Adjuvant Folate with Escitalopram Treatment and Homocystein, Folate, Vitamin B-12 Levels in Patients with Major Depressive Disorder

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ÖZET:

Major depresif bozukluğu olan hastalarda essitaloprama folik asit ekleme tedavisi ve homosistein, folik asit, B-12 vitamini düzeyleri

Amaç: Tıbbi yazında Major Depresif Bozukluğu (MDB) olan ve antidepresan ilaç verilen hastalarda folik asit ekleme tedavisinin klinik iyileşmeye katkısı olduğunu ortaya koyan çalışmalar vardır. Bu çalışmanın öncelikli amacı; ilk kez psikotrop tedavi alacak olan MDB tanısı konan hastalara essitalopram tedavisine folik asit eklemenin tedaviye olan katkısını değerlendirmektir. İkincil amaç ise, MDB hastalarında psikometrik ölçümler, folik asit, total homosistein (tHst), B-12 vitamini seviyeleri arasındaki ilişkiyi araştırmaktır.

Yöntem: Çalışma örneklemini oluşturan toplam 35 MDB hastası iki gruba ayrıldı: Ardışık olarak bir gruba; günlük 10 mg essitaloprama ek olarak 2,5 mg folik asit verilirken (n=20), diğer gruba sadece günde 10 mg essitalopram verildi (n=15). Tüm hastalar DSM-IV'e göre major depresyon tanı kriterlerini karşılamakta olup, kontrol grubu 32 sağlıklı gönüllüden oluşmaktaydı. Depresyon semptomlarının şiddeti, Montgomery-Asberg Depresyon Değerlendirme Ölçeği (MADDÖ) ile değerlendirilirken, hastalığın şiddeti Klinik Global Değerlendirme ölçeğinin (KGD) uygun modülü kullanılarak ölçüldü. MADDÖ de başlangıç skoruna göre %50 azalma sağlanması tedaviye yanıt olarak kabul edildi. Gruplarda depresif semptom şiddeti, Folik asit, B-12 vitamini ve tHst seviyeleri tedavi öncesi ve tedavi sonrası (altıncı hafta) ölçülerek karşılaştırılmıştır.

Bulgular: Tüm MDB hastalarının essitalopram tedavisine klinik yanıtı %53,4 olarak saptanırken (n=35), başlangıçtaki ölçümlerde tüm hastaların serum folik asit ve plazma tHst seviyeleri arasında belirgin negatif korelasyon vardı (Spearman r=-0.426 ve p=0.011). Yalnız essitalopram alan hastaların %80'i tedaviye olumlu yanıt verirken, folik asit ekleme tedavisi alan hastaların cevap oranı %35 olarak tespit edildi (P =0.016). Ayrıca hastaların plazma tHst düzeyleri sağlıklı kontrollere göre daha yüksekken (z= -2.72, p= 0.006) idrar tHst düzeyleri daha düşük saptanmıştır(z= -6.48, p<0.001).

Tartışma: Essitalopramın çok sayıda araştırma ile antidepresan etkisi kanıtlanmış olmasına rağmen, bu çalışmada folik asit ekleme tedavisinin daha önce yapılan çalışmaların tersine antidepresan etkinliğe katkı sağlamadığı anlaşılmıştır. Bununla birlikte bu konuda, daha uzun süreli folik asit kullanımına imkan sağlayan ve daha geniş popülasyonda yapılacak kontrollü prospektif çalışmalara ihtiyaç vardır. Ayrıca MDB olan hastalarda antidepresan tedaviye cevabı değerlendirirken folat düzeylerinin yanında B 12 vitamini gibi diğer değişkenlerin de hesaba katılması gerekmektedir.

Anahtar sözcükler: Essitalopram; folik asit; homosistein; depresyon; B-12 vitamini

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ABSTRACT:

Adjuvant folate with escitalopram treatment and homocystein, folate, vitamin B-12 levels in patients with major depressive disorder

Objective: Folate supplementation in Major Depressive Disorder (MDD) has been investigated in clinical studies with promising results. The primary objective of this trial was to study the effect of adjuvant folate treatment with escitalopram in MDD patients. Secondary objectives were to evaluate the relationships between the levels of three metabolites [Folic acid; total homocysteine (tHcy); vitamin B12] and psychometric measurements in patients with MDD.

