Association of Serum Homocysteine and Methionine Levels with Cognition and Functioning in Bipolar Disorder

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SUMMARY

Objective: The relationship between homocysteine (HCY) levels and cognitive impairments, particularly executive functions in bipolar disorder (BD), has recently been investigated. However, conflicting results were reported. The aim of the present study is to investigate changes in serum HCY, methionine, vitamine B12 and levels in BD patients are relative to controls and to investigate the relationship between HCY, methionine, vitamin B12, and folate levels and clinical features, cognitive functions and psychosocial functioning in euthymic BD patients and controls.

Methods: Sixty BD type I euthymic patients and twenty controls were assessed with Global Assessment of Functioning and a battery of neuropsychological tests including the Wisconsin card sorting test, the Rey's auditory verbal learning test, the Cancellation test, Trail making test A, Trail making test B, and the Stroop test. HCY, vitamin B12, methionine and folate levels were measured together after collecting blood samples from both patient and controls.

Results: Mean serum methionine concentration was different between groups. Low serum methionine was found to be a predictor of BD. However, a statistically significant difference was not detected between groups for mean serum values of HCY, folate, or vitamin B12. HCY levels showed a positive correlation with illness duration, the number of total episodes, and the number of manic episodes. A significant correlation was not found between HCY, methionine, folate, B12 levels with cognitive functions and functioning in the BD group.

Conclusion: Low serum methionine was found to be a predictor of BD, a condition which can lead to a decrease in SAM synthesis and thus to a variety of complications in methylation reactions. Additional studies are needed to clarify the impact of single carbon metabolism on BD.

Keywords: Bipolar disorder, homocysteine, methionine, cognitive functions

INTRODUCTION

HCY is a cytotoxic amino acid (Regland *et al*., 1995). It is considered an important cause of cardiovascular and cerebrovascular diseases because it induces vascular endothelial damage, exhibits a procoagulant effect, and promotes thrombosis (Jensen and Ingerslev, 1998; Tanriverdi *et al*., 2007). HCY contributes to neurodegenerative and psychiatric diseases through oxidative stress pathways, glutamate toxicity, direct excitotoxic effects, the impairment of neurotransmitter metabolism due to inadequate methylation, and impaired DNA repair and biosynthesis which negatively affects regeneration and synaptogenesis in nerve cells (Mattson and Shea, 2003; Obeid and Herrman, 2006). Epidemiologic and longitudinal studies have shown a causal relationship between HCY and cognitive impairment (Obeid and Herrman, 2006).

Dittman *et al*. (2007) found that HCY levels were significantly higher in euthymic BD patients compared to controls. In a study by Dias *et al*. (2009), no differences were found between euthymic BD patients and controls with respect to HCY, folate, and vitamin B12 levels, although the HCY levels of male BD patients were found to be significantly higher compared to female BD patients. On the other hand, growing evidence suggests that functional deterioration arises during the course of bipolar disorder (BD), at least in some patients. Varying degrees of functional impairment were shown in more than 50% of BD patients, and a severe loss of functionality was shown in 10-15% of patients in a follow-up study lasting

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more than eight years (Goldberg and Harrow, 1999). This is consistent with observations of impairment in cognitive functions during the euthymic period in BD patients (Bora *et al*. 2008). Investigations into the role of HCY in impairment of psychosocial and cognitive function in BD following schizophrenia have been accumulating (Levine *et al*. 2002; Reif *et al*. 2003; Applebaum *et al*. 2004; Levine *et al*. 2005; Stahl *et al*., 2005). Several studies of impairments in cognitive function caused by HCY have focused on the executive functions of BD patients (Stahl *et al*. 2005; Dittman *et al*. 2007; Dittman *et al*. 2008).

Initially Osher *et al*. (2004) reported that patients with high HCY levels exhibited increased functional deterioration. In this report the HCY levels of younger male subjects were higher, both in the patient and control groups. With the exception of a single study, none of these reports evaluated whether elevated HCY levels might be a predictor for BD. In this study, the authors have explored the association of MTHFR gene polymorphism with HCY, folate, and vitamin B12 in Turkey. They found that the serum levels of HCY were higher in BD patients and their relatives compared to controls. However, mood episodes were not reported (Ozbek *et al*. 2008).

Dittman *et al*. (2007, 2008) and Osher *et al*. (2008) reported that elevated HCY levels were associated with cognitive impairment. These authors concluded that HCY could play a role in the pathophysiology of executive dysfunction in BD patients. However, Dias *et al*. (2009) found that the HCY level was not useful as a predictor of cognitive impairment, and a relationship was not found between HCY levels and functional deterioration. In the study by Reif *et al*. (2005) HCY was not found to be associated with cognitive function or imaging, and the authors reported that the HCY levels of disabled patients tended to be higher. Similarly, Osher *et al*. (2004) reported that high HCY levels were present in only the one third of BD patients who showed functional deterioration. However Dittman *et al*. (2007, 2008) did not detect a relationship between HCY levels and functioning.

