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ORIGINAL RESEARCH

Investigation of the Relationship of Serotonin, Dopamine and Their Metabolites with α – Synuclein in Obsessive Compulsive Disorder

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Abstract

Objective: Obsessive compulsive disorder (OCD) is a mental disorder characterized by obsessions and/or compulsions. Impulsive features play a role in the etiology, symptomatology and clinical presentation of OCD. α-synuclein which plays a role in dopaminergic and serotonergic neurotransmission has been shown to be effective in impulse control. The aim of this study was to examine the levels of α-synuclein, serotonin (5-hydroxytryptamine; 5-HT), dopamine, dopamine β-hydroxylase (DBH), 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA) and heat shock protein 70 (HSP70) in OCD.

Methods: Twenty-six OCD patients and age-gender matched 23 healthy volunteers were included in this study. Biochemical parameters were analyzed by enzyme-linked immunosorbent assay method. Patients were evaluated with Barratt Impulsivity Scale (BIS) and Dimensional Obsession Compulsion Scale (DOCS).

Results: Compared with the controls, significant reduction of 5-HT, dopamine and DBH levels while significant increase of α-synuclein, HVA and HSP70 levels was found in patients with OCD. Moreover, α-synuclein levels were significantly negatively correlated with 5-HT, dopamine and DBH, while significantly positively correlated with HVA, 5-HIAA and HSP70. HTR and dopamine levels were found to be associated with the subscale of BIS. Dopamine and DBH levels were found to be associated with the subscale of DOCS.

Conclusion: It may be suggested that α-synuclein is associated with dopaminergic and serotinergic pathways, and may provide important contributions for elucidating the etiology of OCD. It can be suggested that the effect of dopamine is more than 5-HT, based on the fact that not only dopamine but also DBH is correlated with OCD symptoms.

Keywords: Obsessive Compulsive Disorder, Impulsivity, α-synuclein, Dopamine, Serotonin

INTRODUCTION

Obsessive compulsive disorder (OCD) is a mental disorder characterized by obsessions and/or compulsions. Impulsive features play a role in the etiology, symptomatology and clinical presentation of obsessive compulsive disorder. Clinical studies reveal that obsessive-compulsive and impulsive symptoms have similar characteristics, both of which have difficulties in delaying or preventing repetitive behaviors (1, 2). The Barratt Impulsiveness Scale (BIS) is one of the most commonly used scales to measure the construct of impulsivity. Findings with BIS showed that the scale was related not only to clinical parameters, but also to biological parameters (3). Factor analytic studies have consistently shown that certain obsessions and compulsions tend to cluster together to form specific symptom dimensions. The most empirically supported symptom dimensions include contamination, symmetry and unacceptable obsessional thoughts. Increasing evidence suggests that a dimensional approach for categorizing symptoms may be of value in genetic, neurobiological, and studies of treatment response (4).

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It was stated more than twenty years ago that serotonin (5-hydroxytryptamine; 5-HT) is closely related to impulse control (5). Reductions in brain 5-HT levels have been shown to reduce inhibition of behavior (6). However, dopamine was thought to play an important role in impulsive behavior because of therapeutic effects of psychostimulant drugs such as amphetamine and methylphenidate in attention deficit hyperactivity disorder. Dopamine transmission in the nucleus accumbens seems to control various aspects of impulsive behavior (7, 8).

Experimental studies show that α-synuclein plays a role in impulsivity (9). These studies reveal that spontaneous deletion of chromosome 6, which leads to the loss of the snca gene, reduces impulsivity in rats. In a different study, impulsivity was found to be reduced again compared to wild-type mice after the conscious deletion of the α-synuclein gene (9, 10, 11). Studies on continuing impulsivity analysis and recombinant mouse lines have revealed the relationship between α-synuclein expression levels in the hippocampus and impulsive response (9). When serum and cerebrospinal fluid levels were examined in different disease groups, it was noted that there was a statistically significant increase in serum levels, similar to the elevated levels of α-synuclein in the cerebrospinal fluid. After these important findings, it was stated by some researchers that the determination of α-synuclein levels and α-synuclein serum/cerebrospinal fluid ratios can serve as a valuable prognostic marker for clinical evaluations (12, 13). Presynaptic α-synuclein protein has been shown to play a role in dopaminergic and serotonergic neurotransmission, and it has also been shown to be effective on impulse control disorders in experimental studies. Moreover, clinical data have reported that an increasing of mRNA and protein expression of α-synuclein in peripheral tissue in presence of impulsivity. However, in a literature review, we could not find any study in which peripheral levels of α-synuclein, dopamine and 5-HT were evaluated together in patients with obsessive compulsive disorder.

Since dopamine and 5-HT have been implicated in OCD and α-synuclein protein has been shown to play a role in dopaminergic and serotonergic neurotransmission, we hypothesized that we would find α-synuclein, 5-HT, dopamine and their metabolites altered by obsessive and impulsive behaviors. Considering these aspects, we aimed to investigate the peripheral levels of α-synuclein, dopamine, 5-HT, heat shock protein 70 (HSP70), dopamine β-hydroxylase (DBH), 5-HIAA and HVA in patients with OCD.

METHODS

Participants

The study was started after receiving ethical approval from the Hitit University Clinical Research Ethics Committee (28.11.2018/19/06). The research was carried out at the Hitit University Çorum Erol Olçok Training and Research Hospital, Department of Mental Health and Diseases, Outpatient Treatment Unit. Twenty six patients aged between 18-65 years who gave verbal and written consent to participate for research were included in the study (Figure 1).

