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N-Terminal Pro-Brain Natriuretic Peptide, Homocysteine and Methylenetetrahydrofolate Reductase Gene Polymorphism in Elderly Depressed and Mild Cognitive Impairment Patients

[Hafif Bilişsel Bozukluk ve Depresyon Görülen Yaşlı Hastalarda N-Terminal Pro-Beyin Natriüretik Peptid, Homosistein Düzeyleri ve Metilentetrahidrofolat Redüktaz Gen Polimorfizmleri]

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ABSTRACT

Purpose: To evaluate the role of N-terminal pro-brain natriuretic peptide, homocysteine and methylenetetrahydrofolate reductase gene polymorphism (TT, CT or CC) in elderly patients with mild cognitive impairment or depression, and to determine the association between TT genotype and both homocysteine and N-terminal pro-brain natriuretic peptide on one-hand and depressive and cognitive scores on other-hand .

Material and Methods: This study included 60 elderly patients, subdivided into, patients with depression, patients with mild cognitive impairment, in addition to the control group. N-terminal pro-brain natriuretic peptide, homocysteine, folate and methylenetetrahydrofolate reductase gene polymorphism were determined.

Results: Both N-terminal pro-brain natriuretic peptide and homocysteine were significantly increased in the patient groups as compared to the control, and were significantly positively correlated with depression scores, but significantly negatively correlated with cognitive impairment. TT genotypes had an increased risk of developing depression and had significant higher plasma level of both N-terminal pro-brain natriuretic peptide and homocysteine than CT or CC patients .

Conclusion: The methylenetetrahydrofolate reductase gene may play a role in the modulation of mood but does not contribute to genetic susceptibility to cognitive performance in later life. It is also associated with N-terminal pro-brain natriuretic peptide and homocysteine levels, which may play a role in linking depression and mild cognitive impairment with increased cerebrovascular and/or cardio-vascular risk.

Key Words: N-terminal pro-brain natriuretic peptide, homocysteine, methylenetetrahydrofolate reductase, cognitive impairment, depression

ÖZET

Amaç: Hafif bilişsel bozukluk ya da depresyon görülen yaşlı hastalarda N-teminal probeyin natriüretik peptid, homosistein ve metilentetrahidrofolat redüktaz gen polimorfizmlerinin (TT, CT, ya da CC) rolünün değerlendirilmesi ve TT genotipi ile hem homosistein ve N-terminal pro-beyin natriüretik peptid hem de depresif ve bilişsel derece arasındaki ilişkinin araştırılmasıdır.

Gereç ve Yöntem: Çalışmaya 60 yaşlı hasta dahil edilmiş, depresyon ve hafif bilişsel bozukluğa sahip olmalarına göre iki gruba ayrılmıştır. N-terminal pro-beyin natriüretik peptid, homosistein, folat ve metilentetrahidrofolat redüktaz gen polimorfizmleri araştırılmıştır.

Bulgular: N-terminal pro-beyin natriüretik peptid ve homosistein düzeyleri hasta grubunda kontrol grubuna kıyasla daha yüksek bulunmuştur. Bu değerler depresyon derecesi ile doğru, bilişsel bozukluk ile ters orantılıdır. TT genotipi depresyonun ortaya çıkmasında riski arttırmaktadır ve bu genotipe sahip hastalarda N-terminal pro-beyin natriüretik peptid ve homosistein plazma düzeyleri CT ve CC genotipine sahip hastalardan daha yüksek olarak saptanmıştır.

Sonuç: Metilentetrahidrofolat redüktaz geni ruh halinin düzenlenmesinde rol oynamaktadır. Fakat yaşamın daha sonraki dönemlerinde bilişsel performansa genetik yatkınlıkta katkısı bulunmamaktadır. Aynı zamanda artmış serebrovasküler ve/veya kardiyovasküler risk ile birlikte depresyon ve hafif bilişsel bozuklukta görev alan N-terminal pro-beyin natriüretik peptid ve homosistein düzeyleri ile de ilişkilidir.