HCY is one of the components of one-carbon metabolism. Single–carbon metabolism represents a candiate mechanism waiting to be explored in relation to cognitive impairment in BD. There are discrepancies in the literature regarding the relationship between HCY levels and cognitive and psychosocial functioning in bipolar patients. These differences have necessitated the need for new studies. Measurement of methionine was added to the study to facilitate a more complete understanding of the effect of single-carbon- metabolism in BD. Additionally to control for other confounding factors TFT, KCT, BFT, CBC, and blood lipid analysis was performed in all subjects. These analyses distinguish our study from previously published reports. Consequently, in this study we aimed first to compare the serum HCY, methionine, folate, and vitamin B12 concentrations in euthymic bipolar patients with those of healthy controls and second to observe the relationship of the abundance of these molecules with clinical features and cognitive and psychosocial functioning in bipolar patients in comparison with healthy subjects.

METHODS

Subjects

This case-control study was conducted in an outpatient clinic of psychiatry in Akdeniz University Medical Faculty. The sample size was estimated as 60 euthymic BD patients and 20 controls, resulting in statistical power of 95%. Patients who were diagnosed as type 1 BD using the semi-structured clinical interview in the DSM-IV-TR, patients who scored a 5 or less on the Turkish version of the Young Mania Rating Scale, and patients who received a 7 or less on the Turkish version of the Hamilton Depression Rating Scale were accepted as euthymic. Patients who had neurologic diseases, cardiovascular diseases, renal diseases, diabetes mellitus, hypo/hyperthyroidism, non-psychotropic drug use that could affect HCY levels, substance abuse, an IQ score below 80 based on the WAIS-R test (Epir and Iskit, 1972), a history of electroconvulsive therapy during the previous 6 months, or patients who tested positive for biochemical markers suggestive of abnormal thyroid, liver or renal functions were not included in the study. Seventy-one patients were considered for this study. Eleven patients were excluded because of cardiovascular disorders (three patients), abnormal thyroid function tests (two patients), and unwillingness to participate (six patients). Finally, 60 patients in the euthymic period who provided informed consent and 20 controls matched for age, gender, and educational level were included in the study. The control group included subjects who had not been diagnosed with a psychiatric disease and did not have a known significant medical condition or mental development disorder, subjects who were matched with the patient group in terms of age and gender, and patients who did not have psychiatric disorders according to a Structured Clinical Interview for DSM III R-nonpatients (SCIDNP). Approval was obtained for this study from the ethics committee of Akdeniz University Medical Faculty. All patients were informed prior to participation, and volunteer consent was obtained.

Materials

Sociodemographic and clinical data questionnaire: Sociodemographic and clinical data were collected from euthymic BD patients who were enrolled in the study as the result of clinical and laboratory assessments through a questionnaire prepared in accordance with clinical experience and information obtained from screened references. The variables evaluated in this questionnaire included age, gender,

educational level, marital status, working condition, history of psychiatric diseases in the family, age of illness onset, illness duration (months), number of total episodes, number of manic and depressive episodes, episodes clinical charecteristics (postpartum onset, psychotic, melancholic, seasonal), drug therapy, cigarette consumption, alcohol and substance addiction, and suicide attempt.

Structured Clinical Interview for DSM-IV (SCID-I): SCID-I is a structured diagnostic interview according to DSM-IV. It has ben re-adopted to DSM-IV-TR by First *et al*. (1997). Validity and reliability study has been reported by Özkürkçügil *et al*.(1999).

Structured Clinical Interview for DSM-III-R, Non-Patient (SCID-NP): A structured form developed by Spitzer *et al*. (1987) was utilized in diagnostic interviews and has been adopted to Turkish by Sorias *et al*. (1990).

Hamilton Depression Rating Scale (HDRS): This scale was developed by Hamilton (1960) to measure the severity of depression and to establish a symptom profile of depression. It has been standardized in Turkey by Akdemir *et al*. (1996).

Young Mania Rating Scale (YMRS): This measurement was developed by Young *et al*. (1978) to measure the severity and evolution of manic condition. Its Turkish version was standardized by Karadag *et al*. (2001).

Revised Wechsler Adult Intelligence Scale/ WAIS-R: This measurement was developed by Wechsler (1955). According to this scale verbal, performance, and total IQ scores were obtained. It has been adapted to Turkish by Epir and İskit (1972).

Global Assessment of Functioning (GAF) (Hall, 1995) was used to evaluate psychosocial functioning in the patient group. GAF was assessed by two clinicians who were familiar with the patients but blinded to the results of the neuropsychological tests and the HCY levels.

Neuropsychological assesment

The Wisconsin Card Sorting Test (WCST): This test used for the follow-up of executive and visual-motor functions was developed by Heaton (1981) and adapted to Turkish by Karakaş *et al*. (1998). It includes four stimulus and 64 response cards. The subject is required to match every response card in the pile with the stimulus card which he thinks to be correct. After 10 correct succesive matches, the next category is revealed. The test is finished when all of the six categories are completed or all of the cards are used. 13 different scores are obtained; these are total number of trials, number of total errors, number of total correct responses, number of achieved categories, number of persevarative responses, number of persevarative errors, number of nonpersevaretaive errors, percentage of perseverative errors, number of trials to complete first category, number of conceptual level responses, percentage of conceptual level responses, number of failures to maintain set, and learning to learn score.

Trail Making test (TMT): This test has two sections. Section A is used to measue psychomotor speed and attention while section B is used to evaluate executive functions. Time to complete the test and number of errors are taken into account for scoring. In our study completion time is also evaluated. This test has been developed by Reitan (1958) and adapted for use in Turkey by Karakaş *et al*. (1998).

