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THE RELATIONSHIP BETWEEN ATTACHMENT AND SERUM OXYTOCIN AND HEAT SHOCK PROTEIN-70 LEVELS IN ADOLESCENTS OF PARENTS WITH SCHIZOPHRENIA AND BIPOLAR DISORDER

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Summary

Background: *The aim of this study was to evaluate serum heat shock protein 70 (HSP70) and oxytocin levels, attachment and perceived social support levels in adolescents with parental bipolar disorder (BD) and Schizophrenia (SCZ).*

Subjects and Methods: *This study included 9 adolescents with SCZ parents, 30 adolescents with BD parents and 31 healthy adolescents. Brief Symptom Inventory (BSI), Relationship Scale Questionnaire-Adolescent Form (RSQ-A) and Multidimensional Scale of Perceived Social Support (MSPSS) were administered to all participants. In addition, serum HSP-70 and oxytocin levels were evaluated.*

Results: *There was no significant difference between the groups in terms of attachment style, psychiatric symptoms and perceived social support. Serum HSP-70 levels were found to be lower in adolescents whose parents had BD. Serum oxytocin levels of the SCZ group were significantly lower than those of the BD group.*

Conclusions: *HSP-70 level was found to be lower in adolescents with BD parents. Oxytocin level was found to be lower in adolescents with SCZ parents. These findings suggest that HSP-70 and oxytocin may be a marker of early life stress in adolescents with parental psychopathology. However, studies are needed to evaluate the relationship between attachment, oxytocin and HSP-70 in adolescents exposed to parental psychopathology in early life.*

Keywords: *Attachment, adolescents, bipolar disorder, heat shock protein-70, oxytocin.*

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INTRODUCTION

Parents with psychiatric disorders experience many problems in meeting their children's needs, such as physical, emotional, social and intellectual development. The most important psychiatric disorders that parents have are Bipolar Disorder (BD) and Schizophrenia (SCZ). SCZ and BD negatively affect parenting and parent-child relationship (Alves et al. 2017). SCZ is a complex chronic neuropsychiatric disorder characterized by positive and negative symptoms and cognitive deficits (Avramopoulos 2018). Bipolar disorder is a psychiatric disorder with a chronic course characterized by manic or mixed periods and may be accompanied by depressive periods (Maziade et al. 2008). Individuals with different neuropsychiatric disorders, such as BD and SCZ, may show similar clinical symptoms suggesting common genetic influences and biological mechanisms. Community-based studies have shown that the risk of development of psychiatric disorders other than BD and SCZ increases in adolescents of parents with a diagnosis of BD and SCZ (Mortensen et al. 2010, Dean et al. 2010).

Recently, researchers have focused on high-risk populations, such as adolescents of parents with BD and SCZ, and the risk of psychiatric disorders that threaten them (Lau et al. 2018). Serious psychiatric disorders such as BD and SCZ usually appear during adolescence and early adulthood. Therefore, adolescents with BD and SCZ in their parents should be monitored for the risk of developing psychiatric disorders (Sandstrom et al. 2019).

Attachment starts from the first days of life and develops as a result of the baby's communication with the caregiver and the environment. The role of the primary caregiver in attachment development is to create a secure basis for the regulation of negative feelings and thoughts (Gumley & Liotti 2018). A healthy child-parent relationship is necessary for secure attachment and one of the most important factors that negatively affect this relationship is the presence of chronic psychiatric disorders in the parents (Raikes & Thompson 2008). In studies conducted

with adolescents whose parents have a diagnosis of psychiatric disorder, it has been reported that these adolescents have a troubled and difficult childhood, have a high risk of developing psychopathology in adolescence, and experience more behavioral problems, attachment and social isolation (Duncan & Browning 2009).

Oxytocin is released from the paraventricular nucleus in the brain and regulates emotional functions by affecting the brain stem and amygdala (Nakash-Eisikovits et al. 2000, Pierrehumbert et al. 2012). Oxytocin is reported to be effective in social attachment together with endogenous opioids and prolactin and is associated with attachment (Nakash-Eisikovits et al. 2000). In the literature, it has been found that endogenous oxytocin levels are lower in individuals with insecure attachment style and emotional distress in childhood. The interactions between adverse early life experiences, parental psychopathology, underdeveloped social ties and oxytocin significantly affect individuals' mental health (Pierrehumbert et al. 2012).