Methods: Thirty five patients with major depression were randomly divided into two groups; receiving either 2.5 mg folic acid in addition to 10 mg escitalopram (n=20) and receiving only 10 mg escitalopram (n=15) daily. The comparison group consisted of 32 healthy volunteers. All the patients were evaluated by using the Montgomery-Asberg Depression Rating Scale (MADRS) and the Clinical Global Impression (CGI) scale. Clinical response was defined by at least a 50 % reduction in baseline MADRS total scores. The depressive symptoms of patients, samples of folate, vit-B12 and tHcy levels were measured at baseline and after 6 weeks of treatment.

Results: The clinical response to treatment was 53.4% (n=35) in all patients. A percentage of 80% of patients, who received only escitalopram, showed a good response (50% reduction in score) as compared to 35% of patients who received adjuvant folic acid supplementation beside escitalopram (P =0.016). There was a negative correlation between serum folate and plasma tHcy levels of patients at baseline (Spearman r=-0.426 and p=0.011). In addition, patients had higher mean plasma tHcy (z= -2.72, p= 0.006) and lower urine tHcy levels (z= -6.48, p<0.001) than controls.

Conclusions: Although escitalopram is proved to have antidepressant effect via numerous studies; conversely to the prior studies, folic acid augmentation had no effect of enhancing the antidepressant action in this study. Furthermore, controlled prospective studies administering folate treatment for longer time that involve a wider range of population are needed. Additionally, while evaluating the antidepressant treatment response of MDD, it is necessary to take variables like vit B-12 deficiency into account besides folate levels.

Key words: Escitalopram; folic acid; homocysteine; depression; vitamin B-12

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INTRODUCTION

Clinical depression is common, debilitating and treatable; one in four people experiences it during their lives (1). The monoamine hypothesis of depression implicates a functional deficiency of noradrenaline (NA) or serotonin (5-hydroxytryptamine, 5-HT) in neurotransmission; virtually all antidepressants are thought to act by prolonging the activity of these neurotransmitters or by modulating receptor sensitivity (2). Folate is an essential cofactor for the biosynthesis of both 5-HT and NA. Thus, folate deficiency leads to impaired 5-HT synthesis in the human brain (3). However, it has not been established whether folate supplementation actually enhances monoamine synthesis or release (4). Moreover, studies demonstrated that up to one-third of patients with depressive illness have decreased plasma and red cell folate levels (5). Patients with low folate levels respond poorly to antidepressant therapy (6-10). A variety of evidence suggests that folate may be a useful adjunct to antidepressant treatment: 1) patients with depression often have a functional folate deficiency; 2) the severity of such deficiency, indicated by elevated homocysteine, correlates with depression severity, 3) low folate is associated with poor antidepressant response, and 4) folate is required for the synthesis of neurotransmitters implicated in the pathogenesis and treatment of depression.

More recently, the total plasma homocysteine level was shown to be a sensitive marker of folate and vitamin B12 deficiency, and higher concentrations of homocysteine were observed in depressed patients (11,12). Homocysteine is an amino acid that is formed by the demethylation of nutritional methionine, and folate and vitamin B12 are cofactors in its metabolism. At high concentrations, it is considered to be a neurotoxic substance, causing activation of NMDA receptors and leading to excitotoxicity (13,14). By impairing neuronal plasticity and promoting neuronal degeneration, homocysteine could contribute to the pathogenesis of neurodegenerative and psychiatric disorders (15,16). An association between depression and folate status has been demonstrated in clinical studies, whereas data are sparse on the relationship between depression and other components of 1-carbon metabolism such as vitamin B12 and homocysteine.