Stroop Test: This is a test that evaluates the ability to direct and sustain attention, to resist interferring and incongruent stimuli, and to repress incongruent reactions (Golden 1978). It is adapted for use in Turkey by Karakaş *et al*. (1998). In our study, the Stroop test (Basic Science Research Group) is used. This test consists of four cards. On each card there are six lines consisting of four items organised nonselectively. When the subject finishes each task, completion time, number of errors, and number of correction are recorded. Stroop Test evaluates especially interference effect (fifth task with the second stimulus card).

Rey's Auditory Verbal Learning Test (RAVLT): This test was developed by Rey (1964) to evaluate verbal learning and memory. It has been adapted to Turkish by Öktem *et al*. (1992). In the administration of the test the subject is required to remember a list of 15 words that are unrelated. The tester reads the words to the subject 10 times or until he repeats the full list without error. After each trial the subject's recall of the words is recorded. The scores that the test provides are immediate memory score (number of words recalled in the first trial), score of reaching ctiteria (the sequence of the trial at which the subject recalls the complete list of the worda), score of maximum learning (the maximum number of recalled words), long-term memory score (number of words recalled after 40 minutes), recognition score (number of words that were not recalled by the subject), error score (total number of faulty recalls in all the trials).

Cancellation test (CT): Developed by Mesulam (1985), this test measures sustained attention, visual scaning, reaction time, visiomotor speed, and adaptation and evaluates hasty reactions as well as repression of these reactions. It is adapted for use in Turkey by Karakaş *et al*. (2004).

Biochemical analysis

For each subject, a total of 16 cc of venous blood was drawn following 12 hours of fasting on the day that neuropsychological tests were administered to all participants. Blood was portioned into Eppendorf tubes after centrifugation at 4000 rpm for 4 min and stored on ice for 15 min. Afterwards, serum samples were stored at -80 °C until analysis. After measurements of urea, creatinine, TFT, AST, ALT, total cholesterol,

TG, LDL, VLDL and HDL cholesterol had been completed, patients whose results were within the normal ranges were included in the study.

Statistical analysis

SPSS 16.0 was used for statistical analysis. Chi-squared and Kruskal Wallis tests were used for categorical variables, and a Student's t-test was used for continuous variables and the comparison of sociodemographic features of the patient and control groups. Because HCY and vitamin B12 levels did not conform to a normal distribution, the Mann Whitney U test was used for these comparisons. Methionine and folate levels conformed to a normal distribution and the t-test was used for comparison of these goups. Pearson's correlation test was used for analysis of the relationships among parametric quantitative variables. The Spearman correlation test was used for analysis of relationships between nonparametric quantitative variables. Bonferroni correction was used for statsitically significant correlation. HCY, methionine, age, valproate use, gender, the gender-methionine relationship, and the effect of the gender-HCY relationship on the likeliness of developing BD were analyzed by logistic regression analysis. A probability of $p < 0.05$ at a 95% confidence interval was considered statistically significant.

RESULTS

Sociodemographic and clinical characteristics

There was no difference between patients and controls with respect to sociodemographic features. The mean duration of illness was 12.13 ± 8.90 (mean ± S.D.) year in the BD patient group, and the mean age of illness onset was 26.28 ± 10.24. No significant difference in age of illness onset or duration of illness was found between male and female patients. The mean HDRS score was 0.75±1.14 and the mean YMRS score was 0.10±0.54 in the BD patient group. A total of 11 patients (18.33%) were being treated with only lithium, 6 patients (10%) were treated with only valproic acid, 30 patients (50%) were being treated with mood stabilizing drugs plus antipsychotic drugs and 12 patients (20%) were being treated with two mood stabilizing drugs. One third of patients were using valproic acid. One patient (1.66%) was not using medication. Seasonal illness was detected in 41.7% of the patients (n=25), postpartum onset was detected in 13.3% (n=8), melancholic illness was detected in 71% (n=22), and psychotic illness was detected in 56.7% (n=34).

Biochemical data

A statistically significant difference was not detected between the bipolar group and the control group for mean serum values of HCY, folate, and vitamin B12. A statistically significant difference was found between the bipolar group and the control group for mean serum methionine concentration (Table 1). A negative correlation was found between HCY and vitamin B12 ($r=-0.263$, $p=0.042$) and folate ($r=-0.312$, $p=0.015$), whereas no significant correlation was detected between HCY and methionine.

A statistically significant difference was detected between males and females in terms of serum HCY levels in the BD group ($Z=-3.211$, $p<0.01$). A statistically significant difference in serum HCY levels was detected between males and females in the control group ($Z=-2.227$, $p<0.05$). HCY was found to be higher in males in all groups (Fig. 1a). A statistically significant difference in serum methionine levels was detected between males and females in the BD group ($Z=-3.259$, $p<0.01$). The serum methionine levels were found to be higher in males. A significant difference in serum methionine levels was not detected between males and females in the control group (Fig. 1b). A significant difference in serum vitamin B12 levels was not detected between males and females

