Circadian rhythm of salivary serotonin in patients with major depressive disorder

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Abstract

OBJECTIVES: The present study aimed to explore the circadian rhythm of salivary serotonin in patients with major depressive disorder before and after treatment with fluoxetine and its relationship with clinical therapeutic effect.

METHODS: This study investigated salivary serotonin in 13 outpatients with major depressive disorder and age- and sex-matched healthy controls. Depressed patients received six weeks fluoxetine treatment (20 mg/day), saliva was collected before and four weeks after treatment. A total of 8 time-point salivary serotonin was measured across the whole day. Multioscillator cosinor model was used to fit the rhythms.

RESULTS: Serotonin concentration in saliva ranged from 0.32 ng/ml to 9.62 ng/ml. Salivary serotonin showed prominent circadian rhythm in 91% depressed patients and 92% healthy subjects. Circadian amplitude tend to be higher after fluoxetine treatment in depressed patients, so as the ultradian cycle amplitude. The Δ serotonin circadian amplitude (After minus Before) was positively correlated with the decrease of Zung Self-Rating Depression Scale(SDS) scores at day 42 whereas there was no such correlation at day 28. There was no significant difference in the parameters of mesor, acrophase, harmonic and area under curve among three groups.

CONCLUSIONS: Salivary serotonin in patients with major depressive disorder showed clear circadian rhythm. The relationship between the increase of salivary serotonin amplitude and clinical response deserve further study.

ORIGINAL ARTICLE

INTRODUCTION

The role of serotonin (5-hydroxytryptamine, 5-HT) system in depression is well recognized. Peripheral serotonergic parameters have been extensively studied in depressed patients. Platelet-free plasma 5-HT and platelet 5-HT was found to be reduced [9,27], or increased [20] in depressed patients, while one research found there was no significant difference of platelet-free plasma 5-HT between patients with unipolar depression and normal control subjects [1]. One of the explanations for these discrepant results might be that these studies only sampled at one time point (different among these studies) during a day, while it has already been clear now that the levels of the whole blood 5-HT, platelet 5-HT and salivary 5-HT showed diurnal rhythmic patterns [16,18,23–25,33]. The present study aimed to explore the circadian rhythm of salivary 5-HT in patients with major depressive disorder before and after treatment with fluoxetine.

METHODS

Subjects

13 depressed outpatients (7 males, 6 females), age range 19 to 35 (27±5 Mean±SD) and 13 age- and sex-matched healthy control subjects (age 27±4 years old) were recruited in the present study. All patients who fulfilled the criteria for major depressive disorder of DSM-IV[2] were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) [11]. All patients scored at least 16 points on the Hamilton Depression Rating Scale (17 items) [14]. Each depression patient was given a physical and psychiatric examination, routine serum chemistry and hematology studies, electrocardiogram and electroencephalography. Each control subject was screened for physical and psychological health by physical examination, clinical interview and laboratory test if there is any doubt on the subject's healthy status. All subjects gave written informed consent after the procedure had been fully explained. The Institutional Review Board of the University of Science and Technology of China approved the study.

Exclusion criteria were as follows: manic or hypomanic episode before, prominent suicide tendency, alcohol or substance abuse, having taken electro-convulsive treatment (ECT) or long-acting antipsychotics within the last six months, having taken any antipsychotic or antidepressant within four weeks before the study, lactation or pregnancy. Sleep abnormalities, any personal or family history of psychiatric disorder were additional exclusion criteria for the healthy control subjects. The study was performed from August to December (summer and autumn) 2004 in Hangzhou (30 degree North), China. During the research period, control subjects had no medication. The depressive patients began to take fluoxetine, 20mg per day, the next day after they finished principal assessment and collecting saliva in the first day. Five patients took estazolam or lorazepam during the first two weeks when they had problem of sleep. The doses ranged from 0.5 mg to 2 mg per night before bedtime.