Anahtar Kelimeler: N-terminal pro-beyin natriüretik peptid, homosistein, metilentetrahidrofolat redüktaz, bilişsel bozukluk, depresyon

Introduction

The pathological mechanisms that lead to the expression of depression and dementia in later life remain largely unknown. Clarification of the role of vascular risk factors in dementia is important because most are modifiable, in contrast to other risk factors such as age and genetics. Thus vascular risk factors may serve as targets for strategies of prevention (1).

Secretion of N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) increases in several cardiac illnesses, making this neurohormone a reliable diagnostic and prognostic biomarker of cardiovascular risk (2). Brain natriuretic peptide (BNP) is produced as a prohormone (pro-BNP) comprising of 108 amino acids and is enzymatically cleaved into physiologically active BNP (77–108) and the amino-terminal portion of the prohormone (1–76) (N terminal (NT)-proBNP) (3). By means of its natriuretic and diuretic properties, as well as its acting as antagonist of renin-angiotensin-aldosterone system, this neurohormone produces a myriad of biological effects, such as vasodilatation and inhibition of the sympathetic nervous system (4).

Homocysteine (Hcy) is a thiol-containing amino acid that is generated during 1-carbon metabolism. Hcy is produced during the metabolism of amino-acid methionine. By receiving a methyl group from 5-methyltetrahydrofolate, Hcy can be remethylated to methionine, which is also the immediate precursor of S-adenosylmethionine (SAM). In the brain, SAM is directly involved in the synthesis and metabolism of dopamine, norepinephrine and serotonin, which are neurotransmitters postulated to play an important role in the pathogenesis of depression and anxiety (5). The plasma level of Hcy can be influenced by factors such as vitamin deficiency, and a common mutation in the methylenetetrahydrofolate reductase (MTHFR) gene (6).

MTHFR is the crucial enzyme in folate-mediated one-carbon transfer reactions. MTHFR gene is localized in the short arm of chromosome 1 (1p36.3). MTHFR catalyses the NADPH-dependent reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This molecule functions as a cofactor for methylation of Hcy to methionine (7). Frosst et al found a MTHFR gene polymorphism 677C→T (a cytosine to thymine substitution at nucleotide 677), which substituted alanine with valine (A222V). This polymorphism may be associated with decreased in MTHFR activity, mild-to-moderate hyperhomocysteinemia, premature cardiovascular disease and neural tube defects (8). Severe deficiency of MTHFR leads to mental and vascular disorders (9). It is conceivable, however that the MTHFR genotype may play an important role in the modulation of mood and cognitive function in humans. Therefore the aim of this study was to evaluate the changes of NT-proBNP, Hcy, folate, and MTHFR C677T gene polymorphism in late life mild cognitive impairment (MCI) and depression and to determine the association between the MTHFR C677T gene polymorphism and plasma concentration of both Hcy and NT-proBNP on one-hand and with depression and cognitive impairment scores on other-hand.

Materials and Methods

Patients

This study was conducted in the neuropsychiatry department, Tanta University Hospital in the period from the 1st of January 2007 to the 31st of December 2007. It included 60 elderly patients with a mean age of 62.25 ± 6.28; 33 of them were females and 27 of them were males. They attended the outpatient clinic for treatment of depression (group I, n=32) or MCI (group II, n=28). In addition, a control group (group III) consisted of 20 healthy volunteers of 15 females and 5 males, with a mean age of 60.25 ± 4.98 years, matched to the patients with respect to age and gender with no previous history of psychiatric diseases were included. All controls were free of chronic and acute physical illness. Exclusion criteria for all patients included: severe cognitive impairment, dementia, severe sensory impairment, history of strokes, history of current or previous hazardous drinking, used hormone replacement therapy during the six months prior to assessment, patients with drug abuse or past history of drug abuse, smoking, renal insufficiency, cardiovascular, liver diseases and other psychiatric disorders were excluded from the present study. Also, patients on any kinds of vitamin substitution were excluded from the study (10). Written informed consents of all the patients who participated were obtained.

All patients were subjected to the following:

1- Diagnosis of major depressive disorder using semi-structured clinical interview of DSM-IV-TR (11).

2- Assessment of severity of depression using Hamilton Rating Scale of Depression (HRSD) (12).

