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The expression features of serum Cystatin C and homocysteine of Parkinson's disease with mild cognitive dysfunction

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Abstract. – OBJECTIVE: To discuss the expression features of serum cystatin C, and homocysteine in patients of Parkinson's disease (PD) with mild cognitive impairment.

PATIENTS AND METHODS: Patients with PD from the neurology department of the Central Hospital of Xuzhou from august 2012 to august 2014 were enrolled in this study. The Hoehn-Yahr (H&Y) grading rating scale was used to rate the degree of severity of PD, and the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) rating scale was used to rate and group their cognitive impairment, test the level of the serum cystatin C (CysC) and homocysteine (HCY) and their variations after treatment with vitamin B and folic acid.

RESULTS: (1) The CysC and HCY in the PD group was higher than that in the normal control ($p<0.05$); (2) As the H&Y rating upgraded, the CysC and HCY in the PD group gradually increased with the progression of the disease ($p<0.05$); (3) The level of CysC and HCY in the PD-mild cognitive impairment (MCI) group increased significantly compared with those in the PD group ($p<0.05$) The correlation analysis showed that there was significant positive correlation between CysC and HCY. (4) After treatment with vitamin B and folic acid, the CysC, and HCY levels were lower than before, but the grades of the repetition measurement scale, MMSE and MoCA had no significant improvement ($p>0.05$).

CONCLUSIONS: A high level of CysC and HCY might be involved in the development of Parkinson disease. Their expression levels were higher in the PD patients with mild cognitive dysfunction and both showed a remarkably positive correlation.

Key words:

Serum cystatin C, Homocysteine, Parkinson's disease, Parkinson's mild cognitive impairment.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by static tremor, rigid muscles, bradykinesia and abnormal posture and pace. Since the pathogenesis of PD is usually concealed, and the disease develops progressively. Once the clinical symptoms appear, it indicates that PD has come into the medium-latter stage[1]. In recent years, the non-motor symptoms of the PD patients have attracted more attention, and about 40% of the PD patients have cognitive impairment of varying degrees, seriously affecting their quality of life and bring a heavy burden to their families and society[2]. Therefore, to discover the cognitive impairments of the PD, finding out the possible factors that resulted in such impairments and made corresponding treatments are of great significance. The pathogenesis of the PD is not very clear. It was thought to be related to certain mechanisms, such as inflammation, oxidative stress, mitochondrial dysfunction, abnormal protein aggregation, and the excessive activation of NMDA receptors[3,4]. Recent studies on PD have been focused on estrogen, uric acid, nitric oxide, TNF-α and IL-1β, etc. Both serum cystatin C (CysC) and homocysteine (HCY) had been involved in the inflammatory reaction[5]. Based on this, our research focused on studying the relationship between CysC level, HCY level, and PD, and further discusses whether there are any correlations between the level of CysC, HCY, and PD MCI (mild cognitive impairment).

Patients and Methods

Grouping criteria: 69 patients confirmed with PD from the neurology department of the Central Hospital of Xuzhou from august 2012 to august

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2014 were enrolled in this study. Amongst the group, there were 37 male and 32 female. The materials were collected strictly in compliance with the clinical diagnostic criteria issued by the British Association of Parkinson's Disease. The age of the 69 patients was between 48-78 years of age, with an average age of (61.4±5.1) years old. Meanwhile, a control group of 74 healthy patients were selected, composed of 38 male and 36 female between 46-72 years old, on average (62.3±6.5) years old. The control group matched with the patient group in age, gender, and education level and the patients of the same disease were excluded. All of the selected cases volunteered to participate in the study.

Patients with PD excluded from this study had the following characteristics: familial PD, merge serious brain organic diseases, other severe body diseases (such as severe hepatic and renal dysfunction, acute coronary syndrome), autoimmune disease, tumors, and acute or chronic infection. The patients, who had been treated with the medicine that would affect the level of HCY, such as vitamin B6, vitamin B12, folic acid, melbine, and so on, in the past three months were also excluded.

