ORIGINAL ARTICLE

Comparison of salivary testosterone levels in different phases of bipolar I disorder and control group

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Background: Testosterone is considered as a primary sex hormone, also known as an important anabolic steroid, that may involve in various mental disorders such as bipolar I disorder (BID). The goal of this study was to compare the testosterone salivary levels between different phases of BID and its association with the clinical features of BID. **Materials and Methods:** In a case–control study, 15 patients in the mania phase, 10 patients in the depression phase, and 16 in the euthymia phase were selected as patient groups. 18 healthy sex- and age-matched individuals were considered as healthy control group. Salivary samples obtained from all patients and control group and levels of testosterone were determined in saliva using an enzyme-linked immunosorbent assay. All statistical calculations were conducted with the software Statistical Package for Social Science version 20 (IBM Inc., Chicago, IL, USA). **Results:** The mean testosterone level in euthymia phase was 186.34 ± 182.62 pg/mL, mania phase was 239.29 ± 273.22 pg/mL, depression was 153.49 ± 222.50 pg/mL, and healthy participants was 155.73 ± 126.0 pg/mL; no significant difference was found between groups ($P = 0.68$.(No statistically significant differences were found between psychotic and nonpsychotic as well as between patients who attempted suicide and nonattempter patients in terms of testosterone levels ($P > 0.1$). **Conclusion:** Our findings do not reveal significant difference between different phases of BID in terms of salivary testosterone levels. However, more comprehensive studies with larger sample size are required to confirm our findings.

Key words: Bipolar disorder, depression, euthymia, mania, testosterone

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INTRODUCTION

Bipolar I disorder (BID) is known as one of the most important mental illnesses characterized by appearance of at least one manic episode; however, most patients with BID also suffer from episodes of depression.[1] Euthymic period is considered as other episode of bipolar disorder is characterized by neither manic nor depressed phase symptoms; however, some manic or depressed clinical manifestations may remain in the phase.[2] Disease also affects energy, mood, activity, and cognition and is in association with lower functional efficacy, lower quality of life, and rate of suicide.[3] The symptoms of BID in people based on mood vary. The etiology of BID remains unknown; however, it has been proposed that some factors including genetic factors, biological traits, and environmental factors contribute to BID pathogenesis.[4] Testosterone is considered as the primary sex hormone, and an anabolic steroid is the major circulating androgen that is secreted mainly by Leydig cells and ovaries.[5] There are enough reliable published data that testosterone has critical roles in sexology, cognition, and reproduction, and its low or elevated level affects the quality of life and behavior aspects.[6] Recent studies have shown significantly higher levels of testosterone in bipolar and similar behavior disorders.[7,8] Other studies also have revealed the decreased level of testosterone in depression phase of bipolar and other types of depression.[9,10] These studies have demonstrated that the low or high levels of testosterone and its association with the risk

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of bipolar and related disorders, also have evaluated the effect of testosterone level on behavioral brain function.[9-12] Few studies have assessed the level of testosterone in different phases of bipolar disorder including depression, mania, hypomania, and mix phases of bipolar disorder continuously.[12,13] It should be noted that in some instances, the results of these studies are in concordant with else, especially about the level of testosterone in manic phase of bipolar. A study evaluated the plasma level of testosterone and its relation with mania and revealed that the level of testosterone was not higher in manic patients compared to the healthy population.[14] Based on previous studies in regard to the critical role of testosterone in bipolar disorder as one of the most important mental illnesses and related behavioral disorders, and according to the complicated role of testosterone and possibility of its neurobiological and pathophysiological effects on bipolar disorder and the need to optimize the level of testosterone,[7,8] we aimed to investigate the salivary levels of testosterone in depression, mania, and euthymia phases of BID. To the best of our knowledge, our study is the first study which has been investigated and compared the salivary level of testosterone in different phases of BID contentiously and its association with the demographic and clinical features of disease as a diagnostic biomarker.