To the best of our knowledge, no study has been reported yet about adjuvant folate treatment in combination with escitalopram in subjects with MDD. Folate supplementation in MDD has been investigated in clinic studies with promising results. However, it has not been established whether folate supplementation is actually useful adjunct to antidepressant treatment or not. The primary objective of this trial is to investigate the effect of adjuvant folate treatment to escitalopram in the new treatment of MDD. Secondary objectives are to evaluate the relationships between the levels of three metabolites (Folic acid; tHcy; vitamin B-12) and clinical measurements in patients with MDD.

MATERIAL and METHODS

Subjects

A total of 59 patients who were diagnosed with MDD according to the fourth edition of DSM-IV (20) and who were consecutively admitted to the outpatient psychiatric unit of GATA Haydarpasa Training Hospital in the year 2008, were included in this study. Subjects were included if they met the following general inclusion criteria: 1) Male or female had a new episode of depression. A new episode was defined as the first depressive episode in a period of at least six months 2) No comorbid major psychiatric disorders 3) Literate, 4) Had been free from folic acid, MAOIs, and all other anti-depressants for the past 9 weeks. Subjects less than 18 years and those with severe physical illness such as thyroid disease were excluded from the study. Patients with concomitant conditions or a history of another psychiatric disease, epilepsy, organic brain disorders, such as head trauma, that might have caused neurological diseases, and patients with endocrine or metabolic diseases were excluded from the study. In addition, patients were excluded from the trial if they: were folate and vitamin B12 deficient, were pregnant or planning to become pregnant as it is important for pregnant women to take folic acid so they cannot be randomized. Five subjects were excluded due to illiteracy and three due to severe physical illness. Nine subjects reported that they were not willing to participate in the study anymore. Thus, 42 patients with MDD participated in the study. Subjects were randomly divided into two separate groups as patients receiving escitalopram (n=19), and patients receiving escitalopram plus folic acid (n=23). Random-number table

was used for randomization. Seven patients failed to complete the study, fourth were withdrawn because of possible treatment-related side effects and three dropped out of the trial and were lost to follow up.

The comparison group consisted of 37 healthy and literate volunteers (19 men and 18 women) with an age range of 18-66 and a median age of 28, recruited from the healthy volunteers at the same hospital. Each of them underwent a standardized diagnostic interview to rule out psychiatric disorders. They had no severe physical illness, an organic condition that could cause psychiatric symptoms, folate and vitamin B12 deficiencies. In addition, they were free from folic acid, MAOIs and all other anti-depressants for the past 9 weeks. Two subjects were excluded due to illiteracy and two due to severe physical illness. One subject reported that he was not willing to participate in the study anymore. Thus, 32 healthy controls participated in the study.

All subjects received a complete explanation of the study procedures, and written informed consent was obtained before the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Local Ethics Committee.

Measurements and Evaluation

The diagnosis of MDD was confirmed based on clinical examination, DSM-IV (17) and MDD section of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (OY, MAA), Turkish version (18). Semi-structured interview forms prepared by the investigators, were used to collect data concerning the sociodemographic characteristics of the participants. Once eligibility is determined, the investigator and the psychiatrist provided standard information about the trial and all patients were given the opportunity to ask any questions regarding the trial. The patients were told that they could withdraw from the study at any time without their usual care being affected. All patients were prescribed 10 mg of escitalopram. Participants were randomized to either escitalopram alone or 2.5 mg of folic acid (folbiol tb 1/2) besides escitalopram treatment. The severity of disease symptoms was evaluated using the Montgomery-Asberg Depression Rating Scale (MADRS), whereas disease severity was evaluated using the relevant module of the Clinical Global Impression (CGI) scale. Clinical response was defined by at least a 50% reduction in baseline MADRS total score. The depressive symptoms were assessed at baseline and 6th week of treatment.