3- Assessment of the cognitive function using the following measures (13):

   a- Mini-mental state examination (MMSE): It is a screening test that can be used to track the changes in the patient's cognitive state (14).

   b- Faces memory (FM): immediate and delayed memory for faces.

   c- Word lists (WL): measures immediate and delayed memory for verbal material.

   d- Verbal Paired Associates (VPA): uses the same test procedures described for WL and produces measures of immediate and delayed recall for semantically unrelated pairs of words.

   e- Block design (BD): is a constructional test in which the subject is presented with four or nine colored blocks. This is a sensitive test of visuospatial organization.

   f- Verbal fluency (VF):

Part 1: was investigated by asking subjects to name as many words as possible rhyming with the word (dog) within 3 minutes and many words as possible rhyming with the word (key) within 3 minutes. Part 2: was investigated by asking subjects to name as many words as possible derived from the word (cupboard) and to name as many words as possible derived from the word (balcony) within 3 minutes. The VF total score represents the sum of the number of words produced for each one of the two parts.

Analytical procedures

Blood samples were collected in vacuum tubes containing EDTA in the morning after an overnight fasting and was centrifuged within one hour of collection at 1500 × g for 20 min at room temperature. Plasma was separated, stored in aliquots, and kept frozen at –70 °C until analysis for determination of the parameters listed below.

A. ***NT-proBNP*** was measured using ELISA kit (Biomedica Laboratories, Vienna, Austria) according to the manufacturer's protocol (15).

B. ***Total plasma Hcy*** was measured using ELISA kit supplied by IBL-Hamburg, Germany (16).

C. ***Plasma folate*** was measured by using the electrochemiluminescene immunoassay (17) .

D. ***Genotype analysis for MTHFR C677T polymorphism:*** DNA was extracted from buffy coat layer of blood cells by using Qiagen Kits according to the manufacturer's recommendations (Qiagen-France, Courtaboeuf, France) and the 677 C→T mutation was determined by use of the polymerase chain reaction (PCR) and *HinfI* restriction enzyme digestion as described by Frosst et al (8). Briefly, PCR amplification of a 198-bp segment containing nucleotide 677 was done using two specific primers, the sense primer, 5`-TGAAGGAGAAGGTGTCTGCGGGA-3`(exonic) and antisense primer 5`- AGGACGGTGCG GTGAGAGTG-3` (intronic) were used. DNA was amplified by using a PCR thermal cycler (Perkin-Elmer, Cetus, Norwalk, CT). PCR reaction was carried out in a total volume of 50 µL contained about 200 ng DNA template, 0.5 µM of each primer, 200 µM each dNTP, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl and 1.25 U of Taq DNA polymerase (Amersham, Bioscience). The reaction conditions were as follows: initial heat activation at 95°C for 4 min and 35 subsequent cycles of denaturation at 94°C for 60 s, annealing at 61°C for 60 s, and extension at 72°C for 2 min. PCR product (198 bp fragment) was digested with *HinfI* restriction endonuclease (MBI Fermentas) for 12 h at 37°C. When a C to T substitution is present, HinfI restriction site is created. The restriction enzyme digests the 198-bp fragment into a 175-bp and a 23-bp fragment and the amplified product derived from the wild-type allele was not affected. These fragments were separated by electrophoresis on a 2% agarose gel and visualized with ethidium bromide.

Statistical analysis

The raw data were fed to the computer program InStat Guide. Descriptive statistics were used to determine frequencies, means and 95% confidence intervals of the mean (CI). Data was presented as range, mean±SD and median. Qualitative data was presented as number and percentage. Fisher's Exact Test and chi-square with two-sided P value was used for comparison between two groups as regards qualitative data and the odds ratio (OR) estimated for 2 ×2 tables. Non-parametric ANOVA is used for comparison between more than two groups. Spearman non-parametric correlation coefficient was used to test correlation between different variables. Results were considered significant at $p \leq 0.05$.

Results

Comparison between the studied groups as regards age and different biochemical parameters

Table 1 shows comparison between the studied groups as regards age and different biochemical parameters in which there was a significant increase in both NT-proBNP and Hcy but significant decrease in folate in the patient groups as compared to the control with no significant difference between both patients groups and no significant differences as regards age in all the studied groups.