It has been reported that early life stress develops during the developmental period in adolescents with parents with chronic psychiatric disorders such as SCZ and BD (Dean et al. 2010). Clinical studies clearly show that early life stress can cause physical and mental health problems later in life. Cellular homeostasis during stress is maintained by increased levels of molecular chaperones or heat shock proteins (HSPs) (Daugaard et al. 2007). HSP-70 has many functions such as regulation of the response to stress in the system, degradation of irreversibly damaged proteins, protein folding and anti-apoptotic effect (Daugaard et al. 2007, Turturici et al. 2011). HSP-70 has been shown to have an important neuroprotective function in central nervous system development under stress conditions that develop during the stages of embryonic development. HSP-70 proteins mediate the response to cellular attacks and have an important role in neuronal integrity in the pathogenesis of some psychiatric disorders (Pae et al. 2009). Some previous studies have shown that serum HSP-70 levels are low in autism, depression, attention deficit hyperactivity disorder and schizophrenia (Özaslan et al. 2022). In another study, HSP-70 was shown to be associated with anxiety, mood disorders and schizophrenia (Solarz et al. 2021). Abnormal expression of HSP-70 is thought to be associated with structural brain abnormalities in schizophrenia patients (Pae et al. 2009). In addition, serum antibody level against HSP-70 was found to be high in patients with schizophrenia (Kim et al. 2001). Similar to SCZ, a difference in HSP-70 levels was found in patients with bipolar disorder (Cheng et al. 2018).

Although there are studies examining the relationship between attachment and oxytocin in the literature, studies investigating attachment in adolescents with SCZ and BD parents are limited. In addition, no study investigating serum HSP-70 and oxytocin levels in adolescents with parents diagnosed with SCZ and BD was found in the literature. The main aim of this study is to evaluate whether serum oxytocin and HSP-70 levels are different in adolescents of parents with BD and SCZ compared to adolescents of healthy parents. The second aim of our study is to examine the differences between comorbid psychiatric symptoms, attachment styles and perceived social support levels in parents with BD and SCZ compared to adolescents of healthy parents.

SUBJECTS AND METHODS

Sample characteristics and assessment

In our study, adolescents aged 12-17 years of parents who applied to Düzce University Faculty of Medicine, Department of Psychiatry for inpatient or outpatient treatment between May 2021 and November 2021 and who were followed up for at least 6 months with SCZ and BD diagnoses according to DSM 5 diagnostic criteria were included. Our study consisted of 3 groups: 9 adolescents with at least one parent diagnosed with SCZ (5 mothers, 4 fathers), 30 adolescents with at least one parent diagnosed with BD (19 mothers, 12 fathers) and 31 healthy adolescents whose parents did not have any psychiatric disorder. Exclusion criteria for adolescents whose parents had SCZ and BD were intellectual disability and history of concomitant neurologic, acute or chronic physical illness.

For the control group, adolescents who applied to the child and adolescents psychiatry outpatient clinic but did not have any psychiatric disorder were included in the evaluation. Those with a parental diagnosis of psychiatric disorder or chronic physical illness were excluded. The exclusion criteria for adolescents in the control group were as follows: psychiatric disorder, psychiatric drug use, intellectual disability, family history of psychiatric disorder, history of accompanying neurological, acute or chronic physical illness. In the sample distribution, care was taken to ensure that the age and gender of the 3 groups were similar.

Adolescents and their parents were verbally informed about the study design before the study. Written informed consent forms in accordance with the Declaration of Helsinki were obtained from the adolescents and their parents who agreed to participate in the study. All participants were evaluated by a Child and Adolescent Psychiatrist. The psychiatric disorders of the participants according

to DSM 5 diagnostic criteria were evaluated with the Turkish Adaptation of Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL-T). Sociodemographic data form, Brief Symptom Inventory (BSI), Relationship Scales Questionnaire-Adolescent Form (RSQ-A) and Multidimensional Scale of Perceived Social Support (MSPSS) were administered to all participants via face-to-face interview. In this study, it was calculated that at least 29 individuals should be studied for each group in order to examine the significance of the 0.5-unit correlation between binding, oxytocin and hsp-70 under 80% power and 5% type I error conditions. Power analysis was done with PASS v.15 demo program.

To measure serum HSP-70 and oxytocin levels, 10 ml venous blood samples were collected from the antecubital areas of all participants between 08:00-10:00 in the morning. For all samples studied, blood samples obtained after fasting for at least 10-12 hours were centrifuged at 4°C and 3000 rpm for 15 minutes. While the parameters measured in the autoanalyzer were processed immediately, serum samples were separated into different eppendorfs for the parameters to be analyzed by spectrophotometric method using commercially available kits and stored at -80°C until the time of the study, and a single protocol was applied when all samples were completed. The principle of operation of the autoanalyzer was performed according to the Biochemistry routine laboratory systematics and the spectrophotometric ELISA method was performed according to the kit package insert study protocol. The data of the SCZ and BD study group and the control group were analyzed comparatively. HSP-70 and Oxytocin levels of the participants included in the study were determined by enzyme-immunoassay method (Gen 5.2.Era) with a specific ELISA kit (ELK Biotechnology, CH, Cat 1749).

The study was approved by the Clinical Research Ethics Committee of Düzce University Faculty of Medicine (05.03.2021-2021/120).

Sociodemographic Data Form: It is an information form organized by us to evaluate the sociodemographic characteristics of the participants.