Methods

The basic information of the patients was recorded, and the severity of their illness was evaluated. The Hoehn-Yahr (H&Y) rating scale was used to grade the degree of their illness severity, and the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) rating scale was used to grade and group their cognitive impairment. A quantity of 5 ml of fasting venous blood was drawn from the patients the next morning and sent to the clinical laboratory. The Siemens BNII automatic analyzer was, then, used to detect the level of C-reactive protein. The Abbott AXSYM automatic immunity analyzer was used to detect the level of homocysteine. The Hitachi 7600 biochemical analyzer was used to detect the level of serum cystatin C, creatinine, cholesterol, triglyceride, uric acid, high-density lipoprotein and low-density lipoprotein. Patients in PD-MCI group and PD treatment group were administered with folic acid and vitamin B (folic acid 5mg, three times a day; mecobalamin 500 μg, three times a day; vitamin B6 10mg, three times a day). Three months later, the patients were reexamined for their level of serum cystatin C and homocysteine, and retested for the grading of MMSE and MoCA and compared to the PD-MCI placebo group.

The Parkinson patients were divided into three stages according to their disease development by means of the H&Y rating scale: early stage (H&Y<2); middle stage (2≤H&Y<3); late stage (H&Y≥3). They were then divided based on the generalized MCI diagnosis standards in the *Diagnostic and Statistical Manual-IV of Mental Disorder (DSM-IV)* issued by American Psychiatric Association's and that issued by the 2004 MCI international working group: (1) with cognitive impairment, but not up to the diagnostic standard of dementia; (2) with cognitive decline, the patients and/or insiders confirmed and the object test also confirmed that cognitive impairment existed; (3) with ability to maintain normal daily life, and with normal or slight impaired ability to maintain complex instrumental daily life. The 69 PD patients were graded by means of MMSE and MoCA rating scale and screened again, then divided into PD-MCI and Parkinson's disease group of normal cognitive ability. PD-MCI group (36 patients): with clinical evidence of declined cognitive ability and symptom lasted for over six months; the overall cognitive ability was normal, the total grades of MMSE were within the normal range; the grades of MoCA were between 19-25 points.

Statistical Analysis

The SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the data and the mean ± standard deviation (± s) to present the data. The *t*-test was used to compare the sample mean between the two groups, and the one-way variance analysis (one-way ANOVA) was used to compare the sample mean amongst the multiple groups. The Spearman correlation analysis was used to analyze the correlations. A $p<0.05$ represented significant differences.

Results

Comparisons of the clinical information between the patient group and the control group

There was no significant differences between the patient and the control groups ($p>0.05$) on the general information and biochemical index, including age, gender, smoking history, hypertension, diabetes mellitus, creatinine, urea nitrogen, triglyceride, cholesterol, high-density lipoprotein, and low-density lipoprotein. But the differences of CysC and HCY between the two groups had statistical significance ($p<0.05$) (Table I).

Table I. Comparisons of clinical information and biochemical index of PD group and control group.

Project	Control group	PD group
Cases	74	69
Age	62.3±6.5	61.4±5.1
Gender (male/female)	38/36	37/32
Smoking	14 (18.9%)	12 (17.4%)
Hypertension	21 (28.4%)	16 (23.2%)
Diabetes	13 (17.6%)	15 (21.7%)
Creatinine (umol/L)	65.1±12.6	67.6±13.2
Urea nitrogen (mmol/L)	5.4±1.4	6.8±1.7
Triglyceride (mmol/L)	1.61±1.25	1.73±0.62
Cholesterol (mmol/L)	4.98±1.05	5.22±1.31
High density lipoprotein	1.59±0.23	1.51±0.49
Low density lipoprotein (mmol/L)	3.16±0.85	3.40±0.67
Serum cystatin C (mg/L)	0.66±0.17	1.82±0.22*
Homocysteine (mmol/L)	9.32±2.13	17.07±3.10*

Remark: In comparison with the control group: *$p<0.05$

The relationship between serum cystatin C and PD rating

The PD patients were divided into three stages: early, middle and late stages, according to their disease development based on the H&Y rating scale. The uric acid, C-reactive protein, homocysteine and cystatin C had significant differences between the PD and the control group ($p<0.05$). As the H&Y grading upgraded, the uric acid detected that there were no significant differences amongst the PD groups ($p>0.05$). As the disease developed, the serum CRP, HCY, CysC increased gradually in the PD groups, and there were significant differences amongst the groups ($p<0.05$) (Table II).

