Study on serum homocysteine level in Alzheimer's disease and its relationship with the stages of this disease

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ABSTRACT

الأهداف: فحص مستوى الهيموسيستين لمصل مرض الزهامير وعلاقته مع شدة المرض.

الطريقة: شملت هذه الدراسة 40 مريضاً يعانون من مرض الزهامير و40 شخصاً لا يعانون منه. أجريت هذه الدراسة بمدينة تابريز – تركيا، في الفترة ما بين مايو 2006م وحتى سبتمبر 2007م. تم اختيار مرضى الزهامير بناءً على تصنيف الاتحاد النفسي الأمريكي. كما تم تحديد شدة المرض بناءً على مقياس رايسبيرج. قيمت الحالة العقلية للمرضى بواسطة فحص الحالة العقلية الصغيرة (MMSE)، وقيست مستويات الهيموسيستين باستخدام طريقة (إليسا).

النتائج: بلغ متوسط مصل مستوى الهيموسيستين لدى مجموعة المرضى الأربعين 23.01±14.40mmol/L، وبلغ في مجموعة التحكم الأربعين 15.40±6.23 (p=0.003). كان معدل مستوى مصل الهيموسيستين في المجموعة الأولى 21.7±12.7mmol/L، وفي المجموعة الثانية 22.3±13.8، وفي المجموعة الثالثة 24.9±14.2. لم تكن العلاقة بين نقاط (MMSE) ومستوى مصل الهيموسيستين للمرضى ملحوظة (p=0.4).

خاتمة: كان مستوى مصل الهيموسيستين لدى المرضى المصابين بمرض الزهامير أعلى من مجموعة التحكم، ولكنه لم يظهر علاقة ملحوظة مع شدة المرض.

Objectives: To investigate homocysteine levels in Alzheimer's disease and its relationship with the severity of disease.

Methods: This investigation was performed as a case-control study on 40 Alzheimer patients and 40 non-Alzheimer patients in Tabriz, Iran from May 2006 to September 2007. Alzheimer patients were selected based on the criteria of the American Psychological Association. The severity of illness was determined based on Reisberg scale. Mental status of the patients was evaluated by Mini Mental State Examination (MMSE). The serum levels of homocysteine were measured by enzyme-linked immunosorbent assay method.

Results: The average serum homocysteine level in the 40 patient group was 23.01±14.40mmol/L, and in the 40 patient control group was 15.40±6.23 (p=0.003). The average serum homocysteine level in the first group of patients was 21.7±12.7mmol/L, in the second group 22.3±13.8, and in the third group 24.9 ± 17.2. The relationship between MMSE score and serum homocysteine level of patients was not significant (p=0.4).

Conclusion: The average serum homocysteine level in Alzheimer patients was higher than in the control group, however, it did not show a significant relationship with the severity of illness.

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Alzheimer's disease (AD) is the most common and important degenerative disease of the brain, and is also the most common cause of dementia. Earlier symptoms are impairment of near memory, and awareness of time and place. In the advanced stage of the disease one could point to psychosis, paranoia, and delirium.[1] One of the probable mechanisms of Alzheimer is vascular disorders, and for this reason the risk factors of vascular disorders increase the appearance and development of AD.[2,3] Hyperhomocysteinemia is

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seen in 25% of patients with brain vascular disease, and is one of the risk factor for vascular disease. So, the relationship of homocysteine level with AD is an important point, and some studies have been carried out in this area.[4-7] Most studies indicate the presence of hyperhomocysteinemia,[3-6] in contrast to others.[8] The aim of this study is assessing the serum homocysteine level in AD, and comparing it with a control group, and also determining the relationship of homocysteine level with severity of the disease.

