Observational Study

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The relationship between cognitive impairment and homocysteine in a B12 and folate deficient population in China

A cross-sectional study

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Abstract

Alzheimer disease (AD) is the most common neurodegenerative disease in the world. The relationship between AD and homocysteine (Hcy) is contradictory.

A community-based investigation was conducted to find patients with AD in a vitamin B deficient population (≥55 years old) in Lüliang area in China. Venous blood samples were collected. Serum Hcy, folate, and vitamin B12 were measured. For each case, 4 controls were selected matched with age to evaluate the relationship between Hcy and AD.

The crude prevalence of AD among people ages 55 years or older in this area was 8.60%. There were significant differences in serum Hcy and B12 between the case and control groups. We found that the higher level of serum Hcy was associated with a high risk of AD, and higher education level, higher folate and B12 concentration were protective factors to AD.

Adjustment of diet structure and supplementation of folate and B12 may offer potential therapeutic measures in this area.

Abbreviations: AD = Alzheimer disease, aOR = adjusted odds ratio, CI = confidence interval, Hcy = homocysteine, IQCODE = the informant questionnaire on cognitive decline in the elderly, MMSE = Mini-mental State Examination.

Keywords: Alzheimer disease, homocysteine, prevalence, vitamin B

1. Introduction

Alzheimer disease (AD), the most common neurodegenerative disorder in the elderly, has tremendous consequences on individuals, families, and society.[1,2] With the increasing proportion of the elderly, the number of AD will grow up to 3-fold by 2050.[3] The World Health Organization estimates that the prevalence of AD in the elderly, older than 65 years, is 4% to 7%, which is about 3.21% in China. Because of large population base, AD has become a major public health problem in China. The etiology of AD, however, remains unknown after decades of efforts. Epidemiological studies have found that many factors are related to AD, such as age, gene, sex, education, metabolic factors, mental factors, and dietary factors. It is meaningful to identify modifiable risk factors for this disease.

Homocysteine (Hcy) is formed by demethylation of methionine. There is a close relationship between Hcy and folate, B6, and B12 vitamin levels, which act as the coenzymes of methionine and Hcy metabolism. Deficiency of these B vitamins have been associated with increased Hcy,[4] which is known to have a neurotoxic effects and associated with many diseases, such as cardiovascular diseases and neurodegenerative diseases.[5,6] Up to now, the relationship between Hcy and cognitive impairment is still controversial. Some studies[7–11] have demonstrated that elevated Hcy is related to cognitive decline, whereas other studies[12] found no association between them.

Lüliang area is a folate and B12 deficient area,[13] which may have a high proportion of hyperhomocysteinemia. Meanwhile, we presumed that there will be more cognitive impairment cases in this region. Thus it is a proper area to investigate the relationship between cognitive impairment and Hcy. Furthermore, the data about prevalence of cognitive impairment in Shanxi province were lacking. Similar researches were performed in large cities, such as Beijing, Shanghai, and Xi'an before.

In the present study, we conducted a cross-sectional design to investigate the prevalence of AD in people older than 55 years in Lüliang area and a case-control study to explore the relationship between serum B vitamins, Hcy, and AD.

Editor: Massimo Tusconi.

Informed consent was obtained from the participants after notifying them about the purpose of the survey.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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How to cite this article: Meng H, Li Y, Zhang W, Zhao Y, Niu X, Guo J. The relationship between cognitive impairment and homocysteine in a B12 and folate deficient population in China: a cross-sectional study. Medicine 2019;98:47 (e17970).

Received: 30 June 2019 / Received in final form: 4 October 2019 / Accepted: 17 October 2019

http://dx.doi.org/10.1097/MD.0000000000017970

2. Materials and methods

2.1. Study design and participants

This was a cross-sectional study to investigate the prevalence of AD and the relationship between Hcy and AD in a B12 and folate deficient population in Lüliang, China. Lüliang is a mountainous region, which is underdeveloped, and 80% of residents live in rural areas. Three towns (Gaojiagou, Kangcheng, Caijiaya) were selected as the target populations according to the geographic location (Center, South, North). There was no running water supply and all people drank well water there. Inclusion criteria are the following: we got a population list from the household registry department. People over 55 years old living in Lüliang area for >2 months per year were included in our study. When studying the relationship between Hcy and cognition, exclude people who usually supplement vitamin B.

All participants signed the informed consent. The study was approved by the Ethics Committee of the First Hospital, Shanxi Medical University.