Correlation between Hcy, NT-proBNP and both depression and cognitive scores in the patients groups

Both Hcy and NT-proBNP were significantly positively correlated with each other ($r=0.46$ $p<0.001$) and with depression scores assessed by HRSD ($r=0.49$, $r=0.40$, $p=0.001$, respectively), but significantly negatively correlated with cognitive impairment assessed by MMSE ($r=-0.52$, $r=-0.39$, $p<0.001$, respectively), face memory ($r=-0.50$, -0.42, $p<0.001$, respectively), verbal fluency part 1 ($r=-0.45$, -0.32, $p<0.001$), and verbal fluency part 2 ($r=-0.39$, -0.35, $p< 0.001$, respectively) so that the higher the plasma Hcy, the NT-proBNP, the more the scores of HRSD and the lower the scores of cognition assessed by MMSE, FM test and VF-parts 1 and 2, so the more the cognitive impairment. No significant correlation was detected between both plasma Hcy, NT-proBNP and scores of WL, VPA, and BD (Table 2).

Comparison between the studied groups as regards gender, family history and MTHFR genotype and alleles

By using chi-square Test, Tables 3 and 4 show no significant differences between the diseased groups and cont-

Table 1. Age and laboratory parameters in the studied groups

	Depression (group I) (n=32)	MCI (group II) (n=28)	Control (group III) (n=20)	p
	Range Mean±SD median	Range Mean±SD median	Range Mean±SD median	
Age (years)	55.5-70 61.72±5.65 64.5	55.2-70 62.86±6.97 65.4	53.5-67 60.25±4.98 63.8	0.34
NT-proBNP (pmol/l)	60-210 135±76[a] 125	65-190 120±55[a] 110	29.4-45 36.5±6.7[b] 39.4	<0.0001*
Hcy(µmol/l)	8.3-19.2 13.06±2.95[a] 15.8	7.8-20.1 12.43±3.52[a] 16.1	6.3-15.1 10.30±1.72[b] 12.6	<0.005 *
Folate (ng/ml)	1.5-8.9 4.5±3.01[a] 6.2	1.7-8.3 4.7±2.25[a] 5.9	2.7-10.2 6.6±2.56[b] 4.7	<0.02*

Mild cognitive impairment (MCI) N-terminal pro-B-type natriuretic peptide (NT. ProBNP), Homocysteine (Hcy)

Groups with different letters are with statistical significant differences

Groups with same letters are not significantly differen**t,** * means significant differences

Table 2. Spearman correlation between Hcy, NT-proBNP and both depression and cognitive scores in the patients groups (N=60).

	Hcy (µmol/l)		NT-proBNP (pmol/l)	
	r	P	r	p
HRSD	0.49	<0.001*	0.40	<0.001*
MMSE	-0.52	<0.001*	-0.39	<0.001*
FM	-0.50	<0.001*	-0.42	<0.001*
WL	-0.23	>0.05	-0.18	>0.05
VPA	0.19	>0.05	0.13	>0.05
B D	0.17	>0.05	0.15	>0.05
VF-1	-0.45	<0.001*	-0.32	<0.001*
VF-2	-0.39	<0.001*	-0.35	<0.001*
NT. Pro. BNP (pmol/l)	0.46	<0.001*	------	-------

Hamilton Rating Scale of Depression (HRSD), Mini Mental State Examination (MMSE), Faces memory (FM), Word lists (WL),verbal paired associates (VPA), Block design (BD), verbal fluency part 1 (VF-1), verbal fluency part 2 (VF-2). *Significant at $p \leq 0.05$

Table 3. Comparison as regards gender, family history, MTHFR genotypes and alleles between depression and control groups.