Turkish version of Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL-T): Past and current psychiatric disorders in children and adolescents aged 6-18 years are questioned with information obtained from the parents and the child, and the diagnosis is made as a result of the clinician's psychiatric examination. The presence and severity of symptoms are decided in consultation with the child, parents and clinician. The Turkish validity and reliability study of this chart was conducted by Gökler et al. (Gokler 2004).

Brief Symptom Inventory (BSI): The validity-reliability study of the scale developed by Derogatis (1992) for psychopathological assessment was conducted by Şahin, Batıgün, and Uğurtaş (2002). The 53-item scale includes five factors: depression (12 items), anxiety (13 items), negative self (12 items), somatization (9 items) and hostility (7 items). The scale is a four-point Likert scale (between 0-4 points). The range of total scores is 0-212. As the total score obtained from the scale increases, the frequency of symptoms increases (Şahin et al. 2002).

Relationship Scales Questionnaire-Adolescent Form (RSQ-A): The scale developed by Griffin and Bartholomew (1994) consists of a total of 17 questions to measure four attachment styles (secure, avoidant, fearful and preoccupied). Turkish validity and reliability studies of the scale were conducted by Sümer and Güngör (1999). Each item in the scale is analyzed on a 7-point scale (1=It does not describe me at all; 7=It completely describes me) to indicate the extent to which individuals evaluate themselves and their attitudes in close relationships. The highest score obtained from the subscales determines the attachment style of the individual. Increased scores in attachment styles other than secure attachment style indicate insecure attachment (Sümer & Güngör 1999).

Multidimensional Scale of Perceived Social Support (MSPSS): Originally developed by Zimet et al. The Turkish adaptation, validity and reliability study was conducted by Çakır and Palabıyık. There are three different sub-dimensions of the MSPSS: family, friends and special person. The scale consists of 12 items. It is scored from 1 to 7 on a Likert scale. A high score indicates that perceived social support is high (Çakır & Palabıyık 1997).

Statistical Analysis

The distribution of the data was examined by Shapiro-Wilk test and the homogeneity of variance was examined by Levene's test. Independent samples t test, One-Way ANOVA, Welch test or Mann-Whitney U test and Kruskal-Wallis tests were used in the comparison of the groups depending on the number of groups and the distribution pattern given. Categorical variables were analyzed with Pearson chi-square, Fisher's exact or Fisher-Freeman-Halton tests depending on the expected value rule. Correlations between numerical variables were

analyzed by Pearson or Spearman correlation analysis depending on the distribution of the data. Descriptive statistics are given as mean±standard deviation, median, quartiles and minimum-maximum values depending on the given distribution, and categorical variables are summarized as number and percentage. Statistical analyses were performed with SPSS v.22 package program and significance level was taken as 0.05.

RESULTS

In our study, there were 3 groups consisting of adolescents with BD, SCZ and healthy parents. There were 30 adolescents in the BD group, 9 in the SCZ group and 31 in the control group. There was no difference between the 3 groups in terms of age and gender (p>0.05). Among the adolescents in the BD group, 19 (63.3%) of the mothers and 12 (40.0%) of the fathers had a diagnosis of BD. Only one adolescent had a diagnosis of BD in both mother and father. In the SCZ group, the mothers of 5 (55.6%) and fathers of 4 (44.4%) adolescents had a diagnosis of SCZ. The mean disease duration of the parents in the BD and SCZ groups was 7 years (min-max: 1-25 years). Sociodemographic characteristics of adolescents and their parents are shown in detail in Table 1.

Psychiatric evaluation revealed that 10 (33.3%) adolescents in the BD group and 5 (55.6%) adolescents in the SCZ group had psychiatric disorders. None of the adolescents included in the study were diagnosed with BD and SCZ. When the psychiatric diagnoses of the adolescents in the BD group were evaluated; attention deficit and hyperactivity disorder (ADHD) was found in 6 (20%), conduct disorder (CD) in 5 (16.6%), anxiety disorders in 1 (3.33%), major depressive disorder (MDD) in 2 (6.66%), and other psychiatric disorders in 1 (3.33%). Six adolescents in the BD group had psychiatric comorbidity. When the psychiatric diagnoses of the adolescents in the SCZ group were evaluated, it was found that 2 (22.2%) had ADHD, 2 (22.2%) had CD, 2 (22.2%) had anxiety disorders, and 1 (11.1%) had MDD. One adolescent in the SCZ group had psychiatric comorbidities.

It was found that 9 (30%) adolescents in the BD group and 5 (55.55%) adolescents in the SCZ group received psychiatric treatment. In the BD group, 5 (55.55%) adolescents receiving psychiatric treatment were found to use antipsychotics, 3 (33.33%) antidepressants, 3 (33.33%) methylphenidate, and 1 (11.11%) other psychiatric drugs. In the SCZ group, 2 (40%) of the adolescents who received psychiatric treatment were found to use antipsychotics, 3 (60%) used antidepressants, and 2 (40%) used methylphenidate.