Methods. Forty patients with AD and 40 healthy people were studied in Tabriz, Iran, from May 2006 to September 2007. This study was performed with patients consent and approved by the Ethics Committee. The diagnosis of AD was based on the diagnostic criteria of American Psychological Association. Determining the intensity of disease was on the basis of Global Deterioration Scale (GDS), or Reisberg scale.[9] In this method, the disease is divided into 7 stages on the basis of the intensity of the symptoms, and the higher the stage, the severer the disability. However, due to the limited number of patients available for this study, the patients were divided into 3 groups (mild, moderate, severe). We included patients in stages one and 2 in the first group, stages 3 and 4 in the second group, and stages 5, 6, and 7 in the third group. Mini Mental State Examination (MMSE) was performed in all patients. This examination consists of various questions on short and long memory, orientation, calculation, and spatial imagination of shapes, for which their maximum score is 30. Demographic and parameters consideration in the selection of patients were over the age of 60 with characteristics of cortical dementia diagnosed by neurologist and psychiatrist. The following tests were performed for all patients to rule out another causes of dementia: thyroid function tests for rule out hypothyroidism, Veneral Disease Research Laboratory for rejecting neurosyphilis, complete cell count hematology (Technicon, USA) for rejecting macrocytosis caused by deficiency of vitamin B12 and folic acid, creatinine to rule out uremia, potassium, and human immuno virus test. Brain imaging (CT-scan or MRI) was carried out to exclude cerebral lesions or cerebral vascular accident. The control group included gender and age-matched healthy participants. Control group was selected among outpatients that did not have Alzheimer's or any other disease increasing homocysteine levels, such as vascular disease or Parkinson's disease. Neither of the groups used vitamin B6 reducing drugs, such as isoniazid, penicillamine, hydralazine, and hormonal therapy, as we are dealing with an elderly population, which may reduce the serum homocysteine level. Blood sampling from patients and control groups was carried out in the afternoon in most cases, as serum homocysteine levels do not depend on fasting or nonfasting state. Samples were frozen at -70°C, and the serum homocysteine levels were measured by the enzyme linked immunosorbent assay method.

The results were analyzed with SPSS 12 software and T-test, ANOVA, Chi square, and the rate of correlation was assigned by Pearson's correlation coefficient. Results are expressed as mean ± SD. Differences with p value less than 0.05 were considered significant.

Results. The mean age of patients was 72.1 ± 7.9 and control group was 70.8 ± 7.7. There were 19 males and 21 females in the case and control groups. Both groups were equal regarding age and gender, and similar risk factors (**Table 1**). The number of patients according to global deterioration scale is shown in **Figure 1**. The average level of serum homocysteine in patients was 23.01 ± 14.40 mmol/lit, and in the control group was 15.40 ± 6.23 mmol/lit. The difference of serum homocysteine level in patients and control group was significant (p=0.0030). The average serum homocysteine level of patients in the first group was 21.7 ± 12.7 mmol/lit in the second group was 22.3 ± 13.8 mmol/lit, and in the third group was 24.9 ± 17.2 mmol/lit (p=0.8). There was a significant difference in the MMSE score among the 3 groups (p=0.003) (**Figure 2**). The correlation coefficient among the MMSE test, and homocysteine level was r=-0.11, indicating the higher the MMSE score the lower the homocysteine level, however, this correlation is not meaningful (p=0.4). With increasing disease duration, the homocysteine level increases (r=0.12), however, this relation is not meaningful (p=0.3). For estimating the homocysteine cut point, the receiver operating characteristic curve was used, and by determining a point with equal sensitivity and specificity on the curve, the number 16.25 mmol/lit was obtained. The odds ratio was 0.17, indicating that the homocysteine higher than 16.25 mmol/lit increases the risk of Alzheimer's

Table 1 - Comparison of patients and control group based on age, gender and risk factors.