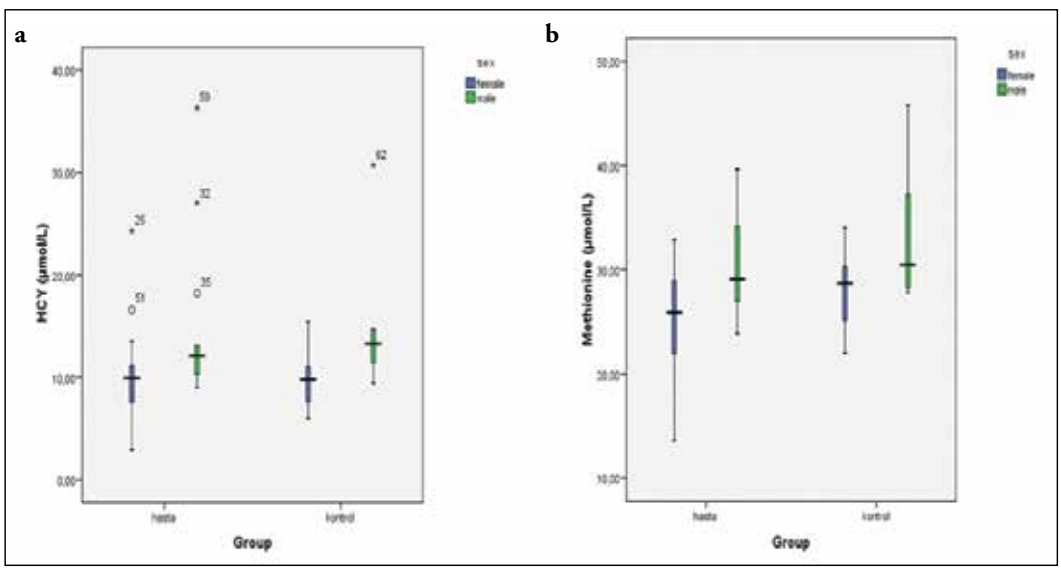

Figure 1.a,b: Serum HCY and methionine levels in female and male in BD and control groups

Table 1. Serum levels of homocystein, methionine, folate, B12 and neuropsychological tests in BD and control groups

		BD (N=60) Mean±SD	Control (N=20) Mean±SD	Z	P*
HCY		10.68 ± 5.45	11.37 ± 5.25	-.089	0.929
Methionine		26.94 ± 5.02	29.62 ± 5.36	-	0.046†
Folate		8.90±3.11	9.78±2.45	-	0.254†
B12		260.01±21.82	241.94 ±93.08	-.267	0.790
WCST	Number of trial	124.13±10.42	114.05±21.19	-2.17	0.030
	Number of correct	64.03±15.22	68.35±10.27	-.95	0.342
	Total errors	60.10±20.61	45.70±23.26	-2.267	0.023
	Number of perseverative responses	36.81±18.70	27.90±16.27	-1.756	0.079
	Number of non-perseverative errors	28.80±15.03	20.90±10.66	-1.968	0.049
	Number of perseverative errors	31.30±14.28	24.75±13.72	-1.601	0.109
	Number of category achieved	2.61±1,91	4.05±1.76	-2.821	0.005
	Percentage of perseverative errors	24.89±10.81	20.34±9.60	-1.595	0.111
	Trials to complete first category	27.80±22.48	23.85±14.19	-.642	0.521
	Percentage of conceptual level responses	36.03±19.36	51.83±21.02	-2.812	0.005
	Failures to maintance set	1.16±1.15	0.75±0.96	-1.409	0.159
	Learning to learn score	-6.09±8.43	-3.28±13.53	-.813	0.416
STROOP	Section 5 total time (interference trial)	29.35±9.43	20.85±5.60	-3.911	0.000
	Section 5 number of error	0.78±2.24	0.45±0.88	-.557	0.577
	Section 5 number of correction	1.40±1.34	0.90±1.02	-1.373	0.170
TRAIL MAKING TEST A	Total time	46.47±28.49	30.00±14.62	-3.485	0.000
TRAIL MAKING TEST B	Total time	132.32±73.93	75.60±30.61	-3.750	0.000
RAVLT	Immediate recall	6.86±1.99	7.30±1.59	-1.149	0.251
	Reaching criteria	6.19±2.17	5.52±1.89	-.858	0.391
	Maximum learning score	14.31±1.20	14.90±0.44	-2.334	0.020
	Learning error score	1.11±1.26	0.30±0.47	-2.566	0.010
	Delayed recall	12.85±2.17	13.80±1.23	-1.648	0.099
	Recognition score	1.71±1.60	0.85±1.03	-2.215	0.027
	Total recognition score	14.58±1.10	14.65±0.48	-1.204	0.228
	Long term memory error score	0.13±0.38	0.05±0.22	-.865	0.387
CANCELLATİON TEST	Total cancelled target number	231.08 ±6.29	231.55 ±7.11	-.518	0.605
	Total missed target number	8.91±6.29	8.45±7.11	-.518	0.605
	Total incorrectly cancelled target number	0.56±1.25	0.40±0.50	-.573	0.567
	Total error	9.48±6.75	8.85±7.19	-.495	0.620
	Total screening time	412.06±130.67	301.15±50.48	-4.017	0.000

*Mann Whitney U testi, †t test (P=0.046, t=-2.031, df=78; P=0254,t=-1.149, df=78)
BD: Bipolar Disorder
HCY: Homocystein
WCST: Wisconsin Card Sorting test
RVALT: Rey's Auditory Verbal Learning Test

in the BD group. A statistically significant difference in serum vitamin B12 levels was detected between males and females in the control group (Z=-2.144, p<0.05). Serum vitamin B12 levels were found to be higher in females. No statistically significant difference was found between genders with respect to serum folate levels. No statistically significant difference was found when levels of HCY, methionine, vitamin B12, and folate in males in both the bipolar group and control group were compared. No statistically significant difference was found when levels of HCY, methionine, vitamin B12,

and folate in females in both the bipolar group and control group were compared.