Differences between serum HSP-70 and oxytocin values of adolescents in BD, SCZ and control groups were evaluated statistically. There was no significant difference in serum HSP-70 levels between adolescents with BD parents (55.48 ng/L, 4.83-110.73 ng/L), adolescents with SCZ parents (70.24 ng/L, 20.01-80.82 ng/L) and adolescents in the control group (80.30 ng/L, 3.35-610.61 ng/L) (Table 2). However, serum HSP-70 levels were found to be lower in the BD and especially in the SCZ group compared to the control group. There was no significant difference was found between adolescents with BD parents (126,89 ng/L, 56,83-193,03 ng/L), adolescents with SCZ parents (91.19 ng/L, 32,68-122,98ng/L) and adolescents in the control group (111.16 ng/L, 15,54-367,35ng/L). Serum oxytocin levels of adolescents in the SCZ group were significantly lower than those in the BD group (p=0.045).

In our study, four attachment styles, namely secure, attachment-avoidant, fearful and preoccupied attachment styles were evaluated with the RSQ-A. When grouping according to four different attachment styles, attachment styles in adolescents were divided into two groups as secure and insecure attachment due to the small number of subjects. Apathetic, fearful and preoccupied attachment styles were considered as insecure attachment. There was no significant difference between the groups in terms of attachment type, but the rate of secure attachment was higher in adolescents in the control group (45.2%) compared to adolescents in the BD (23.3%) and SCZ (22.2%) groups (p=0.148). It was found that the SCZ group had a high rate of indifferent attachment. The results are shown in detail in Table 3.

When Table 3 is examined, there was no significant difference between the adolescents in the BD group, SCZ group and the control group in terms of the total score obtained from the MSPSS (p=0.112) and the social support perceived from family (p=0.363), friends (p=0.065) and special people (p=0.615). However, it was found that the total score and sub-factor scores of the MSPSS were higher in the control group than in the BD and SCZ groups.

When the scores obtained by the adolescents in the BD, SCZ and control groups from the BSI scale were evaluated, no significant difference was found between the 3 groups in terms of the mean scores of the anxiety (p=0.393), depression (p=0.493), negative self (p=0.528), and somatization (p=0.581) subscales and the total score (p=0.278) of the BSI. Only the hostility (p=0.039) subscale of the BSI was found to be significantly higher in the SCZ group compared to the adolescents in the BD and control groups (p<0.05). The results are shown in Table 3.

The correlations between the scores obtained by the adolescents in the BD group from the BSI scale and the

Table 1: Distribution of various characteristics of participants according to BD, SCZ and control groups