Clinical assessment

Clinical effects of fluoxetine treatment were assessed by using Hamilton Depression Rating Scale (HDRS) and Zung Self-Rating Depression Scale (SDS) [35]. The severity of illness item (CGI-S) and the improvement item (CGI-I) of Clinical Global Impression Scale (CGI) were also used before each test. The Apathy Evaluation Scale by a clinician (AES-C) [21] was used for measuring apathy. Beck Depression Inventory (BDI) [4] and Beck anxiety Inventory (BAI) [5] were used for the assessment of global depression and anxiety. State-Trait Anxiety Inventory (STAI) [29] was used for measuring state and trait anxiety. Each control subject was also assessed by these scales (SDS, BDI, BAI, and STAI), their scores were within the normal range.

Saliva collection

The depressed patients and healthy controls were community living. They were instructed to avoid heavy exercise, sexual intercourse or eating cheese, and to avoid drinking alcohol or caffeine on the sampling day. The subjects were instructed to brush their teeth without toothpaste and to rinse their mouths with water 10 minutes before sampling during daytime. All samples were taken under normal light except at 0100 h and 0400 h when subjects were in darkness or the light intensity was <50 lux. Saliva was collected by placing Salivettes (Sarstedt, Numbrecht, Germany) in the mouths without chewing for five minutes, then salivettes were stored at 4 °C immediately during the day time. During the night the salivettes were stored temporarily in a cold box near the subjects' pillows. Saliva was collected from 1900 h to 1600 h the next afternoon, with the time points 1900 h, 2200 h, 0100 h, 0400 h, 0700 h, 1000 h, 1300 h, and 1600 h. One and two hours after supper, saliva was also collected in order to assess the possible effect of food on salivary serotonin level. At 2230 h and 0630 h the next morning, the subjects went to bed/got up, respectively, with the light turned off/on. This schedule is not significantly different from their habitual rest-activity routine. All of the depressed patients got help for sampling from their relatives. At the end of each sampling day the samples were centrifuged for 15 min at 3 000 G , and the supernatants were kept at –20 °C until assayed. In depressed patients, saliva was collected before and after four weeks fluoxetine treatment, respectively.

Salivary serotonin assay

Salivary serotonin was measured by competitive radioimmunoassay (RIA) using commercial kits (Cat No: BA-0900 Labor Diagnostika Nord Gmbh & Co. KG, Nordhom, Germany). Cross-reactivity for tryptamine, melatonin and 5-hydroxyindole acetic acid were 3%, 0.06%, and 0.002%, respectively. The protocol was offered by Labor Diagnostika Nord Gmbh & Co. KG and a modification

of the radioimmunoassay procedure was used. The read concentrations of the saliva had to be divided by 20, and then we got the real concentration. The standard curve of salivary serotonin assay was highly reproducible, with an average correlation coefficient of 0.9987 in this study. The average intra-assay coefficient of variation (CV) was 4.5% and average inter-assay coefficient of variation was 5.1%. Analytical recovery was on average 98%. Linearity was assessed across the range of measurements, with an average recovery of 100% (range 87–110%). The range of standards was 0–2 000 ng/ml and sensitivity was 0.33 ng/ml. All samples were analyzed in duplicate with the operators being blind to any clinical data.

Statistics

Examination of the raw data indicated that both ultradian and diurnal components were clearly present, fitting just a plain 24-hour periodic function [26] will not yield good fit. We used Rao's F-distributed T-statistic [17], which applies a penalty for increasing numbers of parameters, to evaluate several multi-harmonic models and select the best while parsimonious model. Applying Nyquist's sampling frequency rule, our sampling frequency allowed for investigation of ultradian rhythms with periods of 6 hours or more. Curve fitting was performed in each set of data using constrained nonlinear regression analysis (SPSS11.5). Circadian rhythm of serotonin was fitted with complex cosine functions (CCF) [26] with modification. Each set of data was fitted with the following equation:

$$Y(x)=M+A_1 * \cos(x-\phi_1)+A_2 * \cos(u * x-\phi_2)$$

where x represents the time of day (in radians). Y is the predicted value of serotonin at time x. M is the mesor. A_1 and ϕ_1 is the amplitude and acrophase of 24 hours period respectively. A_2 and ϕ_2 is the amplitude and acrophase of the ultradian component, u refers to the harmonic of the ultradian component, where u = 2 or 3, corresponding to rhythms of 12 or 8 h. In 69% of the control, 62% of the depressed patients before treatment and 78% of the depressed patients after treatment, the ultradian rhythm was best described by the 2nd harmonic, corresponding to a period of 12 h. In 23% of the control, 31% of the depressed patients before treatment, and 11% of the depressed patients after treatment, the ultradian rhythm was better described by the 3rd harmonic, corresponding to a period of 8 h, without statistically significant difference among three groups.