No significant correlation was found between age and HCY, methionine, vitamin B12, or folic acid levels in the BD patient group. Logistic regression analysis was used to evaluate HCY levels, methionine levels, age, valproate use, gender, the gender-methionine relationship, the gender-HCY relationship, and the effect of the HCY-valproic acid relationship on the likeliness of having BD. A statistically significant difference in the level of methionine between patients and controls was found (p=0.049, 95% CI=0.790-1000, OR=0.889). The probability of having BD decreased 0.889-fold for each unit increase in methionine. HCY, age, and gender did not have a deterministic effect on diagnostic predictability.

A significant positive correlation was detected between HCY levels and the duration of illness, the number of total episodes, and mania in the BD patient group (r=0.271, p=0.036; r=0.265, p=0.041; r=0.380, p=0.003). When groups with and without psychotic characteristics were compared, a statistically significant difference was detected with respect to vitamin B12 levels (Z=-3.043, p=0.002).

Cognitive functions

A positive correlation was detected between serum HCY levels and the maximum learning score in RAVLT among the BD group (r=-0.334, p=0.009), and a negative correlation was detected between methionine concentration and the long-term memory error score in RAVLT (r=-0.288, p=0.026). A negative correlation was found between vitamin B12 and the total screening time in the cancellation tests, the results of part A of the TMT, and between section 5 total time (interferance trial) of the Stroop test and the reaching criteria scores of RAVLT (r=-0.294, p=0.023; r=-0.322, p=0.012; r=-0.261, p=0.044, r=-0.301, p=0.021), and a positive correlation was found between vitamin B12 and immediate recall (r=0.271, p=0.037). Statistically significant correlations between HCY, methionine, vitamin B12, and cognitive functions were no longer significant subsequent to Bonferroni correction.

Psychosocial functioning

No significant correlation was found between HCY, methionine, folate, or vitamin B12 levels and GAF scores in the BD patient group (p>0.05). A significant correlation was found between GAF scores and the results of the neuropsychological tests (Table 2).

Table 2. The relationship between global assessment of functioning and neuropsychological tests in BD patients.

		r	P
WCST	Number of trial	-0.253	0.051
	Number of correct	0.371	0.004
	Total errors	-0.402	0.001
	Number of perseverative responses	-0.292	0.024
	Number of non-perseverative errors	-0.285	0.027
	Number of perseverative errors	-0.279	0.031
	Number of category achieved	0.393	0.002
	Percentage of perseverative errors	-0.273	0.035
	Trials to complete first category	-0.313	0.024
	Percentage of conceptual level responses	0.328	0.010
	Failures to maintance set	0.074	0.576
	Learning to learn score	0.304	0.063
STROOP TEST	Section 5 total time (interference trial)	-.336	0.009
TRAIL MAKING TEST A	Total time	-.538	0.000*
TRAIL MAKING TEST B	Total time	-.525	0.000*
RVALT	Immediate recall	.344	=0.007
	Reaching criteria	-0.309	0.046
	Maximum learning score	0.378	0.003
	Learning error score	-0.299	0.020
	Delayed recall	0.457	0.000*
	Recognition score	-0.457	0.000*
	Total recognition score	0.229	0.078
	Long term memory error score	-0.123	.351
CANCELLATION TEST	Total cancelled target number	347	0.007
	Total missed target number	-0.347	0.007
	Total incorrectly cancelled target number	-0.202	.121
	Total error	-0.361	0.005
	Total screening time	-.574	0.000*

*Significant after Bonferroni correction
HCY: Homocystein
WCST: Wisconsin Card Sorting Test
RVALT: Rey's Auditory Verbal Learning Test

DISCUSSION

Here we report elevated serum methionine concentration in BD patients relative to control patients. Serum HCY concentration correlated positively with illness duration, the number of total episodes, and the number of manic episodes.

Low serum methionine levels are predictive of BD. Lower levels of methionine can result from lower synthesis of SAM and deficiencies in methylation reactions. On the other hand, SAM administration is known to have antidepressant effects

and to strengthen cognitive functions in dementia patients and Parkinson patients with depressive symptoms (Regland 2005). However, SAM may cause a manic shift (Andreescu *et al*. 2008). Prior studies have reported that SAM regulates tetrahydrobiopterin synthesis and tetrahydrobiopterin contributes to metabolism of phenylalanine and tryptophan, amino acids with related monoamines (DA, NE, 5-HT). They found that tetrahydrobiopterin synthesis is decreased in depressed subjects in comparison with controls (Knapp and Irwin 1989).