		Control group (n=31)	BD group (n=30)	SCZ group (n=9)	P value
Age (year)		13,97±1,52	14,27±1,82	14,11±2,09	0,796#
Gender, n (%)	Female	21 (67,7)	17 (56,7)	4 (44,4)	
	Male	10 (32,3)	13 (43,3)	5 (55,6)	0,388&
Place of residence, n (%)	Village	7 (22,6)	2 (6,7)	2 (22,2)	
	District	9 (29,0)	8 (26,7)	2 (22,2)	
	Province	15 (48,4)	20 (66,7)	5 (55,6)	0,405&
School status, n (%)	No	1 (3,2)	1 (3,3)	2 (22,2)	
	Secondary School	15 (48,4)	12 (40,0)	4 (44,4)	
	High School	15 (48,4)	17 (56,7)	3 (33,3)	0,301&
Mother's Age		40,71±5,06	40,30±4,74	38,44±6,75	0,512#
Mother's occupation, n (%)	Civil Service Employee	1 (3,2)	0 (0,0)	1 (11,1)	
	Tradesmen/Self-employed	9 (29,0)	6 (20,0)	2 (22,2)	
	Not working	21 (67,7)	24 (80,0)	6 (66,7)	0,422&
Mother's education, n (%)	Illiterate	2 (6,5)	0 (0,0)	0 (0,0)	
	Primary School	10 (32,3)	16 (53,3)	5 (55,6)	
	Secondary School	10 (32,3)	4 (13,3)	2 (22,2)	
	High School	7 (22,6)	6 (20,0)	2 (22,2)	
	University and higher	2 (6,5)	4 (13,3)	0 (0,0)	0,448&
Father's Age		44,58±5,98	45,97±7,09	43,44±5,81	0,520#
Father's occupation, n (%)	Civil Service Employee	5 (16,1)	2 (6,7)	0 (0,0)	
	Tradesmen/Self-employed	22 (71,0)	18 (60,0)	7 (77,8)	
	Not working	4 (12,9)	10 (33,3)	2 (22,2)	0,267&
Father's education, n (%)	Primary School	9 (29,0)	7 (23,3)	2 (22,2)	
	Secondary School	5 (16,1)	4 (13,3)	3 (33,3)	
	High School	11 (35,5)	14 (46,7)	3 (33,3)	
	University and higher	6 (19,4)	5 (16,7)	1 (11,1)	0,879&
Psychiatric Disease in Mother, n (%)		0 (0,0)a	19 (63,3)b	5 (55,6)b	<0,001$
Psychiatric Disease in Father, n (%)		0 (0,0)a	12 (40,0)b	4 (44,4)b	<0,001$
Cohabitation of parents, n (%)	Together	28 (90,3)	22 (73,3)	9 (100)	
	Divorced	3 (9,7)	8 (26,7)	0 (0,0)	0,090&
Family type, n (%)	Core	24 (77,4)	23 (76,7)	9 (100)	
	Large	7 (22,6)	7 (23,3)	0 (0,0)	0,320$
Birth Complications, n (%)		1 (3,2)a	4 (13,3)ab	4 (44,4)b	0,010&
Mode of delivery, n (%)	Normal	16 (51,6)	14 (46,7)	3 (33,3)	
	Caesarean section	15 (48,4)	16 (53,3)	6 (66,7)	0,637&
Time of birth, n (%)	Mature	31 (100)	27 (90,0)	7 (77,8)	
	Premature	0 (0,0)a	3 (10,0)ab	2 (22,2)b	0,037&
Course of Pregnancy, n (%)	Normal	25 (80,6)	25 (83,3)	6 (66,7)	
	Threat of miscarriage	2 (6,5)	2 (6,7)	3 (33,3)	
	Trauma	1 (3,2)	0 (0,0)	0 (0,0)	
	Psychiatric symptoms	3 (9,7)	3 (10,0)	0 (0,0)	0,286&
Smoking during pregnancy, n (%)		1 (3,2)	7 (23,3)	2 (22,2)	0,065&
Use of medication during pregnancy, n (%)		2 (6,5)	4 (13,3)	1 (11,1)	0,551&
Primary Caregiver, n (%)	Mother	29 (93,5)	27 (90,0)	7 (77,8)	
	Father	0 (0,0)	2 (6,7)	0 (0,0)	
	Other	2 (6,5)	1 (3,3)	2 (22,2)	0,160&
Parental Hospitalization, n (%)		0 (0,0)a	21 (70,0)b	6 (66,7)b	<0,001$
Duration of Parental Illness (years)		-	7 (8) [2-25]	7 (10) [1-15]	0,482‡
Presence of Psychiatric Disease in the Child, n (%)		0 (0,0)a	10 (33,3)b	5 (55,6)b	<0,001$
Pediatric Drug Use, n (%)		0 (0,0)a	9 (30,0)b	5 (55,6)b	<0,001$

* Descriptive statistics are given as mean±standard deviation or median (interquartile range, Q3-Q1) [minimum-maximum]. #: One-Way ANOVA, &: Fisher-Freeman-Halton test, $: Pearson chi-square test, ‡: Mann-Whitney test

Table 2: Distribution of serum HSP-70, Oxytocin and other parameters of participants according to groups

	Control group (n=31)	BD group (n=30)	SCZ group (n=9)	P value
HSP-70 (ng/ml)	80,30 (3,35-610,61)	55,48 (4,83-110,73)	70,24 (20,01-80,82)	0,108†
Oxytocin (ng/ml)	111,16 (15,54-367,35)ab	126,89 (56,83-193,03)a	91,19 (32,68-122,98)b	0,045†

*Continuous data are presented as median and minimum-maximum.†: Kruskal-Wallis test.

Table 3: Distribution of participants' total scale scores and subscale scores according to BD, SCZ and control groups

	Control group (n=31)	BD group (n=30)	SCZ group (n=9)	P value
RSQ-A Attachment Type				
Secure	14 (45,2)	7 (23,3)	2 (22,2)	
Fearful	5 (16,1)	8 (26,7)	1 (11,1)	0,156[&]
Apathetic	8 (25,8)	8 (26,7)	6 (66,7)	
Preoccupied	4 (12,9)	7 (23,3)	0 (0,0)	
RSQ-A				
Secure	14 (45,2)	7 (23,3)	2 (22,2)	0,148[$]
Insecure	17 (54,8)	23 (76,7)	7 (77,8)	
MSPSS				
Family	27 (7) [16-28]	25 (4) [11-28]	25 (18) [8-28]	0,363[†]
Friend	26 (9) [6-28]	21 (10) [4-28]	22 (13) [5-27]	0,065[†]
Special people	15 (20) [4-28]	11 (11) [4-28]	10 (17) [4-26]	0,615[†]
Total score	61,68±16,60	55,03±11,94	52,56±13,99	0,112[#]
BSI				
Anxiety	6 (10) [0-30]	4,5 (10) [0-30]	10 (10) [2-28]	0,393[†]
Depression	8 (17) [0-39]	6 (11) [0-32]	7 (25) [1-38]	0,493[†]
Negative self	7 (11) [0-29]	5 (12) [0-28]	8 (12) [0-38]	0,528[†]
Somatization	3 (5) [0-24]	3 (4) [0-15]	6 (6) [0-9]	0,581[†]
Hostility	4 (8) [0-19]	4 (6) [0-23]	11 (10) [4-17]	**0,039**[†]
Total score	32 (42) [2-129]	21,5 (40) [0-116]	43 (53) [14-115]	0,278[†]