Risk factors	Control group	Case group	*P*-value
Age (year)(mean±SD)	7.7 ± 70.8	7.9 ± 72.1	0.4
Gender (male/female)	52.5/47.5	52.5/47.5	0.8
Hypertension (%)	52.5	45.0	0.3
Hyperlipidemia (%)	25.0	22.5	0.5
Myocardial ischemia (%)	15.0	17.5	0.5
Diabetes mellitus (%)	7.5	15.0	0.4
Smoking (%)	2.5	2.5	0.5

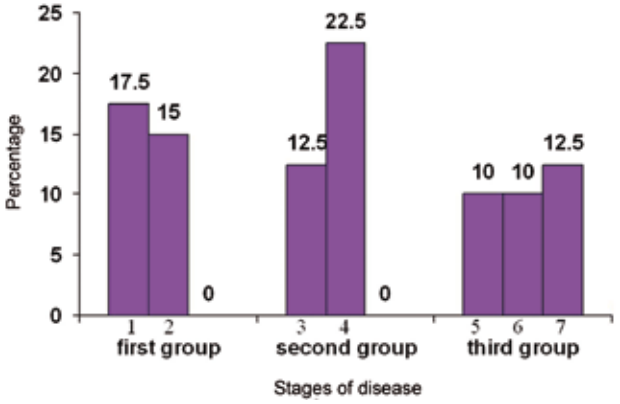

Figure 1 - Distribution of patients in 3 groups based on severity of disease global deterioration scale (GDS).

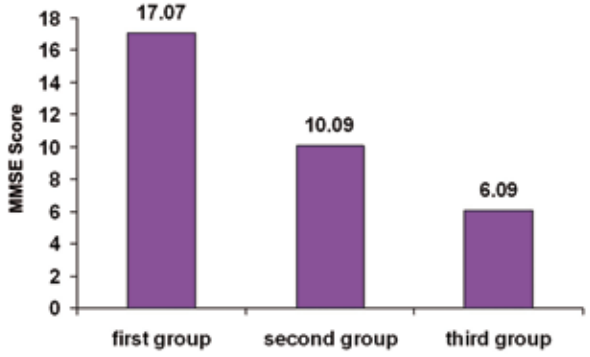

Figure 2 - Mini-mental state examination (MMSE) scores are in percentages.

by 17%. Among the patients, 71% had a homocysteine level higher than 16.25 mmol/lit compared to only 30% in the control group (p<0.005).

Discussion. Any interference for decreasing the risk of disease or to delay the beginning of the disease, has a very important influence on the costs of health care. One factor that has a supportive role for AD is nutrition. In this area, the relationship of the serum concentration of vitamins B12, B6, folic acid, and homocysteine with AD has been shown in various studies.[10] In a study carried out by Seshadri et al,[5] 1092 non-dementia persons (667 females and 425 males with an average age of 65) were followed for 8 years, and 111 individuals were affected ultimately by dementia, with 85 having AD. They concluded that with a plasma homocysteine level higher than 14 mmol/lit, the risk of AD would be approximately 2 times higher. Therefore, homocysteine was considered as a strong and independent risk factor for dementia, and AD. Ravaglia et al,[7] concluded in their study that higher concentration of total plasma homocysteine, and lower concentration of serum folate are independent predicting factors for developing dementia and AD. It was shown by Gallucci et al's, study,[4] that serum homocysteine level had a distinct increase in AD in comparison with the control group. Selley,[3] also showed that plasma homocysteine level in Alzheimer's patients had a distinct increase in comparison with the control group. Against that, there is a considerable decrease in plasma adenosine concentration in Alzheimer's. This is in disagreement with other studies,[8,11,12] which did not find a relationship between homocysteine level and AD. In summary, most studies showed that hyperhomocysteinemia is a risk factor for AD in agreement with ours. The probable mechanisms of effect of homocysteine in Alzheimer's include the cytotoxic effect, which results in damage of vascular endothelium, and accelerate the process of thrombosis, prevention of nitric acid function, which is a vasodilator, and decreasing of adenosine level, which is a supportive molecule against atherosclerosis.[2,3] Furthermore, Pacheco-Quinto et al,[13] reported on the relationship between serum homocysteine level and ß-amyloid peptide in the brain of laboratory mice, which can play an important role in appearing of dementia.

