Homocysteine Contributes to Pathogenesis by Oxidative Stress for Alzheimer’s Disease

Libo Zhao\textsuperscript{1, 2}, Yong Yan\textsuperscript{2}, Yonglong Wang\textsuperscript{2}, Zhiyou Cai\textsuperscript{3*}

Abstract: Background Lipid peroxidation plays an important role in the development of dementia and Alzheimer’s disease (AD). Elevated plasma homocysteine (HCY) has been associated with poor cognition and dementia. Methods Observers were divided into normal blood lipid level group without AD (N), hyperlipidemia without AD (H), AD without hyperlipidemia (A), AD with hyperlipidemia (AH). The level of oxidized low-density lipoprotein (ox-LDL) and HCY were measured by enzyme linked immunosorben assay (ELISA), thiobarbituric acid Determination for MDA, and xanthine oxidase method for SOD. Blood lipid level was measured by biochemistry. Results The serum levels of HCY and MDA in AH group are significantly higher than that of normal group. The serum level of SOD in AH group is significantly lower than that of normal group. The serum levels of SOD and MDA in A and H groups compared with N group have no significant change. The serum levels of ox-LDL, HCY in AH, A and H groups are significantly higher than that of normal group. The serum levels of ox-LDL and HCY in AH and H groups are significantly higher than that of A group. The score of mini-mental state examination (MMSE) in all AD is opposite correlation with the serum levels of ox-LDL and HCY. Relationship between the scores of MMSE and the serum levels of ox-LDL and HCY in AD groups, compared with non-AD groups, has statistical significance. Conclusion HCY and abnormal lipid metabolism participate in pathogenesis of AD, and HCY can enhance oxidative stress in AD.

Keywords: Alzheimer’s disease, homocysteine, oxidative stress, hyperlipidemia

1. Introduction

Abnormal lipid metabolism, lipid peroxidation (oxidized low-density lipoprotein, ox-LDL) plays an important role in the formation and progression of atherosclerosis and Alzheimer’s disease (AD) [1, 2]. Free radical, as a toxic production produced by living organism and inducing lipid peroxidation, is one of central elements of age-related diseases. Malondialdehyde (MDA), the production of lipid peroxidation, is one of the greatest toxic action [3, 4]. Its content responds to the speed and intensity of lipid peroxidation and indirectly responds to the damage degree of free radical [5, 6]. Superoxide dismutase (SOD), a specific enzyme, can enhance the clearance of free radical and plays an important role in the neuroprotection for free radical injury [7, 8]. SOD and MDA level may indirectly respond to the metabolism level of free radical. Recent research discovered that free radical injury, oxidative stress and cerebrovascular risk factors have possibly participated in the process of AD [9, 10].

High serum level of homocysteine has recently emerged as a major vascular risk factor [11, 12]. Elevated total homocysteine levels have been associated with an increased risk of atherosclerotic sequelae, including death from cardiovascular causes, coronary heart disease, carotid atherosclerosis, and clinical stroke [13-15]. These observations led to the hypothesis that elevated plasma homocysteine may be a risk factor for dementia and AD [16, 17]. If this hypothesis is valid, it points to a modifiable risk factor, since plasma homocysteine levels can be lowered by supplementation with folic acid [18, 19]. Elevated plasma homocysteine levels in subjects with cognitive impairment or dementia might be the result of poor nutrition and vitamin deficiencies [18, 20]. A prospective study should be able to show whether elevated plasma homocysteine in cognitively intact adults is associated with an increased risk of dementia and AD on follow-up. Abnormal lipid metabolism, oxidative stress and homocysteine play an important role in the development of dementia and AD, but the pathogenesis was still not clear. Therefore this study tested serum levels of SOD, MDA, ox-LDL, homocysteine and lipid metabolism factors in AD. The relation between oxidative markers and serum levels of homocysteine has been analyzed in this study to clarify the role of homocysteine in AD pathogenesis.

2. Materials and methods

2.1 Subjects

86 AD patients (41 males and 45 females) include 46 cases of AD group without hyperlipidemia and 40 cases of hyperlipidemia group with AD, are respectively from the First Affiliated Hospital of Chongqing Medical University, Chongqing Second Social Welfare Institution and Chongqing Western-suburb Hospital. (1) The clinical diagnoses of AD is according to the following standards: NINCDS-ADRDA ( the National Institute of Neurological Disorders and Stroke and the Alzheimer’s disease and Related Disorders Association ) and CCMD-II ( Chinese Classification and Diagnostic Criteria of Mental Disorders-II ) criteria. MMSE confirms memory and intelligent impairment. CT or MRI scanning removes the dementia caused by other reasons or secondary dementia (e.g. hypothyroidism). Drugs of vitamins and downregulating agents for lipid level has not used for three
months recently. Age of AD patients here chosen is from 55 to 80 years old (average 68±6). Score of MMSE is from 4 to 24 (average 15±6). (2) Non-AD: 40 cases, 20 males and 20 females, above 27 score of MMSE, age between 55 and 80 years old, normal brain CT or MRI scanning, normal liver and kidney function, drugs of vitamins and downregulating agents for lipid level hasn’t used recently. (3) Hyperlipidemia: 40 cases, 20 males and 20 females, above 27 score of MMSE, age between 50 and 73 years old, total cholesterol (TC) ≥5.72mmol/L or/and low density lipoprotein cholesterol (LDL-C)≥3.64mmol/L, 16 samples with hypertension, 8 samples with coronary artery diseases.