The relationship between serum cystatin C and cognitive impairment

The 69 PD patients, amongst which were 36 PD-MCI patients, comprised of 20 male and 16 female, were strictly screened according to the diagnostic criteria. After the test, it was found that the level of CysC and HCY in both the PD group and PD-MCI group were higher than those in the control group, and their differences had statistical significance ($p<0.05$). The level of CysC and HCY in PD-MCI group was higher than that in the PD group ($p<0.05$). After a correlation analysis, it was found that the level of HCY and the level of CysC in the PD patients and PD-MCI patients were positively correlated with each other (PD group r=0.743, PD-MCI group r=0.715□$p<0.01$), which indicated that both might have synergistic effect (Table III).

The variations in the level of HCY and CysC in PD-MCI patients after treatment with folic acid and vitamin B, and their score changes in MMSE and MoCA rating scale

Vitamin B and folic acid could help lower the high level of homocysteine. The data had confirmed that HCY and CysC had a correlation. So could vitamin B and folic acid also lower the level of CysC and improve the patients' cognitive impairment? The PD-MCI group were divided

Table II. Comparisons of serum cystatin C, bilirubin, urin acid, C reactive protein in Parkinson patients of different stage.

Project	Control group	Hoehn-Yahr stage		
		Early stage	Middle stage	Late stage
Cases	30	12	27	30
Uric acid (μmol/L)	312.1±71.2	258.3±69.2	260.6±70.1	254.9±65.8
C reactive protein (mg/L)	2.12±0.21	5.14±1.22	10.02±1.80	14.91±3.16
Homocysteine (μmol/L)	7.94±2.21	10.21±3.02*	14.12±2.64*	19．52±3.79*
Serum cystatin C (mg/L)	0.50±0.19	0.87±0.16*	1.39±0.18*	1.91±0.26*

Remark: In comparison with the control group: *$p<0.05$

Table III. The expression of serum cystatin C and homocysteine in Parkinson patients with mild cognitive impairment.

Project	Control group	PD group	PD-MCI group
Cases	25	22	36
CysC (mg/L)	0.51±0.16	1.31±0.16*	1.83±0.19*#
HCY (μmol/L)	8.12±2.24	14.12±1.64*	18.28±2.76*#

Remark: In comparison with the control group: *p<0.05; in comparison with the PD group: #p<0.05

into a placebo and a treatment group. Then, they were given vitamin B6, vitamin B12, and folic acid for three months. After three months (1 case in placebo group was lost to follow-up), it was discovered that the level of CysC and HCY in all the groups after treatment was lower than before (p<0.05), but the repetition measurement scale and the patients' cognitive impairment had no significant improvement (p>0.05) (Table IV).

Discussion

Mild cognitive impairment, which was put forward by Petersen et al[6], referred to the clinical transition state, which was accompanied by disease characteristics in the early period, between normal aging state and Alzheimer or other dementia state. The PD-MCI referred to the transition state of the Parkinson's patients between the normal state and dementia state[7]. However, there were great differences of the time that they would develop into dementia amongst the PD patients. Dementia might appear in the early period of the disease. There were also patients keeping a perfect cognitive ability after the onset of PD. About 20% of the PD patients had PD-MCI at the time of diagnosis[7,8]. So, early diagnosis and timely treatment of MCI were extremely significant in improving the PD patients' quality of life.

The mechanism of PD cognitive impairment was not quite clear. It was thought from the recent neuropathological studies that it might be related to the cortico-subcortical dopamine circuits damages, which resulted from the prefrontal dopamine depletion resulted in the intrastriatal dopamine depletion[9,10]. Studies from the neurological biochemistry have also confirmed that the brain tissue dopaminergic lesions, the cholinergic lesions, and the norepinephrine neurons damages would lead to cognitive impairments[11]. In general, Parkinson disease was a kind of nigrostriatal dopaminergic neural pathway degeneration disease, which was related to an inflammatory reaction[12]. Our study was focused on two indexes connected with inflammation: serum cystatin C and homocysteine. Through detecting their levels and comparing their variations, we came to discover their correlation.

Serum cystatin C was a kind of low molecular weight cysteine protease inhibitors. It was expressed at a constant speed in all karyocytes, and kidney is the only organ that could clear up the CysC in the circulation, therefore, the CysC in plasma had a better stability, free from the influences of other factors, such as age, gender, diet, inflammation, and serum lipid level[13,14]. So, the previous studies had been mainly concentrated on the clear up of glomerulus. However, recent studies have found that CysC had also been involved

Table IV. Comparisons of the level of HCY, CysC, and the rating scores of MMSE, MoCA on PD and PD-MCI patients before and after treatment with Vitamin B, folic acid.