The parameters of those individual that showed a significant fit ($p<0.1$) to the CCF were used for further calculation of means. To analysis the differences for serotonin between depressed patients (before and after four weeks treatment) and healthy controls, Students Paired T test was used. Circular statistics were applied for the acrophase data, Jupp's Phi and S for mean angle and angular standard deviation. Mardia-Watson-Wheeler χ^2 test for evaluation of acrophase difference between depressed patients and healthy controls [3]. Multiple regressions were performed to determine the correlation between parameters of serotonin rhythm and clinical variables. A level of $p\leq0.05$ was considered to be significant.

RESULTS

Clinical response

Thirteen depression patients entered this study, nine completed the whole study for six weeks. Two changed to taking other antidepressants. One preferred psychotherapy rather than continued antidepressants. One lost connection due to unknown reason. These four patients all quit this program in the first week. There was a 74% mean decrease in HDRS scores after six weeks of treatment. The mean decrease was 36% in SDS scores. Eight out of nine patients responded well to fluoxetine (lowering of HDRS scores >50%). For this part, more details could be found in Tan *et al*. [31].

Serotonin concentration

The read concentration of salivary 5-HT was above the detection limit in all subjects, real concentration we got by the read concentration divided by 20. The 5-HT concentration in healthy control ranged from 0.43 ng/ml to 7.72 ng/ml, and 0.36 ng/ml to 6.13 ng/ml, 0.32 ng/ml to 9.62 ng/ml in depressed patients before and after treatment respectively. 5-HT levels at 1 h or 2 h after supper showed no significant difference from those before supper in three groups (Table 1).

Table 1. Salivary serotonin level (ng/ml) before and after supper in healthy controls and depressed patients at different clock time (mean ± S.D.).

	Before supper	1 hour	2 hour
Before	1.40±0.45	1.69±0.61	1.09±0.34
After	2.12±0.83	2.01±0.92	1.12±0.46
Control	1.09±0.40	0.97±0.45	1.26±0.49

Table 2. Characteristics of circadian and ultradian serotonin rhythm in healthy controls and depressed patients before and after fluoxetine treatment (mean ± S.D.).

	Before (n=12)	After (n=8)	Control (n=12)
Circadian cycle Mesor (ng/ml)	1.39±0.46	1.36±0.48	1.20±0.39
Amplitude(ng/ml)	0.48±0.23	0.66±0.42	0.62±0.36
Acrophase(h:m)	7:00±1:28	6:29±2:01	6:42±1:36
Ultradian cycle Amplitude(ng/ml)	0.41±0.20	0.86±0.49	0.43±0.21
Harmonic	2.54±0.57	2.13±0.35	2.23±0.43
Area under curve	28.43±12.87	27.36±13.05	25.25±9.50

*Paired t-test. Before treatment group vs. Control subjects, n=12; Before treatment vs. After treatment, n=8; After treatment vs. Control subjects, n=8.