Blood samples for folate, vitamin B12 and homocysteine levels and urine samples for homocysteine levels were obtained at baseline and after 6 weeks of treatment. In order to minimize the effects of diet, fasting morning blood samples and first urine samples in the morning were obtained from all study participants. After an overnight fasting period, morning venous blood samples were taken into vacuteiner tubes containing either without additives or with K3EDTA. The researchers involved in the biochemical measurements (OMI, ÖÖ) were blind to the subject group. The assessment of plasma homocysteine, serum folate and serum vitamin B12 concentrations was conducted simultaneously both in patients and controls at the laboratory of Clinical Chemistry of the GATA Haydarpasa Training Hospital. Total homocysteine serum and urine levels were determined quantitatively by a high performance liquid chromatographic (HPLC) kit (Chromsystems, Germany) with an HPLC system (HP Agilent 1100, Agilent Tech. Palo Alto, USA). Urine homocysteine levels were expressed in terms of Ìmol/g creatinine. Quantitative vitamin B12 and folate serum levels were measured with an electro-chemoluminescence immunoassay method performed on an Architect i2000 analyser (Abbott Lab., Abbott Park, IL, USA).

Statistical analyses

Categorical variables were compared with chi-square analysis (chi-square or Fisher's exact test). Homocysteine, folate and vitamin B12 levels in patients and controls did not follow a normal distribution. Thus, the Mann-Whitney U test was used to compare groups on variables, and Spearman's correlation test was used to determine the relationship between homocysteine, folate, vitamin B12 levels and MADRS and CGI scores in patients and controls. Comparison of clinical and biochemical masurements in the study groups at baseline and 6th weeks were done by Wilcoxon signed ranks test. Statistical significance was set at 0.05. Statistical analysis was carried out by the SPSS 15.0 for Windows statistical package (Chicago, IL).

RESULTS

The median age of the all patients was 30.0 (18-66) years, the median duration of education was 11.0 (3-15) years, and 57.1% (n=20) of the patients were married. Sixteen (45.7%) of the all patients were men, and 7 (20%) of those have poor economic status. In summary, there were no significant differences between the patient and control groups in any of the sociodemographic characteristics.

There was no significant difference in serum vitamin B12 and folate concentrations between patients and controls. Patients had higher mean plasma tHcy (z= -2.72, p= 0.006) and lower urine tHcy levels (z= -6.48, p<0.001) than controls. The biochemical measurements of the all patients and controls are shown in Table 1.

Patients were divided into two separate groups as patients receiving only escitalopram (n=15), and patients receiving escitalopram plus folic acid (n=20). Patients receiving escitalopram plus folic acid had lower median plasma tHcy (z= -3,584, p<0,001) and lower urine tHcy levels (z= -2,480, p= 0,013) at 6th weeks measurements than baseline measurements. The other measurements of the study groups are shown in Table 2.

The median age of the escitalopram group was 30 (21-66) years, the median duration of education was 11 (3-15) years, and 46.7% (n=7) of the patients were men. In summary, there were no significant differences between the patients receiving escitalopram plus folic acid and patients who had received escitalopram in any of the sociodemographic characteristics (see Table 3).

The clinical response to treatment was 53.4 % (n=19) in all patients. The patients receiving escitalopram had a better response to treatment than the patients receiving escitalopram plus folic acid (P =0.016) (In Table 3). The clinical response rate to treatment was 80% at the 6th week in the patients receiving escitalopram, but 35% in the patients receiving escitalopram plus folic acid at 6th week.

There were no significant differences between the two treatment groups in any of the baseline characteristics except serum folate levels. Patients receiving escitalopram plus folic acid had lower serum folate levels (z= -2.65, p= 0.008) and higher MADRS scores (z= -2.48, p=0.013) than patients receiving escitalopram at baseline and 6th weeks, respectively. There was no significant difference between the patients receiving escitalopram plus folic acid and patients receiving only escitalopram, although patients receiving only escitalopram showed a trend toward higher vitamin B12 levels. The other

Table 1: Biochemical Measurements in the all Patients and Healthy Controls at Baseline