RSQ-A: Relationship Scale Questionnaire-Adolescent Form, MSPSS: Multidimensional Scale of Perceived Social Support, BSI: Brief Symptom Inventory, [#]: One-Way ANOVA, [&]: Fisher-Freeman-Halton test, [$]: Pearson chi-square test, [†]: Kruskal-Wallis test

Table 4: Correlations between the scores obtained by the participants in the BD group from the BSI scale and the MSPSS scale

		MSPSS Family	MSPSS Friend	MSPSS Friend special people	MSPSS Total score
BSI Anxiety	r_s	**-0,443**	**-0,511**	-0,038	**-0,476**
	p	**0,014**	**0,004**	0,841	**0,008**
BSI Depression	r_s	**-0,513**	-0,309	0,021	-0,315
	p	**0,004**	0,096	0,910	0,090
BSI Negative self	r_s	**-0,427**	**-0,494**	0,143	**-0,363**
	p	**0,019**	**0,006**	0,453	**0,049**
BSI Somatization	r_s	-0,271	-0,278	0,058	-0,256
	p	0,147	0,138	0,759	0,172
BSI Hostility	r_s	**-0,498**	**-0,543**	0,076	**-0,449**
	p	**0,005**	**0,002**	0,689	**0,013**
BSI Total score	r_s	**-0,465**	**-0,491**	0,056	**-0,424**
	p	**0,010**	**0,006**	0,770	**0,020**

MSPSS: Multidimensional Scale of Perceived Social Support, BSI: Brief Symptom Inventory, r_s: Spearman's rho

Table 5: Correlations between BSI and MSPSS scores and serum HSP-70 and oxytocin levels of participants in the BD, SCZ and control groups

		Control group		BD group		SCZ group	
		HSP-70	**Oxytocin**	**HSP-70**	**Oxytocin**	**HSP-70**	**Oxytocin**
MSPSS Family	r_s	0,268	0,093	0,169	-0,256	0,060	**0,712**
	p	0,145	0,619	0,389	0,189	0,879	**0,031**
MSPSS Friend	r_s	-0,071	0,117	-0,133	0,227	0,475	-0,134
	p	0,704	0,531	0,499	0,245	0,197	0,731
MSPSS Friend special people	r_s	-0,060	0,223	0,102	0,256	0,572	-0,354
	p	0,749	0,229	0,607	0,188	0,108	0,349
MSPSS Total score	r_s	0,100	0,176	-0,087	0,217	**0,669**	0,267
	p	0,591	0,343	0,660	0,268	**0,049**	0,488
BSI Anxiety	r_s	-0,235	-0,115	-0,107	0,047	-0,124	-0,443
	p	0,204	0,539	0,589	0,811	0,751	0,233
BSI Depression	r_s	-0,244	-0,088	0,009	0,041	0,256	-0,126
	p	0,186	0,638	0,966	0,838	0,506	0,748
BSI Negative self	r_s	-0,253	-0,069	-0,071	-0,014	0,231	-0,176
	p	0,170	0,711	0,720	0,943	0,550	0,651
BSI Somatization	r_s	0,017	-0,096	-0,262	-0,020	0,004	-0,363
	p	0,927	0,609	0,177	0,921	0,991	0,337
BSI Hostility	r_s	-0,273	-0,107	-0,204	-0,100	-0,109	-0,268
	p	0,137	0,566	0,298	0,614	0,780	0,486
BSI Total score	r_s	-0,245	-0,133	-0,090	-0,038	0,184	-0,300
	p	0,184	0,475	0,648	0,848	0,635	0,433

MSPSS: Multidimensional Scale of Perceived Social Support, BSI: Brief Symptom Inventory, r_s: Spearman's rho

scores obtained from the MSPSS scale were evaluated. No significant correlation was found between the scores obtained from the special person subscale of the MSPSS and the BSI scale. There was a significant negative correlation between the total score of the BSI and the total score of the MSPSS (r=-0.424, p=0.020). Correlations between the scales are detailed in Table 4.

There was no significant correlation between the scores of the BSI and MSPSS scales and serum HSP-70 and oxytocin values of the adolescents in the BD, SCZ and control groups (p>0.05). The results are detailed in Table 5.

In the BD group, no significant correlation was found between the scores of adolescents with and without psychiatric illness in their mothers on the RSQ, MSPSS and BSI scales (p>0.05). In the BD group, no significant correlation was found between serum HSP-70 and oxytocin values of adolescents with and without psychiatric illness in their mothers (p>0.05).