PProject	PD group		PD-MCI placebo group		PD-MCI treatment group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Cases	22	22	16	15	20	20
CysC (mg/L)	1.51±0.16	1.01±0.26*	1.86±0.18	1.87±0.21	1.88±0.21	1.05±0.26*
HCY (μmol/L)	14.12±1.64	9.26±1.29*	18.10±2.66	17.97±2.15	18.21±1.95	13.16±2.10*
MMSE	-	-	27.12±1.91	26.82±1.32	26.93±1.60	27.31±2.01
MoCA	-	-	21.03±1.07	22.19±2.06	22.80±1.97	21.95±2.40

Remark: Comparisons before and after treatment: *p<0.05

in many other physiological and pathological processes in the body, including the occurrence and development of inflammation, invasive growth and metastasis of tumor cells, and cardiovascular disease. Besides, it also has been related to various nervous system diseases[14], such as Alzheimer disease[15,16], Guillain-Barré syndrome[17], amyotrophic lateral sclerosis[18], and multiple sclerosis[19]. But there was still no clear evidence on its relationship with PD. So, in this study, we observed the relationship between serum cystatin C and PD. After comparisons of statistics, we have found that the level of CysC in PD patients was significantly higher than that in the control group.

CysC in the brain was compounded by choroid plexus soft meningeal cells, astroglia cell, neural progenitor cells and mature neurons. The high physiological concentrations of CysC in the cerebrospinal fluid and its effect in promoting the proliferation of neural stem cells fully showed that CysC had the effect of nourishing the brain cells[20]. Current studies have found that high level of CysC would participate in the production and degradation of the extracellular matrix through regulating the activities of cysteine protease, especially through the inhibition of cathepsin. It was related to extracellular matrix remodeling and would affect the phagocytic function and chemotactic function of neutrophils. It would also be involved in the inflammatory reaction and could induce nerve regeneration[5].

When the brain tissue was damaged, and the expression of protease in the brain tissue increased accordingly. Such damage could be attributed to being incurred by antagonism cathepsin. CysC was activated, and its expression increased, which produced a protection effect on the cells. Besides, studies have discovered that after a depletion of the CysC in the brain of the mice, the ability of the damaged dopaminergic neuron in activating the microglia cells decreased significantly, thus inhibiting inflammatory reactions and cells damages. So, CysC might play a certain role in the pathogenesis of PD[21].

In this study, the results showed that CysC level in PD patients was significantly higher than that in the control group. As PD developed, CysC level was on an upward trend according to the H&Y rating scale. CysC was in a positive correlation with the severity of PD and the illness duration. Therefore, a high level of CysC might be involved in the progress of the Parkinson's disease. But the number of patients in the study was relatively small, so their correlation still needs further study.

Homocysteine is a sulfur-containing the amino acid, which is generated by intracellular methionine demethylation. It is a type of multi-functional damage factor. A high level of HCY could result in cellular structure damages through a variety of ways by damaging the vascular endothelial cells, promoting the proliferation of vascular smooth muscle cells, and causing the reaction of macrophage and the fat accumulation in vascular wall, which leads to further damage to the blood vessels in the brain[22]. It could also result in the non-vasculogenic neurotoxicity through strengthening inflammatory reactions and oxidative stress, inducing neurofibrillary tangles, damaging neuronal DNA, incurring calcium overload in the cells and so on. Hyperhomocysteinemia had been confirmed to be related to a series of noninfectious chronic diseases, such as coronary heart disease, diabetes and cerebral infarction[23,24]. However, there was still a lack of evidence on its relationship with Parkinson's disease. In this study, we have found that the change of HCY level was in accordance with the change of CysC level. And HCY level in PD group was significantly higher than that in the control group and with the development of PD, HCY level was on a gradual upward trend.