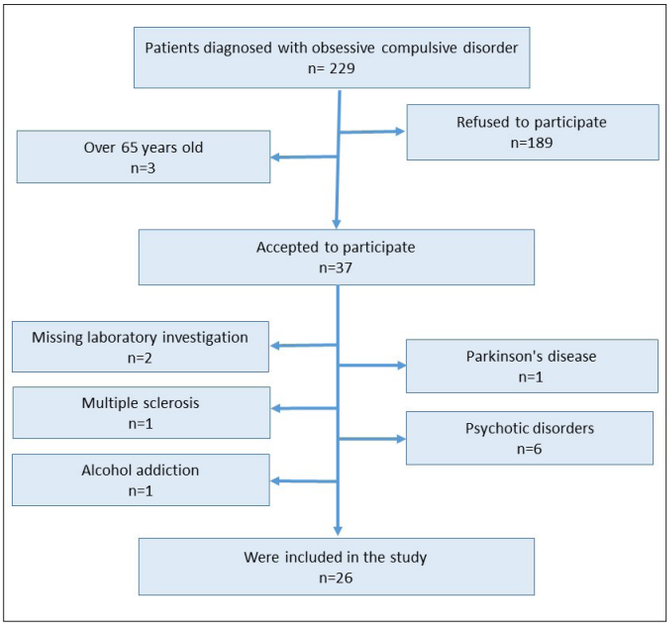

Figure 1. The flow chart shows the selection of participants in the study. In the first phase, 229 patients were informed about the study, and their anthropometric and clinical conditions were started to be determined. Finally, 26 patients who met inclusion and exclusion criteria were included in the study.

The control group of the study consisted of 23 healthy volunteers who were hospital staff, neighbors, friends, as well as compatible with the patient group in terms of age, education, gender, etc.

a) Those who did not have psychiatric disease and were not treated according to DSM-5,

b) Those who do not have neurological disease,

c) Those who were willing to participate in the study and agreed to sign an informed consent form were included in the study.

Patients were evaluated with Structured Clinical Interview for DSM Disorders-5, BIS version 11 and

Dimensional Obsession Compulsion Scale (DOCS), which are detailed below (14-19). It was confirmed that the patients included in the study did not have active additional psychiatric disorders (such as alcohol and substance use disorder) except for obsessive compulsive disorder according to DSM-5 diagnostic criteria. In addition, patients with additional neurological disease (such as degenerative neurological disease, epilepsy, tumor in the central nervous system or cerebrovascular disease), those with a history of head trauma leading to unconsciousness, and mental retardation were not included in the study.

Structured Clinical Interview for DSM Disorders-5

The Structured Clinical Interview for DSM-5 (SCID-5-CV) is an interview guide developed for making DSM-5 diagnoses (14). Turkish validity and reliability studies were performed by Elbir et al. (15).

Dimensional Obsessive Compulsive Scale

It is a 20-item self-report scale developed by Abramowitz et al. (16). It assesses four obsessif compulsive symptom domains (a) contamination, (b) responsibility for harm, injury or bad luck, (c) unacceptable obsessional thoughts and, (d) symmetry, completeness, and exactness. Scores range between 0 to 20 for the subscale(s) (each of the four symptom dimensions), and from 0 to 80 for the total scale. Turkish validity and reliability studies were conducted (17). It was chosen because it is a comprehensive scale that is easy to understand and apply.

Barrat Impulsiveness Scale

It is a self-report scale that provides the opportunity to make a total and dimensional evaluation in terms of impulsivity. This measure consists of 30 items assessing a range of impulsiveness using a four-point scale (1= “rarely/never” to 4= “almost always/always”) and consists of three subscales: attentional, motor, and nonplanning impulsiveness. Turkish validity and reliability studies were conducted (18).

Taking Samples

Blood samples were taken into tubes with cloth activator for serum between 08:00 and 10:00 AM after 12 hours of fasting. The blood kept at room temperature for 30 minutes was centrifuged at 4,000 rpm for 10 minutes and then stored at – 70°C until the analysis.

Analysis of biochemical parameters

Protein and enzyme levels that play a role in synucleopathy were measured with the enzyme-linked immunosorbent method based on the double-antibody sandwich method (Bioassay Technology Laboratory Zhejiang, China; catalog no is E1313Hu for α-synuclein, catalog no is EA0042Hu for 5-HT, and catalog no is EA0041Hu for dopamine, catalog no is EA0014Hu for DBH, catalog no is E1902Hu for HVA, catalog no is E1912Hu for 5-HIAA, catalog no is E1813Hu for HSP70).

Samples of the participants in the patient and control groups were added to the plate wells coated with monoclonal antibody and the plate was incubated as described. Subsequently, an immune complex is formed with biotin-labeled antibody and streptavidin-HRP solution. After the unbound protein and enzymes are cleaned from the medium by washing, the chromogenic reagents A and B solutions are added and incubated at 37 ^{0}C for about 10 minutes in an environment away from light. Finally, a stop solution containing HCl acid is added to inhibit the color change and the optical density of the standard and samples is determined at a wavelength of 450 nm within 10 minutes. By using the optical density and concentration values of the standards, the protein and enzyme levels in the sample are determined.