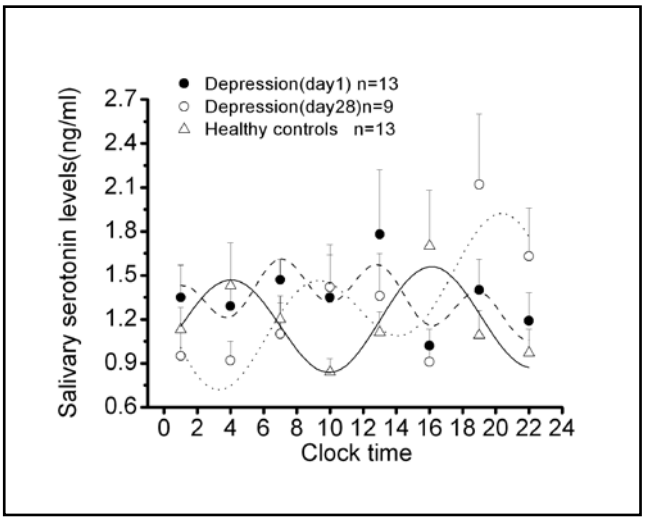

Figure 1. Mean circadian profile of salivary serotonin and fitted model curve for complex cosine functions (CCF). Dashed line, depression (day1), harmonic = 4. Dot line, depression (day 28), harmonic = 2. Solid line, healthy control, harmonic = 2. Vertical lines represent S.D.

Serotonin circadian rhythm

A total of 35 sets of data were gathered in the study. 32 sets data were significant at $p<0.1$ level to fit the CCF (Table 2), other 3 sets data (1 set in each group) were excluded because the p-value was above 0.1 when fitting the CCF. There was also significant consistency of rhythms among healthy subjects and depressed patients by using mean 5-HT levels in each group to fit the CCF (Figure 1). In the control and depressed patients after treatment, the ultradian rhythm was best described by the 2nd harmonic, corresponding to a period of 12 hours. However, the ultradian rhythm was best described by the 4th harmonic, corresponding to a period of 6 hours in depressed patients before treatment.

Figure 2 shows example of raw data and the fitted model of representative individuals. In depressed patients, circadian amplitude tend to be higher after 4 weeks fluoxetine treatment (Before 0.48±0.23; After 0.66±0.42; p=0.13), so as for the ultradian cycle amplitude (p=0.06). There was no significant difference at mesor, acrophase, harmonic or area under curve among three groups.

Relationship between serotonin parameters and clinical response

There was no correlation between depression severity and 5-HT rhythm parameters both before and after treatment. The differences (Δ5-HT) of circadian amplitude (After–Before) was positively correlated with decrease of SDS score ((Before–After)/Before×100%), the correlation reached significance (r=0.80; p=0.016) at 6 weeks but not at 4 weeks (r=0.63; p=0.095) (Figure 3). There was no correlation between Δ5-HT circadian amplitude and decrease of HDRS score (4 weeks: r=0.62; p=0.15; 6 weeks: r= 0.56; p= 0.21).

DISCUSSION

Our results indicate that salivary 5-HT showed clear circadian rhythm in major depression patients. The amplitude tends to be higher after fluoxetine treatment. This effect was associated with the clinical response to treatment after standard oral fluoxetine administration at 6 weeks, but not at 4 weeks.

Salivary 5-HT is a measure of free 5-HT, like platelet free plasma 5-HT including the unconjugated biologically active form of the amine [30]. Though 5-HT in saliva is detectable, its origin is unclear [18,22]. A number of factors have been reported to account for the variability of plasma 5-HT, such as food composition. After a carbohydrate-rich meal, plasma 5-HT increased gradually and reached peak at 2 to 3 hours [7,32]. It is not clear whether salivary 5-HT has such effect. In our research, we compared salivary 5-HT after dinner at 1, 2 hours with base level, there was no significant increase or decrease in patients and control subjects. Seasonal variations were shown for plasma 5-HT and platelet 5-HT [28,34]. In this study, the seasons of each patient and control was same. Age-related decline was reported in platelet 5-HT [12,13], but not in plasma [10,15,30]. No data is available for salivary 5-HT values. In the present study, patients and control subjects are age-matched, and we don't do the correlation between age and salivary 5-HT because the subjects number and range of age are relatively small.