Variables	Depression (group I) (n=32)		Control (group III) (n=20)		Odds ratio (with 95% CI)	P
Sex	No	%	No	%		
Females	20	62.50	15	75	(χ^2=0.4)	
Males	12	37.50	5	25	0.56 (0.16-1.9)	0.53
Family history:						
+ve	9	28.13	0	0	16.6 (0.9-302.9)	0.008*
-ve	23	71.87	20	100		
MTHFR genotypes						
CC	8	25.00	14	70	0.14 (0.04-0.5) (CC vs. other genotypes)	0.003*
CT	14	43.75	5	25	2.33 (0.68-7.98) (CT vs. other genotypes)	0.24
TT	10	31.25	1	5	8.64 (1.01-73.84) (TT vs. other genotypes)	0.03*
MTHFR alleles						
T allele	34	53.13	7	17.5	5.34 (2.06-13.85) (T vs. C)	0.0004*
C allele	30	46.87	33	82.5		

methylenetetrahydrofolate reductase (MTHFR), OR (Odds ratio), confidence interval (CI),*Significant at $p \leq 0.05$

rol as regards gender, but by using Fisher's Exact Test there was a significant difference as regards family history of psychiatric disorders (p=0.008 and 0.0005, respectively). As regards MTHFR genotypes Table 3 shows significant increase in percentages of MTHFR genotypes TT and T allele among depression group as compared to control group (p=0.03,0.0004, respectively). The carriers of MTHFR, TT, genotype and T allele had an increased risk of developing depression (OR = 8.64, 95% CI: (1.01-73.84), OR = 5.34, 95% CI: (2.06-13.85), respectively) on comparing depression with controls. There were no statistically significant differences in the MTHFR genotype and allele distributions in MCI patients compared with controls except for CC genotype vs. other genotypes (Table 4).

Comparison between the different MTHFR genotypes among the studied groups

Upon classification of the patients groups according to MTHFR genotypes no significant difference between the 3 genotypes regarding age but plasma levels of both and NT-ProBNP and Hcy were found to be significantly higher (p<0.001) and plasma folate was non-significantly lower in TT patients than CT or CC patients (Table 5). TT patients have significantly higher scores of depression assessed by HRSD (p=0.008), were more to suffer from significant cognitive impairment as assessed by MMSE (p=0.001), FM (p=0.002), WL (p=0.008) and VF-2 (p=0.001) than CT and CC patients. However, no significant difference was detected between TT patients and the other 2 genotypes regarding VPA, BD, VF-1 (Table 5).

Table 4. Comparison as regards gender, family history, MTHFR genotypes and alleles between MCI and control groups

Variables	MCI(group II) (n=28)		Control (group III) (n=20)		Odds ratio (with 95% CI)	P
Sex	No	%	No	%		
Females	13	46.43	15	75	(χ^2=2.8)	
Males	15	53.57	5	25	0.29 (0.08-1.01)	0.09
Family history:						
+ve	12	42.86	0	0	31.06 (1.7-564.95)	0.0005*
-ve	16	57.14	20	100		
MTHFR genotypes						
CC	11	39.29	14	70	0.28 (0.08-0.94) (CC vs. other genotypes)	0.045*
CT	14	50	5	25	3.00 (0.86-10.52) (CT vs. other genotypes)	0.13
TT	3	10.71	1	5	2.28 (0.22-23.69) (TT vs. other genotypes)	0.63
MTHFR alleles						
T allele	20	35.71	7	17.5	2.62 (0.98-6.99) (T vs. C)	0.06
C allele	36	64.29	33	82.5		

Table 5. Age, laboratory parameters, HRSD, MMSE, FM, WL, VPA, BD, VF-1, and VF-2 in studied patients according to MTHFR genotype

	TT (N=15)		CT (N=27)		CC (N=18)		P	Inter-groups differences
	Mean	±SD	Mean	±SD	Mean	±SD		
Age(years)	62.27	8.01	62.81	5.78	62.85	6.79	0.496	-
NT-proBNP (pmol/l)	170	40	110	30	80	20	0.001*	TT>CT>CC
Hcy(µmol/l)	14.31	1.79	12.15	2.28	11.1	1.01	0.001*	TT>CT=CC
Folate(ng/ml)	5.1	2.4	4.5	1.05	4.4	3.1	0.6	-
HRSD	22.13	3.23	17.74	5.84	17.28	3.89	0.008*	TT>CT=CC
MMSE	17.00	3.57	21.07	2.88	19.89	3.50	0.001*	TT<CT=CC
FM	22.67	4.79	27.85	8.97	32.72	7.33	0.002*	TT<CT=CC
WL	23.93	6.08	25.33	6.26	30.78	7.62	0.008*	TT=CT<CC
VPA	8.27	3.33	10.00	2.48	10.00	2.72	0.122	-
BD	16.13	3.40	14.56	5.29	14.17	4.99	0.469	-
VF-1	16.47	3.52	17.26	4.20	16.11	3.95	0.614	-
VF-2	15.40	2.99	19.15	2.81	19.56	2.90	0.001*	TT<CT=CC