2.2. Questionnaire and cognition evaluation

Trained interviewers (neurologists and senior clinical medical students) administrated a self-designed questionnaire for participants in township hospital. Demographic and medical information was collected, including age, sex, education, marital status, smoking and drinking habits, health situation, and drug use. Education was summarized as illiteracy and literacy. Smoking was divided into 3 categories: never, former, current. Current smokers were defined as participants who smoke ≥1 cigarette/day for ≥6 months; former smokers were defined as participants who smoke <1 cigarette/day or have quit the habit ≥6 months; and participants who never smoke were defined as never. Drinking was also divided into 3 categories: never, former, current. Current drinkers were defined as participants who drink at least once a week for ≥6 months; former drinkers were defined as participants who drink less than once a week or have quit the habit for ≥6 months; and participants who never drink were defined as never.

Cognitive function was evaluated by Chinese versions of the Mini-Mental State Examination (MMSE). MMSE is a global measurement for cognition: orientation in time and place, memory, language, calculation, and constructional praxis. The scores range from 0 (worst) to 30 (best). For people did not appear in the township clinics, investigators conducted household surveys. For someone with hearing or visual impairment, could not fully cooperate, or not at home during home visits, the informant questionnaire on cognitive decline in the elderly (IQCODE) would be used. MMSE (illiteracy ≤17, primary school ≤20, middle school, and above ≤24) and IQCODE ≥3.3 were defined as AD. Hachinski ischemic scale was used to exclude vascular dementia.

2.3. Blood sampling and laboratory tests

Venous blood samples were collected in the morning from participants who agreed. The serum were separated within 30 minutes by centrifugation (4°C, 20 min, 2000 rpm) and transferred in refrigerated boxes to first hospital of Shanxi Medical University, where the samples were stored at −80°C until analyzed. Total Hcy was measured by an enzyme cycling method using a Beckman UniCel DxC 800 Synchron Clinical System Analyzer (Beckman Coulter Inc, CA, USA). Serum folate and vitamin B12 were measured simultaneously by a radio assay kit (MP Biomedical Inc, CA, USA). The concentration unit of serum Hcy, folate, and B12 were μmmol/L, nmol/L, and pmol/L, respectively.

For each case with blood sample, 4 controls were matched with age (±1 years) randomly. Serum Hcy, folate, and B12 were classified into dichotomous variable according to hospital boundaries.

2.4. Quality control

All interviewers were trained strictly by neurologists and psychiatrists. Neurologists and scale evaluator supervised all the progress. Cognitive assessment and blood samples were collected on the same day. The blood samples were taken to our hospital after processing.

2.5. Statistical analyses

The database was set up by the software EpiData3.1, and double input method was adopted. All the data were analyzed by SPSS22.0. Prevalence was calculated and presented as percentages. The Mann-Whitney *U* test was used to compare continuous variables. Differences between categorical variables were calculated using chi-square test. Conditional logistic regression was used to estimate associations between serum tHcy, folate, B12, and AD. The adjusted odds ratios (aORs) were calculated after controlling sex, education, smoking, and drinking status. A *P* value <.05 was considered to be statistically significant.

3. Results

There were 6338 eligible residents in the list of the 3 towns in Lüliang area. A total of 4605 (72.66%) were participated in our study. Age and sex distributions of targeted population and participants are presented in Table 1. Of the participants, 51.1% were women. As the age increased, the proportion of population was getting smaller and smaller, 50.73% between 55 and 64 years, 30.45% between 65 and 74 years, 16.61% between 75 and 84 years, and 2.21% older than 85 years. A total of 2103 (45.67%) were illiterate; 39.07% were current smokers and 6.32% were current drinkers.

In Table 2, 396 were finally considered to be AD. The crude prevalence of AD in people older than 55 years living in Lüliang area was 8.60%. The prevalence of AD increased from 5.31% to 17.64% with advancing age. Moreover, the prevalence of women (11.13%) was about twice that of men (5.60%).

Table 1

Age and sex distribution of targeted population and participants.

	Targeted population (n=6338)	Participants (n=4605)
Age, n (%)		
55–64	3220 (50.80)	2336 (50.73)
65–74	1840 (29.03)	1402 (30.45)
75–84	1121 (17.68)	765 (16.61)
85+	158 (2.49)	102 (2.21)
Sex, n (%)		
Men	3143 (49.59)	2250 (48.86)
Women	3195 (50.41)	2355 (51.14)

Table 2

Prevalence of cognitive impairment in different age and sex groups.