Salivary 5-HT in the present study ranged from 0.32–9.62 ng/ml. This concentration is comparable to the plasma 5-HT concentrations (typically 1 to 2 ng/ml) [10]. In the study of Kennedy *et al.*[19], the normal range of plasma 5-HT is 1.06–8.81 ng/ml. 5-HT concentration in the present study was much lower than that in another report (450±405 ng/ml) [22]. Different collecting and measurement methods might relate to this difference. In this study, saliva was collected by placing a plastic tube in the mouth without chewing for five minutes, then the tube was stored at 4 °C immediately during the day time but placed in a cold box during the night. At the end of sampling day, the samples were centrifuged and the supernatants were kept at –20 °C until assayed by radioimmunoassay. In Marukawa *et al.* [22] research, saliva was collected using self-drainage into sterilized dishes only one time, and the saliva was immediately cooled on ice and centrifuged to obtain the supernatant. The salivary 5-HT was measured using reversed phase high performance liquid chromatography (HPLC) with electrochemical detection. There is a report that norepinephrine was stable in saliva stored at 4 °C for 2 hours but 11% degraded after storage at 25 °C for 1 hour [24]. Both 5-HT and norepinephrine belong to catecholamine neurotransmitters, it is not clear whether the stability of 5-HT be similar to norepinephrine.

In healthy subjects, salivary 5-HT rhythm had two peaks, the highest was at 16:00. This result is accord with 5-HT variation in plasma which was higher at 17:00 than at other clock time [18]. Furthermore, salivary

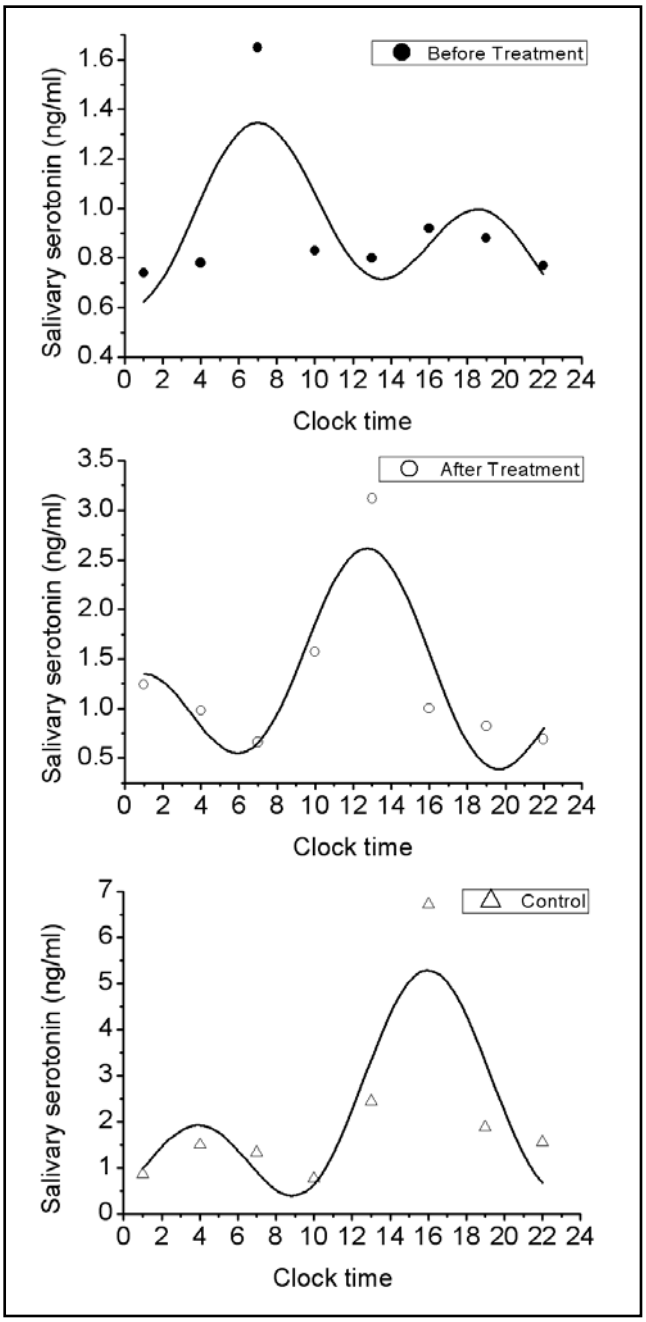

Figure 2. Representative example of raw data points and the model curve of complex cosine functions.