Statistical Analysis

All statistical analyzes were performed using the Hitit University licensed SPSS 23.0 program (IBM Corp., Armonk, New York, USA). Whether the groups showed normal distribution was determined according to the Shapiro-Wilk analysis. Categorical variables were expressed as numbers and percentages. Normally distributed continuous variables were shown as mean±standard deviation, while non-normally distributed continuous variables were shown as median (25^{th}-75^{th} quartiles). Chi-square test was used for the comparison of discrete data of demographic and clinical characteristics. Student’s t-test or Mann-Whitney U test was used as appropriate. Pearson or Spearman correlation analyses were used for correlation analysis. The diagnostic power of molecules associated with synucleopathy in differentiating between subjects with and without OCD was determined using ROC analysis. Sample size was calculated by using G-power program (19). A total sample size of 16 for each group was enough to have 80% power assuming an α value of 0.05. Statistical significance level was determined as 0.05.

RESULTS

The mean ages of the controls and patient group were 35±8.2 and 37±14.8, respectively. When the groups were compared in terms of mean age, there was no statistically significant difference (*P*= 0.706). Eighteen women and 5 men participated in the control group, while 23 women and 3 men participated in the patient group. When the groups were compared in terms of gender, no statistically significant difference was found (Table 1).

When the results of the BIS are examined, the results for primary level factors showed that attention, motor, self-control, cognitive complexity, patience, and cognitive instability were 9.7±2.1, 13.2±3.0, 16.5±3.4, 10.3±2.4, 8.8±1.3 and 6.0±1.3, respectively. The results for secondary level factors showed that attentional impulsivity, motor impulsivity and unplanned impulsiveness were 16.2±2.6, 21.6±2.9 and 27.3±5.0, respectively.

The principal component values of the dimensional obsession scale items in the OCD group are shown in Table 2.

It was found that α-synuclein serum levels of the patients were significantly higher than those of the control group (*P*= 0.027; Figure 2a). Similarly, serum levels of HVA and HSP70 in patients were significantly higher than those of the control group (*P*= 0.043 and *P*= 0.026, respectively). 5-HIAA serum levels of patients were higher than the 5-HIAA serum levels of control group, but this increase was not significantly different (*P*= 0.056). When the study groups were evaluated in terms of 5-HT, dopamine and DBH serum levels: 5-HT (Figure 2b), dopamine (Figure 2c) and DBH serum levels in the patients were significantly lower than those of control group (*P*< 0.001, *P*< 0.001 and *P*= 0.026, respectively; Table 3).

Table 1. Demographic data of controls and patients with OCD

Parameters	Participants		
	Controls	Patients	*P*
Age (Years)	35±8.2	35±13	0.848
Weight (kg)	75 ±16	75±15	0.892
Height (cm)	165±8.3	165±6.9	0.807
Gender M/F	5/18 (21.7/78.3)%	3/23 (11.5/88.5)%	0.448
Marital status S/M/W	7/16/0 (30.4/69.6/0/0)%	12/13/1 (46.2/50/3.8/0)%	0.245
Alcohol use N/R	21/2 (91.3/8.7)%	26/0 (100/0)%	0.215
Smoking N/H/O	15/5/3 (65.2/21.7/13.0)%	24/2/0 (92.3/7.7/0)%	0.050
Living with A/P/F/P&F	2/4/15/2 (8.7/17.4/65.2/8.7)%	1/12/12/1 (3.8/46.2/46.2/3.8)%	0.151
Education P/M/H/U	0/4/3/16 (0/17.4/13/69.6)%	9/3/7/7 (34.6/11.5/26.9/26.9)%	0.001
Employment U/E	10/13 (43.5/56.5)%	18/8 (69.2/30.8)%	0.069
Presence of MD in the family U/P	-	21/5 (80.8/19.2)%	-
Presence of OCD in the family U/P	-	21/5 (80.8/19.2)%	-
Disease onset age	-	22.8±7.99	-
Treatment start age	-	29.6±10.6	-
Number of hospitalizations N/O/TW/TH	-	17/6/2/1 (65.4/23.1/7.7/3.8)%	-
Suicide Story U/P	-	23/3 (88.5/11.5)%	-
Income Level (BMW/MW/AMW)	-	1/10/15 (3.8/38.5/57.7)%	-

Gender (M/F: Male / Female). Marital status (S/M/W: Single / Married / Widowed). Alcohol use (N/R: None / Rarely) Smoking (N/H/O: None / Half pack / One Pack). Living With (A/P/F/P&F: Alone / Parents / Family / Parents and Family). Education (P/M/H/U: Primary/Middle School/High School/University). Employment (U/E: Unemployed / Employed). Presence of MD in the Family (P/U: Unpresence / Presence). Presence of OCD in the family (U/P: Unpresence / Presence). Number of hospitalizations (N/O/TW/TH:None/One Time/Twice/Three Times). Suicide Story (U/P: Unpresence/ Presence). Income Level (BMW/MW/AMW: Below Minimum Wage / Minimum Wage / Above Minimum Wage).