Sample Characteristics	Total patients (N= 35) Median (Range) or n (%)	Healthy Controls (N= 32) Median (Range) or n (%)	z score	p value
Age	30(18-66)	28(18-66)	0.23	0.82*
Plasma tHcy (µmol/L)	12.40 (6.83-56.25)	9.78 (6.02-13.92)	-2.72	0.006*
Urine tHcy(µmol/g crea)	3.18(0.62-51.60)	21(10.14-69.3)	-6.48	<0.001*
Serum Folate (ng/ml)	6.10(2.50-13.10)	5.8(1.5-11.9)	-0.91	0.361*
Serum Vit B-12 (pg/ml)	265.6(118.5-681.4)	246.5(161-405)	-0.54	0.585*

*Mann-Whitney U test; tHcy=Total Homocysteine

Table 2: Comparison of Clinical and Biochemical Measurements in the Study Groups at Baseline and 6th weeks

Sample Characteristic	Escitalopram Group (n=15)		Analysis		Escitalopram+folate Group (n=20)		Analysis	
	Baseline Median (Range)	6th week Median (Range)	z	p	Baseline Median (Range)	6th week Median (Range)	z	p
MADRS	32(47-17)	12(6-35)	-3,411	<0,001	31.50(19-38)	16(10-33)	-3,847	<0,001
CGI	4(3-5)	2(1-4)	-3,473	<0,001	4(3-5)	2(1-4)	-3,930	<0,001
Serum Folate	7.40(4.20-13.10)	5.40(2.50-10.20)	-1,512	0,131	5.40(2.50-10.20)	7(3.70-20)	-3,883	<0,001
Plasma tHcy	9.61(6.83-56.25)	10.05(6.6-60.33)	-1,136	0,256	13.02(8.29-30.40)	10.15(5.40-22.6)	-3,584	<0,001
Urine tHcy	2.87(0.62-51.60)	3.38(0.84-18.30)	-,169	0,866	3.38(0.84-18.30)	2.61(0.66-10.40)	-2,480	0,013

Wilcoxon Signed Ranks Test,, tHcy; Total homocysteine, MADRS; Montgomery-Asberg Depression Rating Scale, CGI; Clinical Global Impression scale.

Table 3: Demographic, Clinical and Biochemical Measurements in the Study Groups

Sample Characteristics	Escitalopram Group (n=15) Median (Range) or n(%)	Escitalopram+folate Group(n=20) Median (Range) or n (%)	Analysis (z or x2)	p value
Age	30(21-66)	29(18-44)	-0.17	0.987***
Education (in years)	11(3-15)	11(5-15)	-0.93	0.350***
Sex				
Men	7(46.7)	9(45)	0.01	0.922*
Women	8(53.3)	11(55)		
Marital status				
Single/Widowed	9(60)	6(30)	3.15	0.076*
Married	6(40)	14(70)		
Occupational Status				
Unemployment	11(73.3)	13(65)		0.721**
Worker	4(26.7)	7(35)		
Economic Status				
Poor	4(26.7)	3(15)		0.430**
Moderate	11(73.3)	17(85)		
Response to treatment	12(80)	7(35)		0.016**
Baseline				
MADRS	32(17-47)	31.50(19-38)	-0.20	0.841***
CGI	4(3-5)	4(3-5)	-0.34	0.732***
Plasma tHcy (µmol/L)	9.61(6.83-56.25)	13.02(8.29-30.40)	-1.80	0.07***
Urine tHcy (µmol/g crea)	2.87(0.62-51.60)	3.38(0.84-18.30)	-0.21	0.834***
Serum Folate (ng/ml)	7.40(4.20-13.10)	5.40(2.50-10.20)	-2.65	0.008***
Serum B-12 (pg/ml)	308.30(128-681.40)	263.45(118.50-469.70)	-0.09	0.368***
6th Weeks				
MADRS	12(6-35)	16(10-33)	-2.48	0.013***
CGI	2(1-4)	2(1-4)	-1.74	0.08***
Plasma tHcy (µmol/L)	10.05(6.6-60.33)	10.15(5.40-22.60)	-0.1	0.920***
Urine tHcy(µmol/g crea)	4.13(1.07-43.8)	2.61(0.66-10.40)	-1.30	0.192***
Serum Folate (ng/ml)	7.30(3.9-12)	7(3.70-20)	-0.18	0.854***

*Chi-square test; **Fisher's exact test; ***Mann-Whitney U test; tHcy; Total homocysteine, MADRS; Montgomery-Asberg Depression Rating Scale, CGI; Clinical Global Impression scale.