We did not encounter any findings regarding serum methionine levels and BD reported in the literature. In previous studies that included schizophrenia patients, methionine load was reported to exacerbate psychotic symptoms (Antun *et al*. 1971), but the reason for this remained unclear until recently. Regland *et al*. (2005) found the CSF-methionine ratio of psychotic patients to be increased relative to controls. In last decade there have been significant findings suggesting that epigenetic modifications may contribute to multifactorial diseases like schizophrenia, BD, and depression. These studies demonstrated that hypermethylation in the brain tissue of schizophrenia, BD, and psychosis patients suppressed expression of reelin and GAD67, and contributed to schizophrenia and BD pathogenesis (Rodenhisar and Mann 2006; Peedicayil, 2007). Our finding that mean serum methionine concentration is lower in bipolar patients suggests that BD must be taken into account seperately with regard to schizophrenia. Additionally, our study includes patients who are in the euthymic stage of the illness, and thus they have been under medication. For instance, valproic acid has been shown to decrease HCY levels and hypermethylation of DNA in animal experiments (Dong *et al.* 2010). Medication can be protective for neurons in terms of hypermethylation. To our knowledge this is the first study in which evaluated the levels of methionine are reported in BD patients. In addition to studies which would explore methionine levels both in serum and CSF, there is a need for sudies investigating the relationship of methionine levels to different stages of the illness.

HCY levels showed a positive correlation with illness duration, the number of total episodes, and the number of manic episodes. We did not encounter this finding in the literature. However, longer illness duration and a higher number of total episodes may be related to more destructive illness. Dias *et al*. (2009) found that HCY levels were higher in patients with advanced age and reported that this could be associated with vascular causes; they also noted that being older corresponds with longer illness duration. In elderly patients, BD-related processes other than HCY levels that affect cognitive functions may also promote decreased cognitive function. Additionally, longer illness duration, conditions of greater undernourishment, a more sedentary life, and substandard living conditions may also lead to higher HCY levels in elderly patients (Stahl *et al*. 2005). Similarly, the observation that HCY levels are correlated with the number of manic episodes suggests that longer illness duration may cause elevation of HCY levels. However, the lack of a correlation between depressive episodes and HCY levels may be the result from poor ability to recall the depressive episodes before diagnosis of BD. On the other hand, if a patient is exposed to consistently high HCY levels, this may increase the number of total episodes. The correlation reported here between a high number of manic episodes and HCY level is the first to the best of our knowledge. Longitudinal studies may be needed to clarify this relationship.

Serum HCY concentration did not differ between BD patients and controls and levels of HCY, folate, and vitamin B12, as well as gender and age, were not predictors of BD in multi-variate analysis. Our results are similar to those of previous studies, with the exception of studies by Dittman *et al*. (2007, 2008) and a study by Ozbek *et al*. (2008). Ozbek *et al.* reported that 1298A>C and c.677C>T polimorphisms of MTHFR enzyme are associated with HS, folate, and B12 levels. They have reported a higher level of HCY in bipolar patients and their relatives when compared to healthy controls, and have suggested that high HCY, together with low levels of folate and vitamine B12, may be risk factors for BD-associated hyperhomocysteinemia. They have also suggested that the neurotoxic properties of hyperhomocysteinemia shows may be due to SAM. In our study, we did not find a significant difference in HCY levels between groups. This may be explained by the fact that the patients included in this study were in the euthymic stage of illness. On the other hand, Ozbek *et al.* did not report the stage of illness for the patients in their study. Be that as it may, both our study and the study by Ozbet *et al.* indicate the importance of SAM in the ethiology of BD. Osher *et al*. (2004) have observed high levels of HCY in one third of BP patients who showed functional impairment, and have related this increase in bipolar paients with functional detoriation to that seen in schizophrenia.

In accordance with previously reported data, there were statistically significant differences in serum HCY levels between males and females in the BD group, the control group and the group as a whole. Our finding of increased HCY levels in males compared to females in both the patient and control groups is consistent with the report by Osher *et al.* (2004). No difference was found when males in the BD and control groups were compared. Dittman *et al.* (2007) and Dias *et al.* (2009) found that HCY levels were high only in males in the patient group. According to our results, HCY levels are higher in males than in females, despite an equal prevalence of BD between gender. This strengthens the hypothesis that there is not a causal relationship between HCY and BD. The higher levels of HCY in males compared to females may be related to protective effects of estrogen in females (Stahl *et al.* 2005), genetic anthropometric differences (Panagiotakos *et al*. 2005), differences in fruit and vegetable consumption and smoking

habits (Ganji and Kafai, 2003), and lower folate and vitamin B12 levels in males (Levine *et al*. 2006). In a multi-variate analysis, gender, the gender-HCY relationship, and the gender-methionine relationship were not found to be predictors for the disease. Undernourishment, smoking, obesity, a sedentary lifestyle and functional deterioration may lead to high HCY levels (Stahl *et al*. 2007). In our study, a slight elevation was seen in HCY levels among smokers. However, no statistically significant difference was found. Additionally, regular alcohol use was evaluated in the patient and control groups.

Similar to the findings of Dias *et al*. (2009), a statistically significant difference was not detected between the two experimental groups in terms of vitamin B12 and folate levels. Consistent with other studies (Ozbek *et al*. 2008), a negative correlation was found between HCY levels and serum vitamin B12 and folate levels.