Table 2. Dimensional obsessive compulsive subscale in the OCD group

		0	1	2	3	4
Subscale	**Basic Components**	**n(%)**				
Contamination	Time Spent	6(23.1)	6(23.1)	11(42.3)	2(7.7)	1(3.8)
	Avoidance	6(23.1)	14(53.8)	4(15.4)	1(3.8)	1(3.8)
	Distress	12(46.2)	-	-	13(50)	1(3.8)
	Impact/ deterioration	6(23.1)	10(38.5)	3(11.5)	6(23.1)	1(3.8)
	Control	4(15.4)	4(15.4)	2(7.7)	15(57.7)	1(3.8)
Responsibility	Time Spent	6(23.1)	10(38.5)	8(30.8)	2(7.7)	-
	Avoidance	7(26.9)	5(19.2)	4(15.4)	10(38.5)	-
	Distress	3(11.5)	13(50)	3(11.5)	7(26.9)	-
	Impact/ deterioration	7(26.9)	5(19.2)	11(42.3)	3(11.5)	-
	Control	5(19.2)	11(42.3)	4(15.4)	6(23.1)	-
Unwanted thoughts	Time Spent	4(16)	11(44)	5(20)	4(16)	-
	Avoidance	3(12)	7(28)	3(12)	12(24.5)	-
	Distress	-	8(32)	3(12)	3(12)	11(44)
	Impact/ deterioration	2(8)	11(44)	6(24)	3(12)	3(12)
	Control	4(16)	7(28)	8(32)	3(6.1)	3(6.1)
Symmetry	Time Spent	8(30.8)	10(38.5)	5(19.2)	2(7.7)	1(3.8)
	Avoidance	6(23.1)	14(53.8)	3(11.5)	2(7.7)	1(3.8)
	Distress	4(15.4)	9(34.6)		12(46.2)	1(3.8)
	Impact/ deterioration	6(24)	12(48)	1(4)	6(24)	-
	Control	4(16)	13(52)	3(12)	5(20)	-

Data were expressed as numbers and percentages for categorical variables.

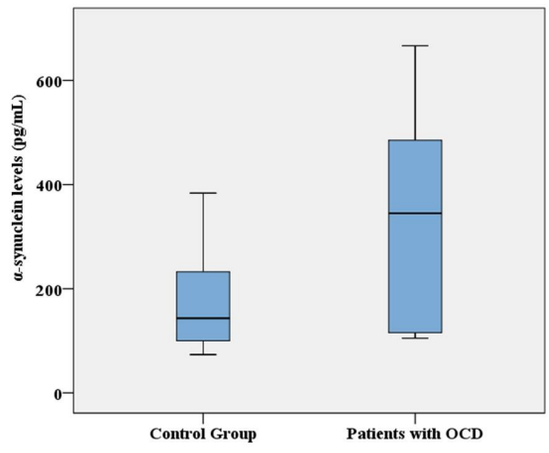

Figure 2a. The box-plot diagram representing the range of serum α-synuclein levels (pg/mL) in patients with OCD versus controls (P=0.027). Lower and upper lines of each box indicate the 25th and 75th percentiles, respectively.

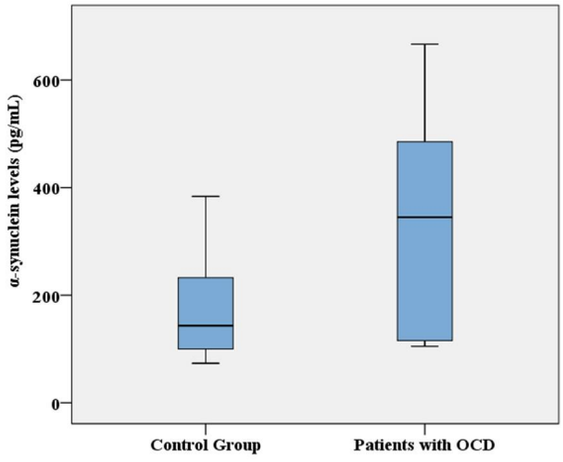

Figure 2b. The box-plot diagram representing the range of serum serotonin levels (ng/mL) in patients with OCD versus controls (P=0.026). Lower and upper lines of each box indicate the 25th and 75th percentiles, respectively.

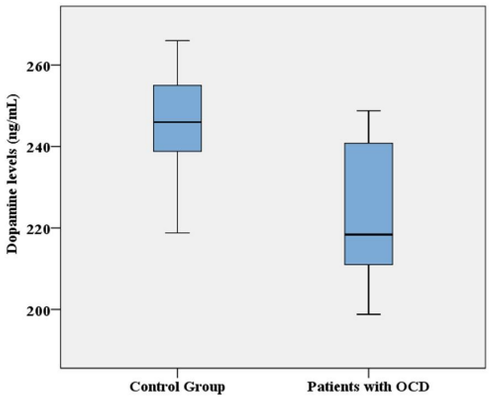

Figure 2c. The box-plot diagram representing the range of serum dopamine levels (ng/mL) in patients with OCD versus controls (P<0.001). Lower and upper lines of each box indicate the 25th and 75th percentiles, respectively.

Table 3. Comparison of groups in terms of biochemical parameters

Parameters	Study Groups			
	Controls n= 23	Patients n= 26	*P*	Effect size
α-synuclein, pg/mL	143(99-233)	144(114-464)	0.027	-
Serotonin, ng/mL	1425±81	1343±91	0.002	0.190
Dopamine, ng/mL	246±13	225±16	<0.001	0.348
Dopamine beta-hydroxylase, ng/mL	1116±37	1027±54	<0.001	0.483
Homovanillic acid, ng/mL	441(340-1112)	540(394-2463)	0.043	-
5-hydroxyndoleacetic acid, mg/L	345(236-891)	426(293-1941)	0.056	-
Heat shock protein 70, ng/mL	9.25(6.19-19.5)	15.9(7.81-41.3)	0.026	-

Data were expressed as numbers for categorical variables, mean±SD, or median (25th-75th quartiles) for continuous variables as appropriate.