Table 4: Correlation Coefficients of the Baseline and 6thweeks Clinical and Biochemical Measurements in the Patients

	MADRS	CGI	Plasma tHcy	Urine tHcy	Folate
Baseline					
Age	0.103	0.208	-0.123	0.119	0.292
MADRS		0.851**	0.011	-0.010	0.064
CGI			0.074	-0.281	0.176
Plasma tHcy (µmol/L)				-0.037	-0.426*
Urine tHcy (µmol/g crea)					0.177
Serum Vit B-12 (pg/ml)	0.123	0.155	-0.049	-0.320	0.113
6thweeks					
E Group					
MADRS		0.605*	-0.197	-0.143	0.012
CGI			-0.112	-0.656	0.066
Plasma tHcy (µmol/L)				-0.393	-0.166
Urine tHcy (µmol/g crea)					0.821*
EF Group					
MADRS		0.781**	0.125	0.289	0.047
CGI			-0.092	0.420	0.306
Plasma tHcy (µmol/L)				-0.054	-0.259
Urine tHcy (µmol/g crea)					-0.055

Spearman correlation; *p<0.05; ** p<0.01, tHcy; Total homocysteine, E; The patients receiving escitalopram, EF; The patients receiving escitalopram plus folic acid, MADRS; Montgomery-Asberg Depression Rating Scale, CGI; Clinical Global Impression scale.

biochemical measurements are shown in Table 3.

Table 4 shows the correlation coefficients of the clinical and biochemical measurements in the patients at baseline and 6 weeks. There was a significant negative correlation between the serum folate and plasma tHcy levels (Spearman r=≥0.426 and p=0.011), and a significant positive correlation between MADRS and CGI scores of patient group (Spearman r=0.851 and p<0.001) at baseline (in Table 4). Whereas the serum folate levels showed a significant positive correlation with the urine tHcy levels (r= 0.821, p=0.023) in patients receiving only escitalopram at baseline; folate, tHcy and vitamin B12 concentrations showed no correlation with clinical response in either treatment group at 6th weeks. The other correlations of biochemical and clinical measurements are shown in Table 4.

DISCUSSION

Although escitalopram improved depressive symptoms; adjuvant folic acid had no enhancing effect on the antidepressant action of escitalopram in this study. The clinical response to treatment was 53.4% (n=35) in all patients. Patients had higher mean plasma tHcy and lower urine tHcy levels than controls. There was a significant negative correlation between serum folate and plasma tHcy levels of patients at baseline.

The trials differed substantially in recruitment criteria and provided little evidence for or against the routine use of folic acid in antidepressant treatment. Whether the putative beneficial effect of folate is limited to those with folate deficiency or not is not clear (19,20). In one of the studies (21), folic acid supplementation greatly improved the antidepressant action of fluoxetine. There is little available information regarding the mechanism that may underlie a possible antidepressant-adjuvant effect (19,21,22). In our study, rates of response to treatment were significantly higher for patients receiving only escitalopram than patients receiving escitalopram plus folic acid (80% vs 35% respectively) at 6th week. As a reason for that, lower serum folate levels of patients receiving escitalopram compared with the other group at the baseline (5.40 ng/ml vs 7.4 ng/ml) should be considered. Several case-control studies since the 1960s have shown a high prevalence of folate and vitamin B12 deficiency in depression (23). However, in our trial, there was no clinical folate deficiency in both study groups initially. In fact, at the sixth week of the study, serum folate levels of patients receiving escitalopram plus folic acid, which already were not significantly lower than healthy control subjects, were equalized with the other group (7 ng/ml vs 7.3 ng/ml). In addition, the biological mechanism of folate augmentation may differ across different antidepressant treatments (24). Besides, compared with the other studies (4,6,19,21), adjuvant folate treatment period might be short (six weeks) in this study.