Experimental studies have shown that the mice on a diet lacking of folic acid would be in a hyperhomocysteinemia state, be susceptible to neurotoxin MPTP, and appear PD like pathological and physiological changes and dyskinesia, which indicated that hyperhomocysteinemia would increase the susceptibility of dopaminergic neurons injuries, and promote the outbreak and development of PD. However, the conditions mentioned above could be greatly improved after a supplementation of folic acid and antioxidant[25,26]. Therefore, hyperhomocysteinemia would also increase the susceptibility of dopaminergic cells to oxidative stress. At present, vitamin B and folic acid have already been used in the clinical treatment of hyperhomocysteinemia[27]. In this study, the selected PD-MCI patients were treated with oral administration of folic acid and vitamin B for half a year, and then rated on the MoSA scale, and their level of HCY and CysC were reexamined. Results showed that the CysC and HCY levels in all groups after treatment were lower than before, but no significant improvement was found in the repetition measurement scale and the patients' cognitive abilities. The possible reason might be that this disease was in correlation with many other diseases, such as genetic factors and environmental factors. More-

over, the number of cases in the study was relatively small and the duration of illness was relatively short. So whether vitamin B and folic acid had any effect in improving the PD cognitive impairment or not still needs further researches.

CysC, a cysteine proteinase inhibitor, inhibits the activity of enzymes in the process of HCY decomposition and then cut off its degradation pathway, which finally resulted in the increase of serum HCY level. Moreover, the CysC could interact with HCY and cathepsin and, thus, caused damages in blood vessel[28,29]. In this study, we have made a correlation analysis of the PD patients' serum HCY level and CysC level, and the results show a positive correlation, which indicates that both might have a synergistic effect.

Conclusions

In recent years, studies on the non-motor symptoms of PD, especially on cognitive impairment, are forever increasing. It is of extreme importance to discover high sensitive predictors, screen out the PD-MCI in the early phases, and provide effective medical and behavioural-based interventions before dementia appears, so as to improve the patients' quality of life. Serum CysC has an important role in predicting the transition from MCI to dementia, which indicates that the level of CysC plays an important part in the prediction of the cognitive decline. But whether it could be a therapeutic target for the neurodegenerative diseases still needs further research. However, the joint detection of homocysteine and serum CysC is conductive to a more sensitive and accurate evaluation of the progress of PD.

Conflict of interest

The Authors declare that they have no conflict of interests.

References

1) Cesaro P, Defebvre L. Drug treatment of early-stage (de novo and "honeymoon") Parkinson disease. Rev Neurol 2014; 170: 237-246.
2) Kouti L, Noroozian M, Akhondzadeh S, Abdollahi M, Javadi MR, Faramarzi MA, Mousavi S, Ghaeli P. Nitric oxide and peroxynitrite serum levels in Parkinson's disease: correlation of oxidative stress and the severity of the disease. Eur Rev Med Pharmacol Sci 2013; 17: 964-970.
3) Mullin S, Schapira AH. Pathogenic mechanisms of neurodegeneration in parkinson disease. Neurol Clin 2015; 33: 1-17.
4) Itoh Y, Kawabata I, Akasaka K, Kino S. Fundamental and clinical aspects of cystatin C. Rinsho Byori 2012; 60: 769-778.
5) Eriksson P, Deguchi H, Samnegård A, Lundman P, Boquist S, Tornvall P, Ericsson CG, Bergstrand L, Hansson LO, Ye S, Hamsten A. Human evidence that the cystatin C gene is implicated in focal progression of coronary artery disease. Arterioscler Thromb Vasc Biol 2004; 24: 551-557.
6) Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999; 56: 303-308.
7) Noh SW, Han YH, Mun CW, Chung EJ, Kim EG, Ji KH, Seo JH, Kim SJ. Analysis among cognitive profiles and gray matter volume in newly diagnosed Parkinson's disease with mild cognitive impairment. J Neurol Sci 2014; 347: 210-213.
8) Petrelli A, Kaesberg S, Barbe MT, Timmermann L, Rosen JB, Fink GR, Kessler J, Kalbe E. Cognitive training in Parkinson's disease reduces cognitive decline in the long term. Eur J Neurol 2014; 22: 640-647.
9) Halliday GM, Leverenz JB, Schneider JS, Adler CH. The neurobiological basis of cognitive impairment in Parkinson's disease. Mov Disord 2014; 29: 634-650.
10) Yarnall AJ, Rochester L, Burn DJ. Mild cognitive impairment in Parkinson's disease. Age Ageing 2013; 42: 567-576.
11) Summerfield C, Gomez-Anson B, Tolosa E, Mercader JM, Marti MJ, Pastor P, Junque C. Dementia in Parkinson disease: a proton magnetic resonance spectroscopy study. Arch Neurol 2002; 59: 1415-1420.
12) Barker RA, Williams-Gray CH. Mild cognitive impairment and Parkinson's disease - something to remember. J Parkinsons Dis 2014; 4: 651-656.
13) Angelidis C, Deftereos S, Giannopoulos G, Anatoliotakis N, Bouras G, Hatzis G, Panagopoulou V, Pyrgakis V, Cleman MW. Cystatin C: an emerging biomarker in cardiovascular disease. Curr Top Med Chem: 2013; 13: 164-179.
14) Niemczyk S, Piotrowska M, Szamotulska K. Usefulness of creatinine and cystatin C in the assessment of renal function in chronic kidney disease and coexisting diseases. Pol Merkur Lekarski 2012; 32: 313-317.
15) Hua Y, Zhao H, Lu X, Kong Y, Jin H. Meta-analysis of the cystatin C(CST3) gene G73A polymorphism and susceptibility to Alzheimer's disease 2012; 122: 431-438.
16) Ghidoni R, Benussi L, Glionna M, Desenzani S, Albertini V, Levy E, Emanuele E, Binetti G.Plasma cystatin C and risk of developing Alzheimer's disease in subjects with mild cognitive impairment. J Alzheimers Dis 2010; 22: 985-991.
17) Yang Y, Liu S, Qin Z, Cui Y, Qin Y, Bai S. Alteration of cystatin C levels in cerebrospinal fluid of patients with Guillain-Barre Syndrome by a proteomical approach. Mol Biol Rep 2009; 36: 677-682.