N-terminal pro-B-type natriuretic peptide (NT. ProBNP), Homocysteine (Hcy)Hamilton Rating Scale of Depression (HRSD), Mini Mental State Examination (MMSE), Faces memory (FM), Word lists (WL),verbal paired associates (VPA), Block design (B

MTHFR gene polymorphisms (TT), (TC) and (CC) were represented in (Figure 1).

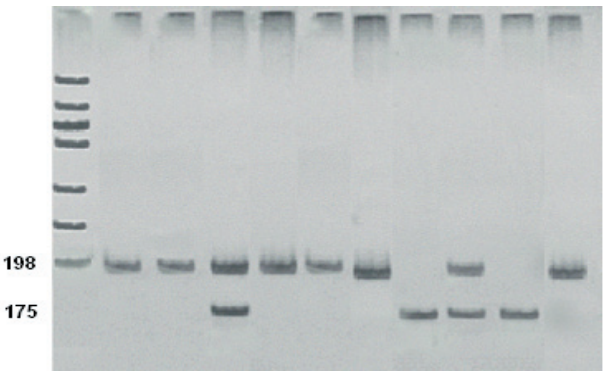

Figure 1. Agarose electrophoresis of the PCR products after cutting with *Hinf1* restriction endonuclease. The bands were visualized using ethidium bromide and 2 % agarose (lanes 1,2,4,5,6 and 10 represent normal homozygote (677CC), lane 7 and 9 represents homozygote (677TT) and lane 3 and 8 represents the heterozygote (C677T).

Discussion

There is increasing evidence that vascular disease contributes to cognitive impairment and depression (1). Although natriuretic peptides have been suggested to exert significant behavioral effects so far few data are available on their circulating levels in relation to negative mood states. The role of B-type natriuretic peptide (BNP) and NT-proBNP in psychiatric conditions remains largely unexplored (10). In the present study higher plasma level of NT-proBNP was detected in patients with depression or MCI as compared with the control group and it was significantly positively correlated with the severity of depressive symptoms and cognitive impairment. The mechanisms underlying the NT-proBNP elevation in depressed patients are not clear. It is possible; however, that endothelium dysfunction, which has been reported in patients with major depressive disorder (MDD), could also be involved in the elevation of NT-proBNP levels. It was also, suggested that increased plasma NT-proBNP may be one of the links between MDD and the increased risk for adverse cardiac events (15). An alternative pathway whereby NT-proBNP values may be altered in depression could be mediated by sex steroid hormones. It is intriguing that patients with depression may show patterns of androgen deficiency, and that androgens can suppress natriuretic peptide release (18). Also, endothelial abnormalities have recently been linked to cerebrovascular disease and reduced cognitive function (19). Nilsson et al found that NT-proBNP was associated with the presence of vascular disease, pathological computer tomography scan (CT) findings and age and they concluded that the control of conventional vascular risk factors and therapy could be guided by the level of plasma Hcy and serum NT-proBNP (10). Also, Nilsson et al observed elevated serum concentrations of NT-proBNP in patients with dementia or vascular disease as a sign of poorer cardiovascular status, and concluded that routine determination of NT-proBNP is valuable for obtaining information about cardiovascular status (20). Furthermore, both Yip et al (4) and Sander et al (21) found higher level of NT-proBNP in stroke patients which can be explained by an increased sensitivity of NT-proBNP secretion in response to enhanced sympathetic activity after stroke. So in their study, they encourage the use of this peptide as a novel biochemical marker for risk stratification in patients after ischemic stroke.