α-synuclein levels showed a significant negative correlations with 5-HT, dopamine and DBH (r= – 0.372, *P*= 0.012, Figure 3a; r= – 0.390, *P*= 0.008, Figure 3b; r= – 0.431, *P*= 0.003, respectively). On the other hand, α-synuclein levels showed a statistically significantly positive correlations with HVA, 5-HIAA and HSP70 (r= 0.959, *P*< 0.001, Figure 3c; r= 0.949, *P*< 0.001, Figure 3d and r= 0.891, *P*< 0.001, respectively). Other correlation values between parameters are summarized in Table 4.

Table 4. Associations between biomarkers

		SNCA	5-HT	DP	DBH	HVA	5-HIAA	HSP70
SNCA	r *P*	1.000 <0.001	-0.372* 0.012	-0.390** 0.008	-0.431** 0.003	0.959** <0.001	0.949** <0.001	0.891** <0.001
5-HT	r *P*	-0.372* 0.012	1.000 <0.001	0.735** <0.001	0.659** <0.001	-0.331* 0.028	-0.312* 0.039	-0.214 0.158
DP	r *P*	-0.390** 0.008	0.735** <0.001	1.000 <0.001	0.859** <0.001	-0.421** 0.004	-0.364* 0.015	-0.309* 0.039
DBH	r *P*	-0.431** 0.003	0.659** <0.001	0.852** <0.001	1.000 <0.001	-0.420** 0.005	-0.411** 0.006	-0.400** 0.006
HVA	r *P*	0.959** 0.000	-0.331* 0.028	-0.421** 0.004	-0.420** 0.005	1.000 <0.001	0.977** <0.001	0.901** <0.001
5-HIAA	r *P*	0.949** <0.001	-0.312* 0.039	-0.364* 0.015	-0.411** 0.006	0.977** <0.001	1.000 <0.001	0.961** <0.001
HSP70	r *P*	0.891** <0.001	-0.214 0.158	-0.309* 0.039	-0.400** 0.006	0.901** <0.001	0.961** <0.001	1.000 <0.001

SNCA: *α-synuclein,* ***5-HT:*** *5-hydroxytryptamine (serotonin),* ***DP:*** *Dopamine,* ***DBH:*** *Dopamine β-hydroxylase,* ***HVA:*** *Homovanillic acid,* ***5-HIAA:*** *5-hydroxyindolacetic acid,* ***HSP70:*** *Heat shock protein.* ***r=*** *Spearman correlation coefficient*

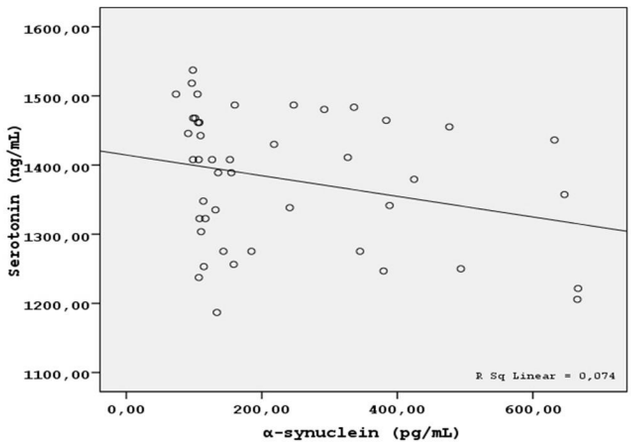

Figure 3a. The scatter plot diagram shows correlation between α-synuclein (pg/mL) and serotonin (ng/mL) in participants (r=-0.372, P=0.012).

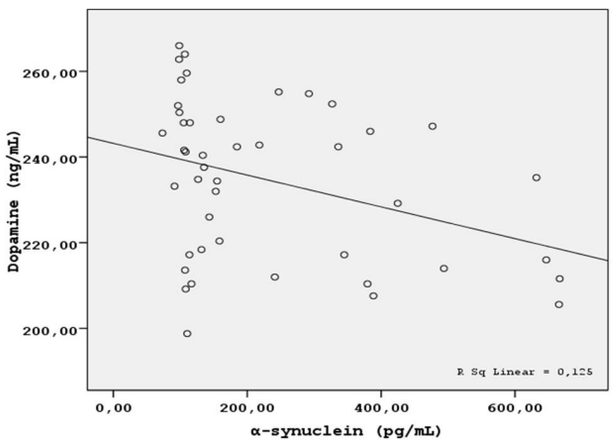

Figure 3b. The scatter plot diagram shows correlation between α-synuclein (pg/mL) and dopamine (ng/mL) in participants (r= – 0.390, P=0.008).