MATERIALS AND METHODS

Study design and participants

In a cross-sectional 41 type I bipolar patients including 15 patients in the mania phase, 10 in the depression phase, and 16 in the euthymia phase were selected, based on inclusion and exclusion criteria, using convenience sampling from who referred to the psychiatric wards and hospitals affiliated to Isfahan University of Medical Sciences during June–October 2016. Furthermore, 18 healthy age- and sex-matched people were selected as hospital controls among patients' relatives who agreed to participate in our study. After explaining all aspects of study objectives, a written informed consent was obtained from all study participants. The ethics committee of Isfahan University of Medical Sciences approved the study' protocol (study project number: 295067).

The inclusion criteria for BID patients were definite BID according to psychiatrist's diagnosis based on structural and clinical interview for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM5),[1] obtaining the minimum score of Young mania rating scale[15] and Hamilton rating scale for depression,[16] being 20–40 years old, being in the depression, mania or euthymia phase, lack of any associated disorders, lack of substance abuse, not being mentally retarded, not having any oral or dental diseases, not using any kind of drug at least 12 h before the test, and not being pregnant. Healthy participants were matched with patients based on age and sex and had the same condition as patients except not being diseased with BID.

Procedure, study instruments, and assessment of variables

DSM5 of the American Psychiatric Association was used for evaluating BID based on clinical and structural interview.[1]

Hamilton rating scale was used for assessing the severity of depression among patients in depression phase, one patient obtained score as 22 and remaining obtained the scores more than 23 indicating severe and very severe depressive condition. The internal consistency of this instrument in the present study was evaluated using Cronbach's α resulted 0.711 indicating high reliability. Validity and reliability of Hamilton rating scale has been approved previously.[17]

For evaluating the mania phase, Young mania rating scale was used. All 15 participants in this phase got the score more than 17, which approved the manic phase for these patients. The internal consistency of this instrument in the present study was evaluated using Cronbach's α resulted 0.716 indicating high reliability. Validity and reliability of the instrument has been evaluated in Iranian population.[18]

Mental health status of study participants in the control group was evaluated using computerized version of SCL-90-R test.[19] The range of overall severity index for all the participants in the SCL-90-R test was between 0.01 and 0.09 (<1) indicating normal status. The internal consistency of this instrument in the present study was evaluated using Cronbach's α resulted 0.783 indicating high reliability. Validity and reliability of SCL-90-R test has been approved in earlier studies in Iranian population.[20] Furthermore, mental component of short form of health-related quality of life (SF-36)[21] was used for mental status evaluation in the control group. All 18 participants in the control group got the score 200 (50%) and more. The internal consistency of this instrument in the present study was evaluated using Cronbach's α resulted 0.841 indicating high reliability. The validity and reliability of SF-36 in Iranian general population has been evaluated.[22]

Data included age (year), gender (male/female), educational attainment (less than diploma, diploma (12 formal year education) and university graduate, marital status (married/single), smoking habit (smoker/nonsmoker), employment (homemaker, employed, unemployed), and medication use.

Testosterone assay

Participants were asked to brush their teeth and wash their mouth after eating food and do not use any drugs at the night before the test and fasting before the time of the

test. The samples should not have been polluted by any foodstuff, cosmetics, blood, or other external materials. Saliva was gathered between 7 and 8 A. M. Saliva samples were gathered using glass pipes and funnels. 2–5 cc of saliva was gathered from each participant and the samples were transferred to the laboratory. To evaluate the level of salivary testosterone, enzyme immunoassay method was administered using testosterone saliva enzyme-linked immunosorbent assay kit (Diametra, Italy). This kit is only designed for being used at the laboratory and measures the testosterone level from 10 pg/mL to 1000 pg/mL (Picograms per milliliter). Samples with concentration of more than 1000 pg/mL should be diluted. Samples were centrifuged for 15 min by 3000 rpm and then kept at a −20°C for an hour; then again were centrifuged for 15 min by 3000 rpm. Samples were kept at a temperature of 2°C to 8°C for a week or −20°C for longer periods. The standard level of salivary testosterone in this kit for healthy women is between 10 and 55 and for healthy men is between 50 and 210 pg/mL.[23]

Statistical analysis

Quantitative and categorical data were expressed as mean (standard deviation[SD]), median (minimum-maximum), and frequency (percentage), respectively. Normality of continuous data was evaluated using Kolmogorov–Smirnov test and Q-Q plot. Positively skewed testosterone was subjected to logarithmic transformation. Independent samples *t*-test and one-way ANOVA were used for comparing normally distributed data between groups, and Chi-square test was used for categorical data. All statistical analyses were conducted using Statistical Package for Social Science version 20 (IBM Inc., Chicago, IL, USA).