The link between elevated plasma Hcy and vascular disease is well established with numerous studies confirming that hyperhomocysteinemia is a risk factor for atherosclerosis. In the present study higher plasma Hcy and lower folate were detected in patients with depression or MCI as compared with the control group. These results came in accordance with several studies which revealed that late life depression is associated with high plasma Hcy (10,22,23). The mechanisms that underlie the association between plasma Hcy and depression remain largely unknown, but it is possible that Hcy is harmful to neurons and blood vessels, including the cerebral microvasculature so that such effects may contribute to the cascade of events that leads to cognitive decline, dementia, and depression in later life (10). Furthermore, it has been suggested that homocysteic acid and cysteine sulfinic acid, as metabolites of Hcy, may inhibit the *S*-adenosylmethionine–dependent methylation of biogenic amines and phospholipids, thus, an elevated plasma Hcy concentration may merely be a marker of impaired monoamine metabolism, which causes depression through reduced CNS methylation (22, 23).

The atherosclerotic and thrombogenic promoting effect of Hcy may also increase the risk for stroke and cerebrovascular disease, which in turn are related to cognitive impairment and dementia (24). The results of the present study came in accordance with Wehr et al (25), Sala et al (26), Russo et al (27), Vidal et al (28) and Kim et al (29) who stated that hyperhomocysteinemia has been associated with cognitive impairment in various neurological diseases. Hcy might influence cognition through a direct toxicity on glutamate neurotransmission and cerebrovascular endothelium, an indirect inhibition of transmethylation reactions in brain, potentiation of amyloid neurotoxicity and promotion of tau phosphorylation (30). It has also been hypothesized that inadequate B vitamin status and high Hcy concentrations may contribute to cognitive decline through silent brain infarction (31). Haan et al (32), Troen and Rosenberg (24) added that if elevated Hcy promotes cognitive dysfunction, then lowering Hcy by means of B-vitamin supplementation may protect cognitive function by arresting or slowing the disease process. In contrast, Reitz et al (33) and Kalmijn et al (34) failed to confirm an association between plasma Hcy and cognitive decline. Various possible explanations for the lack of an association have been offered based largely on methodological differen-

ces. In the present study it was observed that the severity of cognitive decline assessed by the MMSE was significantly negatively correlated with hyperhomocysteinemia. Similar associations between plasma Hcy and the MMSE have been reported by other investigators studying patients with dementia (35).