Another part of our study was on the relationship of serum homocysteine with severity of AD. A few studies have been carried out in this area. Nilsson et al,[14] pointed out the correlation between plasma homocysteine concentration and disease intensity. However, we concluded in our study that although with increasing disease intensity, the average homocysteine level increases, however, this relationship was not significant (p=0.8). Morris,[15] pointed to high homocysteine level as a risk factor for AD, which can be prevented by group B vitamins. Contradictory results have been reported by studying mice. Pacheco-Quint et al,[13] showed that homocysteinemia acts as a risk factor for Alzheimer's. However, Santiard-Baron et al,[16] did not show any changes in plasma homocysteine levels in Alzheimeric mice.

In conclusion, this study showed that the serum homocysteine level in AD was significantly higher in comparison with the control group, however, there is no relationship between serum homocysteine level and intensity of the disease. We suggest that this study should be carried out on a large number of blood samples taken from AD patients, especially with severe disease.

References

1. Lewis PR. Merritt's Neurology. 10th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2000. p. 633-636.
2. Graban A. Hyperhomocysteinemia in patients with dementia. ***Neurol Neurochir Pol*** 2003; 37: 879-887.

3. Selley ML. Increased homocysteine and decreased adenosine formation in Alzheimer's disease. ***Neurol Res*** 2004; 26: 554-557.
4. Gallucci M, Zanardo A, De valentine L, Vianello A. Homocysteine in Alzheimer disease and vascular dementia. ***Arch Gerontol Geriatr Suppl*** 2004; 9: 195-200.
5. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. ***N Engl J Med*** 2002; 346: 476-483.
6. Miller JW. Homocysteine and Alzheimer's disease. ***Nutr Rev*** 1999; 57: 126-129.
7. Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, et al. Homocysteine and folate as risk factors for dementia and Alzheimer disease. ***Am J Clin Nutr*** 2005; 82: 636-643.
8. Luchsinger J, Tang MX, Shea S, Miller J, Green R, Mayeux R. Plasma homocysteine levels and risk of Alzheimer disease. ***Neurology*** 2004; 62: 1972-1976.
9. Gauthier S, Panisset M, Nalbantoglu J, Poirier J. Alzheimer's disease: current knowledge, management and research. ***Can Med Assoc J*** 1997; 157: 1047-1052.
10. Nourhashemi F, Gillette-Guyonnet S, Andrieu S, Ghisolfi A, Ousset PJ, Grandjean H, et al. Alzheimer disease: protective factors. ***Am J Clin Nutr*** 2000; 71: 6435-6495.
11. Mizrahi EH, Jacobsen DW, Debanne SM, Traore F, Lerner AJ, Friedland RP, et al. Plasma total homocysteine levels, dietary vitamin B6 and folate intake in AD and healthy aging. ***J Nutr Health Aging*** 2003; 7: 160-165.
12. Nilsson K, Gustafson L, Hultberg B. Relation between plasma homocysteine and Alzheimer's disease. ***Dement Geriatr Cogn Disord*** 2002; 14: 7-12.
13. Pacheco-Quinto J, Rodriguez de Turco EB, Derosa S, Howard A, Cruz-Sanchez F, Sambamurti K, et al. Hyperhomocysteinemic Alzheimer's mouse model of amylodosis shows increased brain amyloid beta peptid levels. ***Neurobiol Dis*** 2006; 22: 651-656.
14. Nilsson K, Gustafson L, Hultberg B. Plasma homocysteine concentration relates to the severity but not to the duration of Alzheimer's disease. ***Int J Geriatr Psychiatry*** 2004; 19: 666-672.
15. Morris MS. Homocysteine and Alzheimer's disease. ***Lancet Neurol*** 2003; 2: 425-428.
16. Santiard-Baron D, Aupetit J, Janel N. Plasma homocysteine levels are not increased in murine models of Alzheimer's disease. ***Neurosci Res*** 2005; 53: 447-449.

STATISTICS

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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of *P* values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.