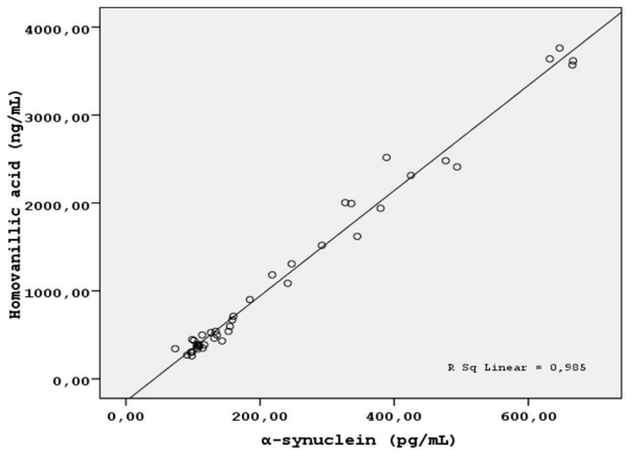

Figure 3c. The scatter plot diagram shows correlation between α-synuclein (pg/mL) and homovanillic acid (ng/mL) in participants (r= 0.959, P<0.001).

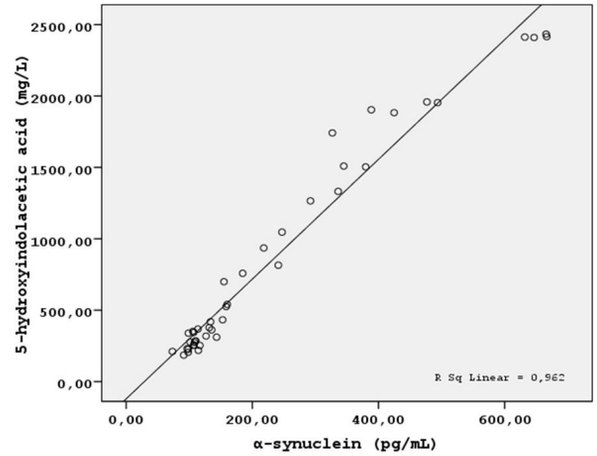

Figure 3d. The scatter plot diagram shows correlation between α-synuclein (pg/mL) and 5-hydroxyindolacetic acid (mg/L) in participants (r= 0.949, P<0.001).

The correlation values between biochemical and clinical parameters are shown in Table 5. DBH levels showed a significantly negative correlations with unwanted thoughts and symmetry (r= – 0.412, *P*< 0.041; r=-0.395, *P*< 0.046, respectively). HTR levels showed a significantly negative correlations with attention (r=-0.513, *P*< 0.007), while positive correlations with motor impulsivity (r= 0.455, *P*< 0.019). Dopamine levels showed a significantly negative correlations with attention (r=-0.399, *P*< 0.043), while positive correlations with motor impulsivity (r=0.461, *P*< 0.018). On the other hand, dopamine levels showed a significantly negative correlations with contamination, responsibility, unwanted thoughts, symmetry (r= – 0.422, *P*< 0.032; r=-0.417, *P*< 0.034; r=-0.530, *P*< 0.006; r=-0.411, *P*< 0.037, respectively, Table 5).

Whether synucleopathy-related molecules could be used in the diagnostic discrimination of OCD was determined by ROC analysis. The values obtained according to this analysis are summarized in Table 4. The differentiation of OCD disorder are shown in Table 6. ROC analysis showed that especially dopamine (*P*< 0.001), 5-HT (*P*< 0.001) and DBH (*P*< 0.001) may be statistically significant biomarkers in the diagnosis of OCD (Table 6).

Table 5. Correlations between biomarkers and obsession and compulsion levels in patients with OCD

	DBH		HTR		DP	
Barratt Impulsivity Scale	**r**	***P***	**r**	***P***	**r**	***P***
Attention	-0.319	0.112	**-0.513****	**0.007**	**-0.399***	**0.043**
Motor Impulsivity	0.372	0.062	**0.455***	**0.019**	**0.461***	**0.018**
Dimensional Obsessive-Compulsive Scale	**r**	***P***	**r**	***P***	**r**	***P***
Contamination	-0.222	0.275	-0.278	0.169	**-0.422***	**0.032**
Responsibility	-0.378	0.057	-0.220	0.280	**-0.417***	**0.034**
Unwanted thoughts	**-0.412***	**0.041**	-0.177	0.398	**-0.530****	**0.006**
Symmetry	**-0.395***	**0.046**	-0.106	0.606	**-0.411***	**0.037**

DBH: *Dopamine β-hydroxylase*, ***HTR:*** *Serotonin*, ***DP:*** *Dopamine.* ***r****=Spearman correlation coefficient*

Table 6. Areas under the ROC curve of parameters for the diagnosis of patients with OCD

Parameters	Area	*P*	Confidence Interval (95%)
α-synuclein	0.750	0.006	0.598-0.902
Serotonin	0.863	<0.001	0.748-0.978
Dopamine	0.910	<0.001	0.824-0.995
Dopamine beta-hydroxylase	0.969	<0.001	0.927-1.000
Homovanillic acid	0.726	0.013	0.572-0.881
5-hydroxyindolacetic acid	0.717	<0.018	0.559-0.874
Heat shock protein 70	0.726	<0.013	0.573-0.880

DISCUSSION

Experimental studies have shown that presynaptic α-synuclein plays a role in impulse control disorders, dopaminergic and serotonergic neurotransmission. Clinical data have reported an increase in mRNA and protein expression of α-synuclein in peripheral tissues with the presence of impulsivity (20, 21). In the literature review, no study was found in which peripheral levels of α-synuclein, dopamine and 5-HT molecules were evaluated together in patients diagnosed with OCD. Therefore, in the present study, we aimed to evaluate the relationship of synuclepathy with dopamine, 5-HT and peripheral levels of metabolites of these important neurotransmitters in patients with OCD.