In the BD group, there was no significant difference between the scores of adolescents with and without psychiatric illness in their fathers in the RSQ, MSPSS and BSI scales (p>0.05). No significant difference was found between the serum HSP-70 and oxytocin values of adolescents with and without psychiatric illness in the BD group (p>0.05).

In the BD group, no significant difference was found between the scores obtained by adolescents with and without parental hospitalization status on the RSQ-A, MSPSS and BSI scales and serum HSP-70, oxytocin values (p>0.05).

In the BD group, there was a significant difference between the scores of adolescents with and without psychiatric illness on the BSI somatization scale (p=0.019), whereas there was no significant difference between the scores on the other sub-scales of the RSQ-A, MSPSS and BSI (p>0.05). No significant difference was found between the serum HSP-70 and oxytocin values of

adolescents with and without psychiatric illness in the BD group (p>0.05).

The relationship between attachment style and serum oxytocin and HSP-70 levels in the BD group was evaluated. There was no statistically significant correlation between secure attachment style (n=7) and insecure attachment style (n=23) and serum oxytocin and HSP-70 levels (p>0.05).

DISCUSSION

Our study is the first study to examine the relationship between attachment and serum HSP-70 and oxytocin levels in adolescents aged 12-17 years of parents with BD and SCZ. The main aim of this study was to examine the differences between serum oxytocin and HSP-70 levels in adolescents of BD and SCZ parents and healthy controls. The second aim of our study is to evaluate the accompanying psychiatric symptoms, attachment styles, and perceived social support level in these adolescents. In our results, no difference was found between the groups in terms of attachment type, psychiatric symptoms and perceived social support. There was no statistically significant difference between the groups in terms of serum HSP-70 levels, but HSP-70 levels were found to be lower in adolescents with SCZ and BD parents. Serum oxytocin levels of adolescents with SCZ parents were significantly lower than those of adolescents with BD parents. Since the sample size in the SCZ group was small, no correlation could be made between the scales and serum parameters.

Our study revealed significantly higher rates of psychopathology in adolescents of parents with BD and SCZ. Similarly, previous studies have found increased rates of psychiatric diagnoses in adolescents of parents with BD and SCZ. In a study, the rate of being diagnosed with a psychiatric disorder in adolescents with a parent diagnosed with BD was found to be 44% (Wals et al. 2004). In another study conducted with 26 adolescents of parents with BD and 28 adolescents of parents with schizophrenia, psychopathology was observed at a rate of 60% and no significant difference was found between the groups (Maziade et al. 2008). Our findings support the literature in this respect. In studies conducted with adolescents whose parents have psychopathology, it has been shown that the most commonly used drugs are antipsychotics, methylphenidate, antidepressants and mood stabilizers (Akdemir & Gokler 2008). Similarly, in our study, antipsychotic, antidepressant and methylphenidate were found to be the drugs most commonly used by adolescents with psychiatric disorders in both the BD group and SCZ group.

The first 2 years of infancy are the most important period for the development of secure attachment. This period may be negatively affected by the current psychiatric disorder of the parent (Hipwell et al. 2000). Parents diagnosed with chronic psychiatric disorders such as BD and SCZ experience some problems in the protection and care of their adolescents and in the safe parent-child relationship due to impaired psychosocial functioning and recurrent periods of illness. Therefore, insecure attachment may develop in the adolescents of these parents (Hillegers et al. 2005). In a study conducted with 25 adolescents with BD parents, RSQ-A was used and it was found that indifferent attachment style was more common in adolescents in the BD group. In this study, no significant difference was found for other attachment styles (Erkan et al. 2015). In our study, indifferent attachment style was found to be more frequent in the BD and SCZ group compared to other attachment types, although not significantly. No other study investigating attachment in adolescents of parents with psychiatric disorders was found in the literature. Therefore, our study is valuable in terms of literature.

Parental psychopathology is linked to poor cognitive and social outcomes in adolescents, such as low school achievement and poor personal, social and emotional development. Features of the social environment, such as social support, are important for the mental health of adolescents whose parents have psychopathology. In a study by Hoefnagels et al. social support was shown as a factor predicting mental health in adolescents of parents with psychopathology (Hoefnagels et al. 2006). In the study conducted by Plass et al. no relationship was found between parental psychopathology and social support (Plass-Christl et al. 2017). In our study, no difference was found in the perceived social support of adolescents with BD and SCZ parents compared to adolescents in the control group. The reason for this result may be that all the participants in our study were in adolescence. As a result of hormonal irregularity in adolescence, children reduce their relationship with their parents, prefer to be alone at home and spend more time with their peers. Although there is evidence that social support and family communication play an important role in the negative impact of parental psychopathology on child mental health, few studies have examined these factors. However, there is no study examining social support in adolescents of parents diagnosed with BD and SCZ. Large-scale studies investigating this issue are needed.