RESULTS

The mean and SD of age of the participants in the euthymia phase was 33.59 ± 4.8 years, in the mania phase was 29.93 ± 7.2 years, in the depression phase was 32.4 ± 3.39, and in the control group was 31.22 ± 9.13 years; no significant difference was observed. The mean and SD of the age of disease's onset in the euthymia phase was 20.68 ± 3.11 years, in the mania phase was 20.86 ± 7.28 years, and in the depression phase was 22.5 ± 4.67 years; the three subgroups of BID patients were not statistically different in terms of age at onset. Table 1 presents the full features of study participants in all study groups; all studied variables are comparable between groups except psychosis number of hospitalization and suicide attempting among bipolar's subgroups ($P < 0.01$).

Figure 1 depicts the mean and 95% confidence interval for mean of salivary testosterone levels in four study groups. The mean ± SD (median [minimum-maximum]) was in euthymia 186.34 ± 182.62 (136.35 [40.70–695.0]), mania 239.29 ± 273.22 (133 [31.3–1025.0]), depressed 153.49 ± 222.50 (175.50 [28.50768.0]), and healthy participants 155.73 ± 126.0 (131.10 [47.3–619.0]); no significant difference was found between groups ($P = 0.68$). Furthermore, no significant difference was found between bipolar patients and healthy participants in terms of mean levels of salivary testosterone – BID patients: 197.69 ± 225.75 (131.10 [28.50–1025.0]) versus control group: 155.75 ± 125 (131.10 [47.30–619]) [$P = 0.46$, Figure 2].

Figure 3 presents the mean salivary testosterone levels in three subgroups of bipolar patients according to psychosis status. As can be seen, the mean levels of testosterone are lower in those who had experienced psychosis than other group; however, the observed differences were not statistically significant ($P > 0.1$, in all subgroups). Furthermore, the comparison of testosterone levels between those who had attempted suicide and who had not, showed no statistically significant difference (for all subgroups of bipolar disorder $P > 0.1$, [Figure 4]).

DISCUSSION

This study compared the salivary level of testosterone between euthymia, depression, and mania phases of BID and healthy control group and its relationship with demographic and clinical features of BID. Our findings revealed that the highest salivary level of testosterone belonged to mania phase and the lowest salivary level of testosterone was related to depression phase. However, the mean of salivary level of testosterone between three groups of patients did not show statistical significance. Furthermore, the difference between salivary level of testosterone in three groups of patients compared to healthy control group was not significant.

During the recent decades, many evidences have been provided on the neuroendocrinology of testosterone in relation to brain activities, cognitive, emotional, and environmental behavior functions, sexual functions, mental health, suicidal behaviors, aging and pathophysiology of mood disorders, psychosis and aggression unobtrusively, and the testosterone's deficiency that may negatively affect the quality of life.[10,24-26] However, a few studies have evaluated the relationship between testosterone levels and bipolar disorder.

Sher *et al*. evaluated the plasma level of testosterone hormone in suicidal women with BID in mixed and depression phases and resulted that the level of testosterone had a positive relationship with bipolar disorder and the number of manic episodes. The level of testosterone also

Table 1: Demographic and basic clinical characteristics of participants in study groups