A few studies have investigated the relationship between HCY and cognitive functions in bipolar disorder, with inconsistent results. In our study, no significant correlation was found between serum HCY, methionine, folic acid, B12 levels, and neuropsychological test results. In our study, executive dysfunction was not observed, although it has been reported in previous studies (Dittman *et al*. 2007; Dittman *et al*. 2008; Osher *et al*. 2008). In a study by Dias *et al*. (2009), a significant relationship was only detected between high HCY levels and the reaction numbers in the Hanoi tower test and the preservation number in the Stroop color test. However, the authors do not suggest that HCY level is a predictor for cognitive impairment when using a linear regression model. Our study supports the results of Dias *et al*.

No significant correlation was found between HCY, methionine, folate, and vitamin B12 levels, and psychosocial functioning in the BD group. However, consistent with previous reports, a relationship was found between some cognitive functions and psychosocial function. Altought a strong relationship exists between psychosocial functioning and cognitive functions, no significant relationship has been detected between HCY levels and psychosocial functioning in these studies. (Dittman *et al*. 2007; Dias *et al*. 2009). Our study supports these findings.

That all patients in this study, with the exception of one, were on medication complicates interpretation of the study results. To eliminate the effects of drugs on cognitive functions, drug-free euthymic patients should be examined. However, such patients are rare. This study did not control for factors such as genetic features, lifestyle of the patient, poor nutrition, and obesity. This adds to the limitations of the study. Furthermore, unlike other studies, the patient and control groups were screened for medical conditions that may contribute to HCY elevation. This was an advantage of our study.

To our knowledge this is the first study to show that mean methionine level is significantly different in BD patients when compared to controls. Additionally, low serum methionine was found to be a predictor for BD. HCY was associated with increased duration of illness and the number of total episodes. Evidence has shown that alterations in the abundance of single-carbon mechanism markers are frequently seen in psychiatric disorders. However, a causal relationship was not detected. Studies of single-carbon mechanisms have focused on low folate and high HCY, whereas psychiatric disorders are multifactorial. The findings of this study suggest a role for both methionine balance and epigenetic mechanisms in the patogenesis of BD. New studies are needed to clarify the role of methionine in distinct episodes of BD. In the future, folate, vitamin B12, HCY levels, and the SAM/SAH ratio may be measured and treated in both BD and the other psychiatric diseases.

REFERENCES

Akdemir A, Önsel S, Dağ I et al. (1996) Hamilton Depresyon Derecelendirme Ölçeğinin geçerliği, güvenirliği ve klinik kullanımı. 3P Dergisi, 4: 251-9.

Andreescu C, Mulsant BH, Emanuel JE (2008) Complementary and alternative medicine in the treatment of bipolar disorder-A review of evidence. J Affect Disord, 110: 16-6.

Antun FT, Burnett GB, Cooper AJ et al. (1971) The effects of L-methionine (without MAOI) in schizophrenia. J Psychiatr Res, 8: 63-71.

Applebaum J, Shimon H, Sela BA et al. (2004) Homocysteine levels in newly admitted schizophrenic patients. J Psychiatr Res, 38: 413-6.

Bora E, Vahip S, Akdeniz F (2008) Bipolar bozuklukta bilişsel belirtilerin doğası ve önemi. Turk Psikiyatri Derg, 19(1):81-93.

Dias VV, Brissos S, Cardoso C et al. (2009) Serum homocysteine levels and cognitive functioning in euthymic bipolar patients. J Affect Disord, 113: 285-90.

Dittmann S, Seemuller F, Schwartz MJ et al. (2007) Association of cognitive deficits with elevated homocysteine levels in euthymic bipolar patients and its impact on psychosocial functioning: Preliminary results. Bipolar Disord, 9: 63-70.

Dittmann S, Seemüller F, Grunze HC et al. (2008) The impact of homocysteine levels on cognition in euthymic bipolar patients: A cross-sectional study. J Clin Psychiatry, 69: 899-906.

Dong E, Chen Y, Gavin DP et al. (2010) Valproat induces DNA demethylation in nuclear extracts from adult mause brain. Epigenetics, 5:730-5.

Epir S, Iskit Ü (1972) Wechsler Yetişkinler Zeka Ölçeği Türkçe çevirisinin ön analizi ve üniversite danışmanlık merkezlerindeki uygulama potansiyeli. Hacettepe Sosyal ve Beşeri Bilimler Dergisi, 4: 198-205.

First MB, Spitzer RL, Gibbon M et al. (1997) Structured Clinical Interview for DSM-IV Clinical Version (SCID-I/CV). Washington DC, American Psychiatric Press.

Ganji V, Kafai MR (2003) Demographic, health, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr, 77: 826-33.

Goldberg JF, Harrow M (1999) Poor Outcome Bipolar Disorders. Bipolar Disorders, Clinical Corse and Outcome, JF Goldberg, M Harrow (ed), Washington DC. American Psychiatric Pres, s. 1-19.

Golden, CJ (1978) Stroop Color and Word Test: A Manual for Clinical and Experimental Uses. Chicago, Illinois. s. 1-32.

Hall RC (1995) Global assessment of functioning. A modified scale. Psychosomatics, 36: 267-75.

Hamilton MA (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry, 23: 56-62.

Heaton R (1981) The Wisconsin Card Sorting Test Manual. Psychological Assessment Resources, Odesa FL.

Jensen OK, Ingerslev J (1998) Increased p-homocysteine a risk factor for thrombosis. Ugest Laeger, 160: 4405-10.