	Men, n (%)	Women, n (%)	Total, n (%)
Age, y			
55–64	44 (3.90)	80 (6.62)	124 (5.31)
65–74	40 (5.95)	94 (12.88)	134 (9.56)
75–84	43 (10.72)	77 (21.15)	120 (15.69)
85+	7 (14.00)	11 (21.15)	18 (17.64)
Total, n (%)	134 (5.60)	262 (11.13)	396 (8.60)

A total of 1845 residents donated blood samples. The proportion of high Hcy, low folate, and low B12 was 71.70%, 57.74%, and 49.81%, respectively. One hundred eighty-two cases were included from the 1845 residents. For each case, 4 controls were matched with age (±1 years) randomly. The characteristics of cases and controls are shown in Table 3. The mean age of cases and controls were 68.84±7.63 and 68.86±7.69 years. There were much more women in case group (72.53%) than in the controls (41.76%) ($P<.001$). The cases had a lower education level ($P=.001$), in other words, there was 68.13% illiteracy in cases compared with the controls (47.8%) ($P<.001$). The proportion of smokers and drinkers was lower in cases than that in controls ($P=.003$, $P=.001$). There were significant differences in serum Hcy and B12 between the 2 groups ($P<.001$). The cases had a higher proportion of abnormal Hcy and B12 ($P=.009$, $P=.002$). These, however, had not been observed in folate ($P=.353$, $P=.140$).

Table 3

The characteristics of cases and controls.

Variables	Case (n=182)	Control (n=728)	*P*
Age, mean±SD	68.84±7.63	68.86±7.69	.992*
Women, n (%)	132 (72.53)	304 (41.76)	<.001†
Education, mean±SD	1.99±3.19	3.01±3.39	.001*
Illiteracy	124 (68.13)	348 (47.80)	<.001†
Literacy	58 (31.87)	380 (52.20)	
Smoking, n (%)			
No	112 (61.54)	320 (43.96)	.003†
Former	8 (4.40)	60 (8.24)	
Current	62 (34.06)	348 (47.80)	
Alcohol drinking, n (%)			
No	174 (95.60)	616 (84.62)	.001†
Former	3 (1.65)	20 (2.75)	
Current	5 (2.75)	92 (12.63)	
Serum tHcy, med (25th–75th)	20.94 (15.06, 31.30)	17.20 (13.62, 22.96)	.001*
≤15	45 (24.73)	272 (37.36)	.009†
>15	137 (75.27)	456 (62.64)	
Serum folate, med (25th–75th)	8.65 (6.00, 14.60)	9.35 (5.96, 15.84)	.353*
≤10	108 (59.34)	376 (51.6)	.140†
>10	74 (40.66)	352 (48.4)	
Serum B12, med (25th–75th)	122.86 (85.65, 168.23)	146.04 (107.97, 218.70)	<.001*
≤133	101 (55.5)	288 (39.6)	.002†
>133	81 (44.5)	440 (60.4)	

Concentration unit of age and education is year, tHcy is μmmol/L, folate is nmol/L, and B12 is pmol/L.
The cutoff of serum tHcy, folate, and B12 was hospital boundaries.
Data are presented as n (%) or med (25th–75th) or mean±standard deviation (SD).
SD=standard deviation.
* *P* value determined by Mann-Whitney *U* test.
† *P* value determined by chi-square test.

Table 4

Conditional logistic regression analyses of cognitive impairment and plasma tHcy, folate, and B12.

Variables	Univariate		Multivariate	
	OR (95% CI)	*P*	OR (95% CI)	*P*
tHcy				
≤15	1	.011	1	.006
>15	1.821 (1.149, 2.888)		2.083 (1.238, 3.505)	
Folate				
≤10	1	.142	1	.016
>10	0.731 (0.481, 1.110)		0.538 (0.324, 0.893)	
B12				
≤133	1	.006	1	.003
>133	0.574 (0.387, 0.850)		0.493 (0.311, 0.783)	

ORs were adjusted by sex, education, smoking, and drinking status.
Concentration unit of tHcy is μmmol/L, folate is nmol/L, and B12 is pmol/L.
CI=confidence interval, Hcy=homocysteine, OR=odds ratio.