Psychiatric symptoms such as depression and anxiety were found to be more common in adolescents with parental psychopathology. Children and adolescents experiencing early life stress show more signs of hostility than

normal children (Soylu et al. 2013). In our study, depression, anxiety, negative self and somatization symptoms of the adolescents in the BD and SCZ groups were similar to the control group. However, it was determined that the signs of hostility were higher in adolescents whose parents had a diagnosis of SCZ. It is thought that this situation may develop due to psychosocial deprivation as a result of the psychopathology of its parents. There is a need for detailed studies to investigate the causes of hostility in these adolescents.

Oxytocin increases social relationships by stimulating dopamine D2 receptors in the nucleus accumbens and associated reward centers. Thus, it is thought that there is a reciprocal relationship between attachment and oxytocin. Serum oxytocin level is higher in individuals with secure attachment (Tops et al. 2007). In our study, serum oxytocin level was found to be significantly lower in adolescents in the SCZ group compared to adolescents in the BD group. However, this was not found to be related to adolescents' attachment style. In our study, no significant difference was found between secure and insecure attachment types and oxytocin levels in adolescents with parents diagnosed with BD.

It has been shown that there are abnormalities in oxytocin level in SCZ, and higher plasma levels are associated with milder positive symptom severity and milder social deficit (Rubin et al. 2011). It has been observed that oxytocin infusion acts as selective serotonin reuptake inhibitors and increases the release of serotonin in raphe nucleus. In one study, it was found that oxytocin levels were high in individuals with BD (Turan et al. 2013). In our study, oxytocin level was found to be low in the SCZ group, while oxytocin level was found to be high in the BD group.

The results of studies investigating serum oxytocin levels in schizophrenia are mixed. In some studies, serum oxytocin levels were found to be lower in patients with schizophrenia than in healthy participants (Liu et al. 2019). In one study, oxytocin level was found to be high in the cerebrospinal fluid of patients with schizophrenia (Beckmann et al. 1985). In another study, no difference was found between serum oxytocin levels in participants with and without SCZ (Glovinsky et al. 1994). In our study, no significant difference was found in serum oxytocin levels in the SCZ group compared to healthy adolescents. However, serum oxytocin levels were significantly lower in the SCZ group compared to the BD group. This was considered as a risk for the development of SCZ in these adolescents in the future.

Clinical and experimental studies have shown that HSPs play a critical role in the regulation of immune response and oxidative stress response. HSP-70 exhibits the properties of ATP-dependent enzymes and protects enzymes and proteins that develop against oxidative stress from the harmful effects of reactive oxygen species (ROS) (Kurashova et al. 2020). Decreased level of HSP-70 in the system may cause oxidative damage and increase in ROS (Wang et al. 2014). In our results, lower serum HSP-70 levels in BD and SCZ groups compared to control groups may indicate an impaired response to oxidative stress in these adolescents. HSP70 has recently been shown to be potentially associated with the development of anxiety, mood disorders and schizophrenia. Recent data have shown that polymorphism of HSP70 may be associated with suicidal behavior in SCZ patients (Kowalczyk et al. 20209. In another study, HSP70 was identified as one of the serum and brain biomarkers that may be involved in the development of anxiety disorders (Yang et al. 2013). Our data show that compared to healthy adolescents, serum HSP-70 levels are significantly reduced, especially in adolescents diagnosed with BD. We also showed that it was lower in adolescents in the SCZ group compared to the control group. Therefore, we suggest that HSP70 may be a potential biomarker for early life stress-related psychopathologies in adolescents whose parents have chronic psychiatric disorders such as BD and SCZ.

In the literature, there is no study examining the effects of HSP-70, oxytocin levels and perceived social support on attachment in adolescents whose parents have psychopathology. More detailed prospective studies are needed to elucidate the link between attachment, oxytocin and HSP-70 in adolescents exposed to parental psychopathology in early life.

Our study had some limitations. The sample size of the groups in our study was small and the number of adolescents whose parents had schizophrenia was quite small. The reason for the small number of SCZ patients with SCZ parents was thought to be that SCZ patients never marry or, even if they marry, they never have adolescents. Moreover, the cross-sectional nature of our study prevents the development of causal interpretations.

CONCLUSION

Adolescents of parents with BD and SCZ have a high risk of developing psychiatric disorders. Therefore, the adolescents of these patients should be screened for psychiatric disorders and necessary psychosocial interventions should be made. In our study, HSP-70 level was found to be lower in the BD group than in the SCZ group. Oxytocin level was found to be significantly higher in the BD group compared to the control group, while it was found to be lower in the SCZ group compared to

the control group. As a result of our findings, it can be assumed that HSP-70 and oxytocin changes may play an important role in psychopathology and may be a clinical marker in psychiatry. Regardless of the limitations of our study, HSP-70 and oxytocin dysregulation may play a role in individuals experiencing early life stress. More comprehensive and detailed longitudinal studies are needed to understand whether changes in HSP-70 and oxytocin levels are a cause or consequence of early life stress.

Disclosure statement

All of the authors declare that they have no conflicts of interest.

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