18) Pasinetti GM, Ungar LH, Lange DJ, Yemul S, Deng H, Yuan X, Brown RH, Cudkowicz ME, Newhall K, Peskind E, Marcus S, Ho L. Dentification of potential CSF biomarkers in ALS. Neurology 2006; 66: 1218-1222.

19) Yamamoto-Watanabe Y, Watanabe M, Jackson M, Akimoto H, Sugimoto K, Yasujima M, Wakasaya Y, Matsubara E, Kawarabayashi T, Harigaya Y, Lyndon AR, Shoji M. Quantification of cystatin C in cerebrospinal fluid from various neurological disorders and correlation with G73A polymorphism in CST3. Brain Res 2010; 1361: 140-145.

20) Gauthier S, Kaur G, Mi W, Tizon B, Levy E. Protective mechanisms by cystatin C in neurodegenerative diseases. Front Biosci (Schol Ed) 2011; 3: 541-554.

21) Tizon B, Sahoo S, Yu H, Gauthier S, Kumar AR, Mohan P, Figliola M, Pawlik M, Grubb A, Uchiyama Y, Bandyopadhyay U, Cuervo AM, Nixon RA, Levy E. Induction of autophagy by cystatin C: a mechanism that protects murine primary cortical neurons and neuronal cell lines. PLoS One 2010; 5: 98-119.

22) Sun Y, Chien KL, Hsu HC, Su TC, Chen MF, Lee YT. Use of serum homocysteine to predict stroke, coronary heart disease and death in ethnic Chinese. 12-year prospective cohort study. Circ J 2009; 73: 1423-1430.

23) Kennedy DO, Haskell CF. Vitamins and cognition: what is the evidence. Drugs 2011; 71: 1957-1971.

24) Ansari R, Mahta A, Mallack E, Luo JJ. Hyperhomocysteinemia and neurologic disorders: a review. J Clin Neurol 2014; 10: 281-288.

25) Brosnan JT, Jacobs RL, Stead LM, Brosnan ME. Methylation demand: a key determinant of homocysteine metabolism. Acta Biochim Pol 2004; 51: 405-413.

26) Szadejko K, Szabat K, Ludwichowska A, Slawek J. Homocysteine and its role in pathogenesis of Parkinson's disease and other neurodegenerative disorders]. Przegl Lek 2013; 70: 443-447.

27) VITATOPS Trial Study Group. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. Lancet Neurol 2010; 9: 855-865.

28) Boysen G, Brander T, Christensen H, Gideon R, Truelsen T. Homocysteine and risk of recurrent stroke. Stroke 2003; 34: 1258-1261.

29) Dzielinska Z, Januszewicz A, Wiecek A, Prejbisz A, Zielinski T, Chudek J, Makowiecka-Ciesla M, Demkow M, Tyczynski P, Januszewicz M, Ruzyllo W, Naruszewicz M. Reduced kidney function estimated by cystatin C and clinical outcomes in hypertensive patients with coronary artery disease: association with homocysteine and other cardiovascular risk factors. Kidney Blood Press Res 2010; 33: 139-148.