Both MDD and MCI are complex disorders that thought to result from multiple genes in combination with environmental and developmental components (36). The MTHFR C677T gene polymorphism has been shown to be a risk factor for premature cardiovascular disease and neural tube defect. Deficient activity of MTHFR has also been implicated in the pathogenesis of psychiatric conditions such as schizophrenia and affective disorders (37). In the present study, MTHFR TT homozygous was significantly more common among depression patients group as compared with control. Elderly people carrying TT genotype of MTHFR gene had higher serum level of Hcy and NT-proBNP as compared with CT and CC genotypes. Moreover, TT genotype subjects are more depressed and had higher scores on HRSD than those carrying CT or CC genotypes. The results of the present study came in accordance with Słopien et al who found a significant contribution of the MTHFR C677T polymorphic variants to depression in postmenopausal women (38). Also, with Gilbody et al (36) and Kempisty et al (37) who demonstrated an association between the MTHFR C677T variant and depression, schizophrenia, and bipolar disorder (BD). The association of 677TT genotypes with BD and schizophrenia may be linked to the excitatory amino-acids hypothesis and/or decreased SAM concentration of blood plasma in neuropsychiatric disorders. Such association may suggest the shared genetic defects in these disorders, but Gaysina et al found no significant differences in genotype or allele frequencies between depressive patients and controls and stated that homozygosity for the T677 allele of the MTHFR gene is unlikely to play a major role in the pathogenesis of schizophrenia or affective disorders in their samples (39). Such discrepancies between different reports may be partially due to socio-economic status. Also, it could be explained by low statistical power due to the limited number of cases, combined with the low frequency of MTHFR T/T homozygosity. So that a further exploration of the involvement of the MTHFR gene in the susceptibility to affective disorders, with larger sample sizes, are needed to fully establish the role of the MTHFR gene. The present data do not provide evidence for an association between the MTHFR C677T gene polymorphism and MCI which did not coincide with McIlroy et al who stated in their study that possession of the T allele of the MTHFR C677T polymorphism significantly increases risk for vascular dementia, leading to the possibility that this allele confers increased risk for dementia after stroke (40). It is feasible that this increased risk could be mediated by the effect of the reduction in activity of the enzyme associated with the substitution of valine for an alanine residue, leading to an increase in Hcy levels. But the present study came in accordance with the results of Almeida et al (6), Religa et al (41), Brunelli et al (9), who found no relation between the MTHFR gene polymorphism and cognitive impairment in older persons. Therefore, their data showed that homozygosity for the MTHFR C677T gene polymorphism is not a genetic risk factor for cognitive impairment in the oldest old. A possible explanation of increased plasma concentrations of Hcy are associated with cognitive impairment in older persons, whereas there is no association with the MTHFR C677T gene polymorphism, is that the increased plasma concentration of Hcy is a phenomenon associated with cognitive impairment or its treatment, instead of being part of the causal mechanism. Another possibility could be that the number of persons was too small to find significant differences between the MTHFR genotypes and the cognitive measurements (41). In the present study an association between the MTHFR C677T gene polymorphism and plasma Hcy levels, regardless of folate status, was shown. This result suggested that the MTHFR C677T gene polymorphism and Hcy concentrations are so closely associated that folate levels cannot compensate for the reduced activity of the MTHFR enzyme and hyperhomocysteinemia. These data are supported by the study of Husemoen et al in which plasma Hcy levels were significantly higher in TT individuals compared to CC and CT individuals with normal folate status (42). Almeida et al added that the MTHFR 677TT genotype, is associated with a significant elevation in the circulating concentrations of Hcy and a decrease in serum folate concentrations (6). This may parallel a similar reduction in 5-methyltetrahydrofolate in the CNS, leading to a potential reduction in monoamine neurotransmitter function and an elevated risk of depressive disorder. Religa et al stated that moderate homocysteinaemia was found in subjects with the TT genotype when the level of folic acid was low, suggesting that individuals having the TT genotype should obtain higher folate intake to minimize the risk of developing dementia (41). Gorgone et al suggested that TT677 MTHFR genotype promotes plasma Hcy increase which in turn may favors intima-media thickening in patients with cognitive impairment thus may promote neuronal damage (43). Also, Khandanpour et al (44) and Trabetti (45) have found a strong association between raised Hcy, the TT genotype, and peripheral arterial disease. In contrast Kim et al found no association between MTHFR C677T gene polymorphism and Hcy levels (46).

Finally in the present study, NT-proBNP concentrations were significantly higher in patients with the MTHFR C677T gene polymorphism compared to patients without such polymorphism. This is supported by Cho et al who found an association between the CT genotype and vascular disease in mild hyperhomocysteinemia(7). There is insufficient data about this point so that it needs further investigations.

Conclusion

It has been shown that increased Hcy and NT-proBNP are frequently present in elderly patients with depression or MCI. Fully elucidating the link between depression or MCI and elevated levels of both Hcy and NT-proBNP concentrations may prove an important step toward understanding the association between major depression and/or MCI with either cerebrovascular or cardiovascular upsets. The MTHFR C677T gene polymorphism may play an important role in the modulation of mood but does not contribute to genetic susceptibility to cognitive performance in later life. The MTHFR C677T gene polymorphism is associated with both plasma Hcy and NT-proBNP levels. In depression or MCI patients with MTHFR C677T gene polymorphism, prospective observation of the development of cerebrovascular or cardiovascular upsets, involving periodic repeated measurement of Hcy and NT-proBNP concentrations may be needed.

Recommendations : Whether the elevated levels in Hcy and NT-proBNP in both depression and MCI may be due to vascular pathogenesis of both disease entities or it may be due to accompanying silent cardiovascular disease, needs further investigations with further performing a correlation between both Hcy and NT-proBNP with routine markers for cardiac injury. Also, as a continuation of this study it remains to be shown if supplementation with B vitamins and/or homocysteine lowering therapy, can influence the rate of cognitive decline and/or depression.

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