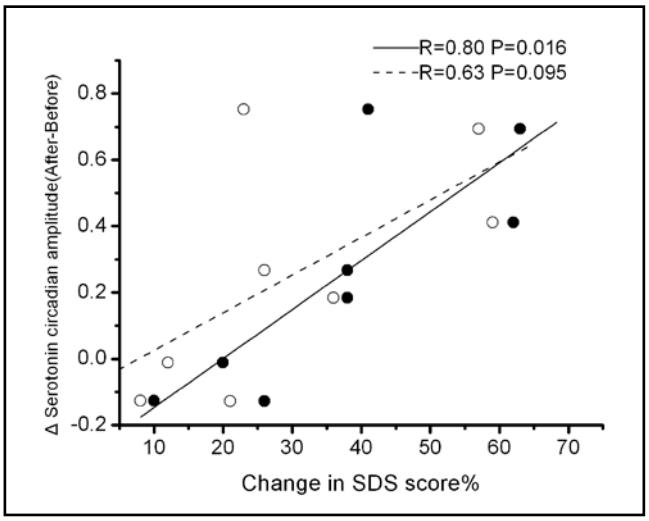

Figure 3. Δ 5-HT circadian amplitude show positive relationship with change in SDS score following fluoxetine treatment. Δ 5-HT amplitude = After–Before. Change in SDS score = (Before–After)/Before×100%. Dot and solid line means day42. Circus and dash line refers to day 28. Note clinical improvement at day 42 was significantly associated with increase of serotonin amplitude.

5-HT rhythm had two peaks in depressed patients after treatment. And the highest peak was around 20:00. 5-HT rhythm in depressed patients before treatment had 3 peaks. It seems salivary 5-HT rhythm in depressed patients tend to be normal after treatment in the context of group cosinor test.

To our knowledge, this study is the first to find that salivary 5-HT show clear circadian rhythm in patients with major depressive disorder. In the present study cosinor analysis of the circadian rhythm revealed a significant rhythm of salivary 5-HT in 91% of the depressed patients and 92% of healthy control subjects. This rhythm was also significant in the group cosinor test. We did not find significant difference in circadian parameters (mesor, amplitude, acrophase), ultradian amplitude and harmonic between depressed patients and control subjects, which implicates salivary 5-HT circadian rhythm might not affected by depression. Alvarez *et al.*[1] studied plasma 5-HT levels in 27 drug-free unipolar depressed patients and also did not reveal any significant difference between patients and healthy controls.

After fluoxetine treatment, the circadian amplitude and ultradian cycle amplitude tend to be higher. Some researches reported that plasma 5-HT increased and platelet 5-HT decreased after fluoxetine treatment for 30 to 40 days in depressed patients [6,8].

We have found Δ5-HT circadian amplitude (day 28–day 1) was positively related with clinical improvement (day 42). This means higher increasing 5-HT amplitude predict better treatment effect in depressed patients treated with a standard dose of fluoxetine. However, the long term prognosis still need to be tested, and the reasons for such association are unclear, the relationship between 5-HT in plasma and saliva with serotonergic system in central nervous system remains to be clarified. Spreux-Varoquaux *et al.* [30] studied 27 depressed patients received either a 25 mg clomipramine slow infusion or a 25 mg clomipramine tablet on day 1. The daily dose was subsequently titrated up to 75 mg i.v. or 150 mg orally. They found plasma 5-HT increased in both i.v. group and oral group on day 1. But only in i.v. group, the increase of 5-HT correlated with Montomery-Asberg Depression Rating Scale (MADRS) decrease over 14 days. They thought the initial plasma 5-HT increase might predict

the clinical response to clomipramine, though its median and long-term predict efficacy remains to be determined.

In conclusion, salivary 5-HT in patients with major depressive disorder shows clear circadian rhythm, no matter being drug-free or after fluoxetine treatment. The relationship between the increase of salivary 5-HT amplitude and clinical response deserve a large sample and prospective study.

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