Variable	Group (study groups)				*P*
	Euthymia (*n*=16)	Mania (*n*=15)	Depression (*n*=10)	Healthy participant (*n*=18)	
Age	33.56±4.80	29.93±7.21	32.40±5.40	31.22±5.14	0.34
Age at diagnostic	20.69±3.12	20.86±7.29	22.50±4.67	-	0.67
Number of hospitalization	6.94±1 0.5 (0-2)	2.86±2.42 2 (1-8)	2.20±1.39 2 (1-5)	-	0.013
Gender					
Male	7 (43.8)	6 (40)	3 (30)	8 (44.4)	0.89
Female	9 (56.2)	9 (60)	7 (70)	10 (55.6)	
Educational level					
Under diploma (12 years formal education)	5 (31.3)	7 (46.6)	2 (20)	3 (16.8)	0.74
Diploma	5 (31.3)	6 (40)	2 (20)	5 (27.8)	
University graduate	5 (37.6)	2 (13.33)	6 (60)	10 (56)	
Employment status					
Homemaker	6 (37.5)	4 (26.7)	3 (30)	6 (33.3)	
Unemployed	2 (12.5)	6 (40)	3 (30)	3 (16.7)	
Employed	3 (18.8)	1 (6.6)	2 (20)	5 (27.8)	
Self-employed	5 (31.2)	4 (26.7)	2 (20)	4 (22.2)	
Marital status					
Single	6 (37.5)	9 (60)	5 (50)	7 (38.9)	0.56
Married	10 (62.5)	6 (40)	5 (50)	11 (61.1)	
Cigarette smoking					
Yes	-	3 (20)	2 (20)	-	
No	16 (100)	12 (80)	8 (80)	18 (100)	
Suicide attempt					
Yes	3 (18.3)	11 (73.3)	9 (90)	-	<0.001
No	13 (81.7)	4 (26.7)	1 (10)	-	
Psychosis					
Yes	0	14 (93.3)	5 (50)	0	<0.001
No	16 (100)	1 (6.7)	5 (50)	18 (100)	
Medicine use					
Lithium carbonate					
Yes	8 (50)	5 (33.3)	2 (20)	-	0.29
No	8 (50)	10 (66.7)	8 (80)	-	
Sodium valproate					
Yes	9 (56.3)	11 (73.3)	6 (60)	-	0.59
No	7 (43.8)	4 (26.7)	4 (40)	-	
Carbamazepine					
Yes	4 (25)	1 (6.7)	1 (10)	-	0.32
No	12 (75)	14 (93.3)	9 (90)	-	
Olanzapine					
Yes	4 (25)	6 (40)	4 (40)	-	0.61
No	12 (75)	9 (60)	6 (60)	-	
Quetiapine					
Yes	3 (18.8)	4 (26.7)	3 (30)	-	0.78
No	13 (81.3)	11 (73.3)	7 (70)	-	
Clonazepam					
Yes	2 (12.5)	2 (13.3)	2 (20)	-	0.86
No	14 (87.5)	13 (86.7)	8 (80)	-	
Lorazepam					
Yes	1 (6.3)	3 (20)	3 (30)	-	0.27
No	15 (93.8)	12 (80)	7 (70)	-	
Haloperidol					
Yes	1 (6.3)	3 (20)	-	-	0.21
No	15 (93.8)	12 (80)	10 (100)	-	

had a positive relationship with major depressive episodes in men.[8]

Sher *et al*. also evaluated the relationship between blood level of testosterone hormone and suicide attempts in

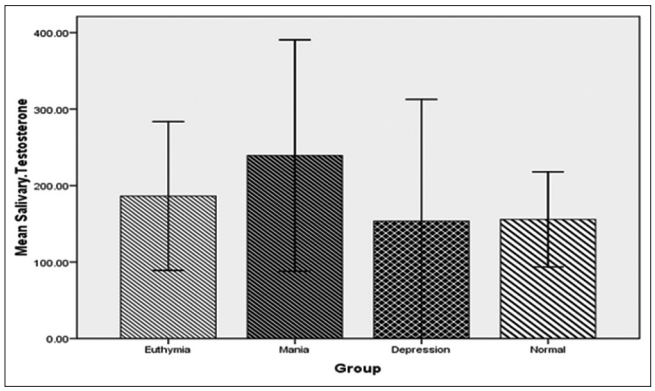

Figure 1: The mean (95% confidence interval for mean) level of salivary testosterone in study groups

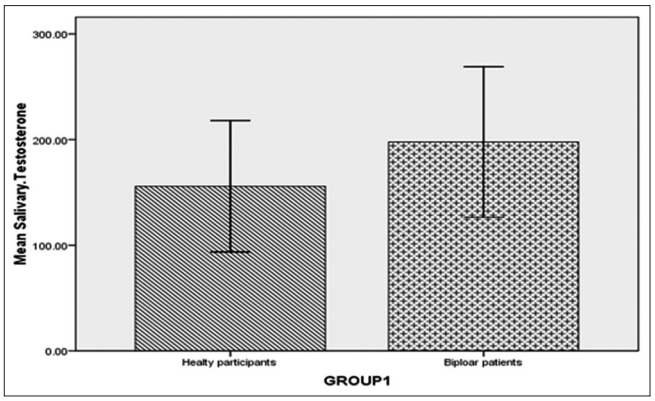

Figure 2: The mean (95% confidence interval for mean) level of salivary testosterone in bipolar patients and control groups

