output) and cognition in individuals with schizophrenia-spectrum disorders. Accumulating evidence from both preclinical and clinical studies highlights the role of melatonin in cognitive and behavioural mechanisms. Melatonergic pathways may therefore be a promising treatment target to enhance cognition and reduce adverse metabolic effects in patients with schizophrenia.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest in this work.

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