In conditional logistic regression analysis, after controlling sex, education, smoking, and drinking status, we found that the higher level of serum Hcy was associated with a high risk of AD [aOR: 2.083, 95% confidence interval (CI): 1.238–3.505, $P=.006$], and high folate and B12 levels were protective factors (aOR: 0.538, 95% CI: 0.324–0.893, $P=.016$; aOR: 0.493, 95% CI: 0.311–0.783, $P=.003$). Furthermore, high education level was also a protective factors ($P<.05$). Smoking and drinking were not associated with AD in this population ($P>.05$) (Table 4).

4. Discussion

In the present study, the participants represented the entire population well according to the sex ratio and age distribution (Table S1, http://links.lww.com/MD/D362). As the age increases, the number of people was getting smaller and smaller, which was in line with the general law. Because of underdevelopment, the level of education in this area was relatively low, nearly half of participants were illiterate. Smokers were also very common in this area. During our investigation, the diagnosis of AD was made by 2 neurologists to ensure the accuracy.

The prevalence of AD in the European elderly population was 4.4%.[14] The prevalence of AD ≥65 years old in Japan were 2.9% to 3.6%.[15] In our country, Zhang et al[16] surveyed Beijing, Shanghai, Xi'an and Chengdu, the prevalence of AD was 3.5%, north was higher than the south (4.2% vs 2.8%). Jia et al found that the prevalence was 3.21%, 4.25% in rural area compared with 2.44% in city. Our findings showed that the prevalence of AD of people older than 55 years in Lüliang area was approximately 8.60%, which were higher than previous studies and may have a lot to do with rural area, poor transportation, and low education level. The prevalence of AD in the present population increased with age, meanwhile, the prevalence of women was about twice that of men, which were consistent with previous studies.

Lüliang, a mountainous region, is located in the midwest of Shanxi province. As early as many years ago, epidemiology found the high incidence of neural tube defects in the area was related to B vitamin deficiency.[13] This is a B vitamin deficiency region, where the main diet is chiefly staple food until now. Among participants with blood samples in our study, the median of tHcy was 19.30 μmmol/L, which were much higher than other studies conducted in China before.[17,18] The majority (71.70%) of them were hyperhomocysteinemia and half of them were B vitamins deficiency. The high prevalence and high Hcy level indicated that Hcy may be involved in the pathogenesis of AD. The previous results about the relationship between Hcy and AD were contradictory. Next, we conducted a case-control study to better study the relationship. There were significant differences between Hcy and vitamin B12 levels among cases and controls in this population. In conditional logistic regression analysis, hyperhomocysteinemia was a significant risk factor of AD; folate and vitamin B12 were protective factors, as well as the high education level. Hcy may induce Aβ deposition, Tau protein hyperphorylation, neuronal DNA damage, neuronal autophagy abnormalities, and other mechanisms, eventually leading to the occurrence of AD.[19–22]

The potential benefit of B vitamins has been demonstrated in many studies.[5,23–26] Durga et al[27] reported that 3-year folate supplementation can improve the cognitive function. Folate may be effective for mild to moderate AD.[28] The combination of folate and B12 may achieve more obvious effects in mild cognitive impairment elderly in Ma et al' study. Animal experiments have also confirmed that supplementation with multivitamin B can improve cognitive function in mice.[29] Several studies, however, reported that the treatment of folate and B12 was uncertain.[30–33] The role of B vitamins need to be further studied. The supplement of vitamins and adjustment of diet structure in this area is still not up to standard, and the low education level may affect implementation.

We investigated the relationship between Hcy and AD in a folate and B12 deficient population for the first time, and provided data supporting the association. Moreover, we proposed valuable interventions. Our study also had some limitations. The blood samples were less than half of the entire population, which may lead to bias; this was just a cross-sectional study, and cannot fully assess the temporal relationship between Hcy and cognitive outcome. A longitudinal study should be conducted to observe the effect of B vitamins supplement.

5. Conclusion

In conclusion, the crude prevalence of AD of people older than 55 years old in Lüliang area was higher than earlier studies. We observed a significant association between serum Hcy concentration and AD in a Chinese folate and B12 deficient population, the result of our study imply that hyperhomocysteinemia was a risk factor of AD. People with higher education level may understand more about reasonable diet structure. Adjustment of diet structure and supplementation of folate and B12 may offer potential therapeutic measures.

Acknowledgment

The authors are grateful to all the participants in this study. The authors appreciate the strong support of the local government and township health centers.

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