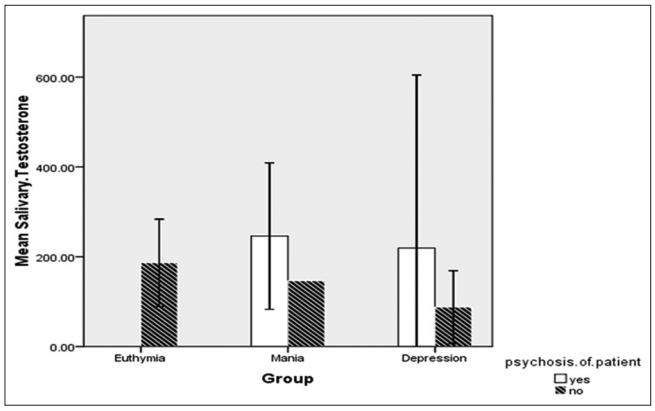

Figure 3: The mean (95% confidence interval for mean) level of salivary testosterone in bipolar patients with and without psychosis

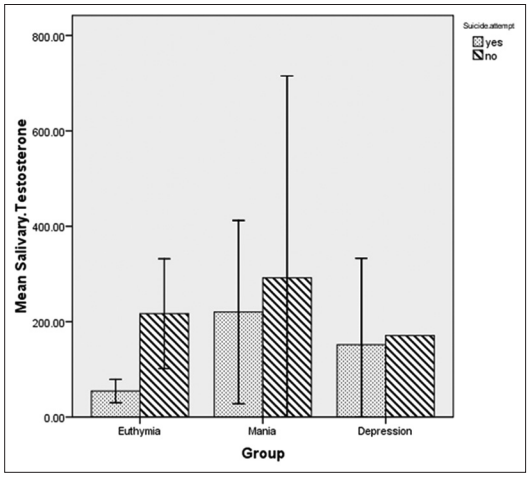

Figure 4: The mean (95% confidence interval for mean) level of salivary testosterone in bipolar patients with and without suicide attempt

bipolar women. It was revealed that the level of testosterone had a positive relationship with major depressive episodes in bipolar women.[12]

However, in our study, we did not observe any significant correlations between testosterone levels and demographic and clinical parameters of BID, but similar to previous studies, testosterone levels in BID patients in males were higher compared to females.

Wooderson *et al*. evaluated testosterone levels in patients with bipolar disorder compared to a healthy control group.[7] The results of this study showed that testosterone levels were significantly lower in male patients with bipolar disorder compared to male controls; women with bipolar disorder had significantly higher testosterone levels than female controls.

Contrary to this study, our findings revealed elevated salivary testosterone levels in BID patients compared to healthy control group, but this difference was not significant [Figure 2].

The most important limitations of the present study were small sample size, time constraints and low budget, addiction of a large number of hospitalized patients, reluctance or withdrawal of a number of patients.

CONCLUSIONS

This is the first study to compare the salivary levels of testosterone between the phases of BID and control group. Previous studies have examined plasma levels of testosterone.

This study revealed that there was no significant difference in the salivary levels of testosterone between mania, depression, and euthymia phase in bipolar disorder and in healthy control group. There was no significant difference in any of the demographic and clinical features. Furthermore, there was no significant difference between psychotic and nonpsychotic patients and suicidal attempters and nonattempters. Authors' reason to select a salivary testosterone was to employ a simple, easy, and noninvasive method for all participants, especially patients in acute phases. The difference between this study and previous studies may be related to the method, sample size, or research environment.

Therefore, the results are not generalizable and should be interpreted with caution; more comprehensive studies with larger sample size are required to confirm our findings.

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Conflicts of interest

There are no conflicts of interest.

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