In this study, α-synuclein levels of individuals diagnosed with OCD were found to be significantly higher than those of the controls. α-synuclein levels showed a negative correlation with 5-HT, dopamin and DBH. However, in the correlation analysis, there was no significant correlation between α-synuclein and clinical parameters.

α-synuclein is mainly localized at presynaptic terminals and has been shown to constitute around 1% of total proteins in the neuronal cytosol. α-synuclein consists of the amphipathic N-terminus, which mediates the lipid-binding properties of the protein, the non-amyloid component defined as the aggregation site, and the acidic C-terminus associated with the calcium binding and inhibition (22).

Many studies have shown that α-synuclein is associated with the regulation of synaptic vesicle reserves, the mobilization of vesicles, and the release of neurotransmitters (23-25). Kanaan et al. showed that α-synuclein may also play a role in the regulation of synaptic transmission (26).

In our study, the findings obtained from dopamine levels in patients with OCD support the results reported in the literature. We found α-synuclein levels significantly higher, while dopamine levels significantly lower in patients with OCD than those of the control group.

It has been raported that the effects of α-synuclein specifically at dopaminergic synapses are seen in the synthesis, release, reuptake, and storage phases of dopamine. Studies show that α-synuclein exerts these effects through the modulation of dopamine external release (9). α-synuclein negatively affects dopamine synthesis by inhibiting tyrosine hydroxylase and amino acid decarboxylase enzyme activities (27, 28). It has been observed that overexpression of the protein decreases dopaminergic release in vitro and in vivo, and increases the release of dopamine in knock-out mouse models. α-synuclein has also been shown to alter the expression and activities of the dopamine transporter and vesicular dopamine transporter (29, 30).

On the other hand, in our study HVA levels were significantly higher, while DBH levels were significantly lower in patients with OCD than those of the control group. In addition, α-synuclein levels showed a statistically significantly positive correlation with HVA, 5-HIAA and HSP70.

Homovanillic acid is an important catecholamine metabolite produced by the sequential action of monoamine oxidase and catechol-O-methyltransferase

on dopamine. Plasma levels of HVA, a metabolite of dopamine, may be a useful measure of brain HVA production by the central dopamine systems. Experimental manipulations that alter brain HVA produce parallel changes in plasma HVA levels (31). Dopamine beta-hydroxylase is the enzyme that converts dopamine to norepinephrine (32). It has been suggested that diurnal variations of DBH activity and HVA concentrations reflect the altered activity of catecholaminergic neurons in the peripheral and central nervous systems. Homovanillic acid levels may also change in catecholamine metabolism disorders. While monoamine oxidase-A deficiency causes a decrease in urinary HVA values, DBH can cause an increase in urinary HVA values (32). Considering the literature, it may be concluded that decreased levels of DBH and increased levels of HVA levels in the present study overlap with finding of "DBH deficiency may cause increased urinary HVA values" (32). On the other hand, α-synuclein levels were significantly positively correlated with HVA, while significantly negatively correlated with dopamine and DBH.

Another important finding in our study was that the 5-HT levels in patient with OCD were found to be statistically significantly lower than those of the control group. In addition, as a result of the statistical evaluations, it was observed that there was a significantly negative correlation between α-synuclein levels and 5-HT levels. α-synuclein levels were significantly positively correlated with 5-HIAA.

It is known that 5-HT is closely related to impulse control. It has been known that the decrease in brain levels reduces the inhibition of behavior and low levels of 5-HIAA, a 5-HT metabolite, in the cerebrospinal fluid are associated with impulsive aggression, violence and suicidal behavior in humans (33). It has been shown that inhibition of 5-HT reuptake with clomipramine and selective 5-HT reuptake inhibitor may be more effective in the treatment of OCD. The decrease of 5-HIAA level in the cerebrospinal fluid, and the decrease in OCD symptoms with clomipramine while it does not respond to desipramine, which has similar antidepressant activity, indicate that 5-HT reuptake has a significant effect in OCD. Researches have reported that the antiobsessional effect in OCD is related to 5-HT receptor blockade that the response to treatment decreased as the values decreased (33).

Rio et al. showed that α-synucleinopathy in 5-HT neurons negatively affects brain circuits that control mood and emotions, resembling the expression of neuropsychiatric symptoms occurring at the onset of Parkinson's disease. Early preservation of 5-HT function by reducing α-synuclein synthesis/accumulation may alleviate Parkinson's disease-related depressive symptoms (34).

In the present study, we found that HSP70 levels in patient with OCD were found to be statistically significantly higher than those of the control group.

Mitochondria is an organelle at the center of the cell's response to ischemia, due to its roles in energy production, free radical formation, and regulation of apoptosis. Heat shock protein 70 is an important chaperone localized in the mitochondria (35). Additional overexpression and accumulation of α-synuclein has been implicated in the etiology and pathology of Parkinson's disease associated with synucleinopathies. Heat shock protein 70 has been reported to protect against α-synuclein-driven neurodegeneration, while high levels of HSP70 cause α-synuclein misfolding in cell and animal models, but little is known about the mechanism of this important protective pathway. It is generally assumed that HSP70 binds to α-synuclein using the canonical and random substrate binding cleft to limit aggregation. Heat shock protein 70 binds translocase proteins to form an ATP-dependent transporter that transfers mitochondrial proteins into the matrix (36). Lewy bodies contain fibrillary α-synuclein protein aggregates that are biochemically similar to the fibrillar protein aggregates found in other neurodegenerative diseases. There have been several studies investigating the interaction between α-synuclein and heat shock proteins, particularly HSP70 (35, 36).