Weak response to treatment in the patients receiving escitalopram plus folic acid compared to the other group may be a result of relative vitamin B-12 deficiency at baseline (263.45 pg/ml vs 308.3 pg/ml). However, because distribution of groups didn't match the normal distribution in this study, we could not perform regression analysis. Investigations on a possible role of vitamin B12 status in neuropsychiatric disorders have been motivated by the central nervous system damage caused by overt or subtle vitamin B12 deficiency (25). Data regarding the association between vitamin B12 status and depression are scarce (6,26). Hyperhomocysteinemia, vitamin B12 deficiency, and to a lesser extent, folate deficiency were all related to depressive disorders (12). For folate deficiency and hyperhomocysteinemia, the association with MDD was substantially reduced after adjustment for functional disability and cardiovascular disease, but for vitamin B12 this appeared independent (12). The association of vitamin B12 and folate with depressive disorders may have different underlying mechanisms.

An association between depression and folate status has been demonstrated in clinical studies, whereas data are sparse on the relationship between depression and other components of 1-carbon metabolism such as vitamin B12 and homocysteine. Overall, hyperhomocysteinemia, but not low plasma folate or vitamin B12 levels, were significantly related to depression (12,27). Another study found that low folate and high tHyc, but not low vitamin B12 levels, were correlated with depressive symptoms (28). However there were no significant correlation between the biochemical measurements (tHcy, folate, vitamin B-12) and depressive symptoms in our study. In addition, we detected decreased urine tHcy levels in depressive patients. Although tHcy concentrations are known to be increased in chronic renal insufficiency (29), the observation that the rat kidney

takes up and metabolizes homocysteine in vitro (30) and in vivo (31) could not be confirmed in humans (32,33). The altered renal handling of tHcy could also occur without renal insufficiency. This may explain why our study group had increased plasma tHcy levels. A combination of decreased renal Hcy filtration and possibly down-regulation of intrarenal degradative pathways would lead to decreased plasma clearance in depressive patients (34). In addition, the patients with MDD had higher plasma tHcy levels than controls in this study (12.40 µmol/L vs 9.78 µmol/L, $p<0.01$). Similar to previous studies (34,35), there was a significant negative correlation between the baseline serum folate and plasma tHcy levels of the all patients in this study. The conclusion was that daily dosages of ≥0.8 mg folic acid, in addition to dietary intake, are typically required to achieve the maximal reduction in plasma tHyc concentrations (about 25%) (36,37). Folic acid 2.5mg/day reduced plasma tHcy concentrations (about 25%, from 13.02 to 10.15) six weeks later in our study, too.

The main limitation of this study was the small sample size. Second, this study was open-label and non placebo-controlled and psychotherapy during the study period was not controlled. Third, the medication treatment was short and lasted only six weeks. Fourth, treatment groups were not homogeneous although not shortcoming of folate and vitamin B12 levels. Besides, other variables that might have an influence on homocysteine plasma levels (e.g. cigars-alcohol-caffeine consumption, diet, exercise, menopause) were not evaluated.

CONCLUSIONS

In conclusion, the current findings have important theoretical and clinical implications for understanding the folic acid adjuvant treatment in MDD. To our knowledge, this is the first study of adjuvant folate treatment added to escitalopram treatment in patients with MDD. Consequently, while escitalopram is an effective antidepresssant in major depressive patients, adjuvant treatment with folic acid was not found to have an enhancing impact antidepressant efficacy. Patients had higher mean plasma tHcy and lower urine tHcy levels than controls. There was a significant negative correlation between serum folate and tHcy levels of patients at baseline. Further controlled prospective studies providing folate treatment for longer time and involving a wider range of population are needed. Additionally, while evaluating the antidepressant treatment response of MDD, it is necessary to take variables like vitamin B-12 deficiency into account besides folate levels.

"Abbreviations: MDD; Major Depressive Disorder, tHcy; total homocysteine, MADRS; Montgomery-Asberg Depression Rating Scale, CGI; Clinical Global Impression scale."

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