Karadağ F, Oral ET, Aran Yalçın F et al. (2001) Young Mani Derecelendirme Ölçeğinin Türkiye' de geçerlik ve güvenirligi. Turk Psikiyatri Derg, 13: 107-114.

Karakaş S, Irak M, Ersezgin ÖU et al. (1998) Wisconsin Kart Eşleme Testi (WCST) ve Stroop Testi TBAG formu puanlarının test içi ve testler-arası ilişkileri. X. Ulusal Psikoloji Kongresi özet kitabı, p. 44.

Karakaş S (2004) BİLNOT Batarayası El Kitabı: Nöropsikolojik Testler İçin Araştırma ve Geliştirme Çalışmaları. Ankara Dizayn Ofset.

Knapp S, Irwin M (1989) Plasma levels of tetrahydrobiopterin and folate in major depression. Biol Psychiatry, 26: 156-62.

Levine J, Stahl Z, Sela BA et al. (2002) Elevated homocysteine levels in young male patients with scizophrenia. Am J Psychiatry, 159: 1790-2.

Levine J, Agam G, Sela BA et al. (2005) CSF homocysteine is not elevated in schizophrenia. J Neural Transm, 112: 297-302.

Levine J, Stahl Z, Sela BA et al. (2006) Homocysteine reducing strategies improve symptoms in chronic schizophrenic patients with hiperhomocysteinemia. Biol Psychiatry, 60: 265-9.

Mattson MP, Shea TB (2003) Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends in Neurosci, 26: 137-46.

Mesulam MM (1985) Principles of Behavioral Neurology. Philadelphia FA Davis Company.

Obeid R, Herrman W (2006) Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. Febs Lett, 580: 2994-3005.

Öktem Ö (1992) Sözel Bellek Süreçleri Testi (SBST): Bir ön çalısma. Noropsikiyatri Ars, 29: 196-206.

Osher Y, Sela BA, Levine J et al. (2004) Elaveted homocystein levels in bipolar disorder patients showing functional deterioration. Bipolar Disord, 6: 82-6.

Osher Y, Bersudsky Y, Silver H et al. (2008) Neuropsychological correlates of homocysteine levels in euthymic bipolar patients. J Affect Disord, 105: 229-33.

Ozbek Z, Kucukali CI, Ozkok E et al. (2008) Effects of the methylenetetrahydrofolate reductase gene polymorphisms on homocysteine, folat and vitamin B 12 in patients with bipolar disorder and relatives. Prog Neuropsychopharmacol Biol Psychiatry, 32: 1331-7.

Özkürkçügil A, Aydemir Ö, Yıldız M et al. (1999) DSM-IV eksen I bozuklukları için yapılandırılmış klinik görüşmenin Türkçeye uyarlanması ve güvenilirlik çalışması. İlaç ve Tedavi Dergisi, 12: 233-6.

Panagiotakos DB, Pitsavos C, Zeimbekis A et al. (2005) The association between lifestyle-related factors and plasma homocysteine levels in healthy individuals from the "ATTICA" Study. Int J Cardiol, 98: 471-7.

Peedicayil J (2007) The role of epigenetics in mental disorder. Indian J Med Res, 126: 105-11.

Regland B, Johansson BV, Grenfeldt B et al. (1995) Homocystinemia is a common feature of schizophrenia. J Neural Transm, 100: 165–9.

Regland B (2005) Schizophrenia and single-carbon metabolism. Prog Neuropsychopharmacol Biol Psychiatry, 29: 1124-32.

Reif A, Schneider MF, Kamolz S et al. (2003) Homocysteinemia in psychiatric disorders: Associations with dementia and depression but not schizophrenia in female patients. J Neural Transm, 110: 1401-11.

Reif A, Pfuhlmann B, Lesch KP (2005) Homocysteinemia as well as methylenetetrahydrofolate reductase polymorphism are associated with affective psychoses. Prog Neuropsychopharmacol Biol Psychiatry, 29: 1162-8.

Reitan RM (1958) Validity of the Trailmaking Test as an indicator of organic brain damage. Percept Motor Skills, 8: 271-6.

Rey A (1964) L'examen Clinique en Psychologie. Paris Presse Universitaire de France,

Rodenhisar D, Mann M (2006) Epigenetics and human disease: translating basic biology into clinical applications. CMAJ, 174(3): 341-348.

Sorias S, Saygılı R, Elbi H et al. (1990) DSM-IIIR yapılandırılmış klinik görüşmesi, Türkçe versiyonu. Bornova, Ege Üniversitesi Basımevi.

Spitzer RL, Williams JBW, Gibbon M (1987) Structured Clinical Interview for DSM-III-R (SCID). New York State Psychiatric Institute-Biometrics Research, New York.

Stahl Z, Belmaker RH, Friger M et al. (2005) Nutritional and life style determinants of plasma homocysteine in schizophrenia patients. Eur Neuropsychopharmacol, 15: 291-5.

Tanriverdi H, Evrengul H, Enli Y et al. (2007) Effect of homocysteine induced oxidative stres on endotelial function in coronary. Slow-Flow. Cardiology, 107: 313-20.

Wechsler D (1955) Manual for the Wechsler Adult Intelligence Scale. New York, The Psychological Corporation.

Young RC, Biggs JT, Ziegler VE et al. (1978) A rating scala for mania: reliability, validity, and sensitivity. Br J Psychiatry, 133: 429-35.