Considering the literature information and the findings obtained in our study, especially the high levels of HSP70 and the positive relationship between α-synuclein and HSP70, strengthens the findings that these two molecules are associated in the etiology of OCD.

In the present study, dopamine and HTR showed a negative correlation with attention while positive correlations with motor impulsivity. Attention and motor impulsivity are two important parameters that are sub-factors of the BIS (37). In the literature review, no study was found that examined the relationships between blood dopamine levels and attention and motor impulsivity in patients with OCD. On the other hand, the findings of experimental studies on brain parts showed that dopamine and 5-HT levels in different structures of the brain are related to attention and motor impulsivity (38-41). Previous studies showed that the dopaminergic system is involved in aggravating or inducing these symptoms (38). Yates et al. found that higher levels of impulsive

action and hyper dopaminergic tone were related in prefrontal subregion (41). Animals exhibiting low levels of impulsive action has higher dopamine D2 receptor mRNA levels in the prelimbic portion of medial prefrontal cortex (42). Moreover, experiments using positron emission tomography showed that low dopamine D2/D3 receptor availability in the ventral tegmental area and substantia nigra is associated with a highly impulsive phenotype (40). The results show that different dopaminergic signals are involved in pathological repetitive behaviors (41).

Impulsivity is defined as the tendency to exhibit behavior without adequate mental assessment of possible outcomes (43,44). Brain imaging and pharmacogenetic studies have demonstrated that 5-HT dysfunction is associated with impulsive behaviors (45).

Dopamine and 5HT have also been shown to play an important role in the regulation of attention and response control in frontal cortex by animal models (46). By this means, it has been concluded that central 5-HT levels are related to waiting impulsivity rather than stopping impulsivity (46).

On the other hand, DBH levels showed a negative correlation with subscale of DOCS including unwanted thoughts and symmetry. Similary, dopamine negative correlations with subscale of DOCS including contamination, responsibility, unwanted thoughts and symmetry. DOCS is a measurement tool that examines the severity of thematically distinct symptom domains of OCD. The most consistently replicated OCD symptom dimensions include contamination, responsibility, symmetry and unwanted thoughts (47). Over the past decade, the DOCS has become a widely used self-report measure to assess OCD around the globe. In fact, researchers have culturally adapted and validated the DOCS for Turkish populations (48). Animal studies have indicated that the dopamine neurotransmitter system is involved in symmetry (49). OCD patients with primary symmetry related symptoms had significantly higher OCD severity scores, longer illness duration, and increased psychiatric comorbidity (50) This condition is characterized by a chronic maladaptive pattern of excessive perfectionism in OCD. This is consistent with a range of data pointing to an important role for the dopamine system in OCD (38). Considering the link between symmetry symptoms and OCD severity noted earlier, as well as the human and animal studies both confirm the involvement of dopamine circuitry in OCD, it is interesting that treatment-resistant OCD specifically may exhibit improved response following adjunctive treatment with a D2 receptor antagonist (51). According to the results of previous studies, positive treatment responses to the dopaminergic antagonists were shown. These results suggest that other neurotransmitter systems, such as dopamine, are involved in the pathophysiology of OCD (38). It may be suggested that changes in the dopaminergic system may reflect the influence of contamination risk, asymmetry and unwanted thoughts in patients with OCD.

The diagnostic power of the analyzed molecules in differentiating people diagnosed with OCD was determined by ROC analysis. According to this evaluation, it was determined that especially DBH, 5-HT, dopamine and α-synuclein could play a significant role in the diagnosis of OCD.

This study has some limitations; such as the absence of cerebrospinal fluid levels of HSP70, DBH, 5-HIAA, α-synuclein, dopamine, HVA and 5-HT. Secondly, the number of participants is relatively low. Thirdly, lack of evaluations for lifetime ADHD and Tic disorder comorbidity among patients with OCD. Lastly, there is no similar serum study in patients with OCD, therefore it is difficult to compare the results of our study. For these reasons, our findings need to be confirmed by the future studies.

CONCLUSION

In the present study, it was found that α-synuclein, HVA and HSP70 levels were significantly higher, while 5-HT, dopamine and DBH levels were significantly lower in patients with OCD than those of the control group. On the other hand, α-synuclein levels were significantly positively correlated with HVA, 5-HIAA and HSP70 while significantly negatively correlated with 5-HT, dopamine and DBH. In conclusion, it may be suggested that α-synuclein is associated with dopaminergic and serotinergic pathways in patients with OCD, and may play a significant role in diagnosis and better understanding the etiology of OCD. On the other hand, HTR levels were found to be associated with the subscale of BIS. DBH levels were found to be associated with the subscale of DOCS. Dopamine levels were found to be associated with the subscales of both BIS and DOCS. It can be suggested that the effect of dopamine is more than 5-HT, based on the fact that not only dopamine but also DBH is correlated with OCD symptoms.

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