2.2 Reagents

Hcy was obtained from U.S. Abbott company and ox-LDL ELISA kit from Bender Medsystems, USA. MDA and SOD kits were purchased from Nanjing Jiancheng Biology Science Company, China. 721 spectrophotometer was from FACS caliber, Becton Dickinson, USA.

2.3 Groups

Observers were divided into four groups: normal group without AD (N), hyperlipidemia group without AD (H), AD group without hyperlipidemia (A), hyperlipidemia group with AD (AH).

2.4 Measurement of ox-LDL and HCY by ELISA

Peripheral venous blood was drawn into blood collection tubes containing sodium citrate. Citrated blood samples were centrifuged (200 g for 10 min) to separate the plasma. Plasma was stored at -80℃ until analysis. ox-LDL and HCY level was determined using ELISA kit according to the manufacturer’s instruction.

2.5 SOD, MDA and serum lipid measured by biochemistry

Levels of MDA and SOD were determined using a commercial kit and standard methods according to the manufacturer’s instruction and thiobarbituric acid Determination for MDA, xanthine oxidase method for SOD.

2.6 Statistical analysis

Quantitative data were expressed as mean±SD. Statistical comparisons were conducted using SPSS11.0 software package for intergroup. For statistical evaluation one-way analysis of variance (ANOVA) were employed. Pearson correlation analysis was also performed to some index. P<0.05 was considered as statistically significant.

3. Results

3.1 Serum level of MDA and SOD

The serum levels of MDA in AH group are significantly higher than that of in normal group (P < 0.05); The serum levels of SOD in AH group are significantly lower than that of in normal group (P < 0.05); The serum levels of SOD and MDA in A and H groups compared with N group have no significant change; SOD and MDA levels have no significant change among A and H groups (Tab. 1). This demonstrated that hyperlipidemia may enhance reaction of free radical and effects of oxidative stress involved in the pathogenesis of AD. At the same time, linear correlation analysis showed that MMSE score for AD has no correlation with serum SOD and MDA levels (r = 0.201, P > 0.05; r = - 0.214, P > 0.05 ) (Figure 1, 2 ). This indicated that the effect of oxidative stress in the pathogenesis of AD has no specificity.

3.2 Serum level of ox-LDL

The results showed that the serum levels of ox-LDL in AH, A and H groups are significantly higher than that of in normal group (P < 0.01 ); The serum levels of ox-LDL in AH and H groups are significantly higher than that of in A group (P < 0.05 ). There was no significant difference between A group and AH group (Tab.1). MMSE score for AD has negative correlation with serum ox-LDL level (r = -0.368, P<0.05 ) (Fig. 3 ). The score of MMSE in AD is opposite correlation with the serum level of ox-LDL. Relationship between the score of MMSE and the serum levels of ox-LDL in AD groups and non-AD groups has statistical significance (P < 0.05).

3.3. Correlation analysis between ox-LDL and SOD, MDA

To further explore the mechanism of that hyperlipidemia contributes to Alzheimer’s disease by oxidative stress, correlation analysis between ox-LDL and SOD, MDA had been done here. The ox-LDL level in AD is positive correlation with the serum level MDA (r = 0.441, P < 0.05). The ox-LDL level in AD is negative correlation with the serum level SOD (r = 0.441, P < 0.05). This indicated that AD patients with hyperlipidemia can enhance lipid peroxidation involving in the pathogenesis of AD.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The serum level results of HCY, SOD, MDA, ox-LDL and serum lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>n</td>
</tr>
<tr>
<td>N</td>
<td>40</td>
</tr>
<tr>
<td>H</td>
<td>40</td>
</tr>
<tr>
<td>A</td>
<td>40</td>
</tr>
<tr>
<td>AH</td>
<td>46</td>
</tr>
</tbody>
</table>

aP<0.05, compared with N group; bP<0.01, compared with N group; cP<0.01, compared with N group.
Figure 1 SOD and MMSE scores. *P>0.05, compared with group A.

Figure 2 MDA and MMSE scores. *P>0.05, compared with group A.

Figure 3 ox-LDL and MMSE scores. *P<0.05, compared with group A.

Figure 4 HCY and MMSE scores. *P<0.05, compared with group A.
3.3 Serum level of HCY

The results showed that the serum levels of HCY in AH, A and H groups are significantly higher than that of in normal group (P < 0.01). The serum levels of homocysteine in AH and H groups is significantly higher than that of in A group (P < 0.05). There was no significant difference between A group and AH group (Tab.1). MMSE score for AD has negative correlation with serum level of HCY (r = -0.402, P < 0.05). It showed that HCY was involved in behavioral symptoms of AD. At the same level of serum level of HCY value, MMSE scores for AD group without hyperlipidemia was significantly lower than non-AD with hyperlipidemia (P < 0.01) (Figure 4). These results indicate that hyperlipidemia contributes to AD by elevated plasma homocysteine.

3.4 Correlation analysis between oxidative markers and HCY

The results showed that the serum levels of HCY in AD patients are positive correlation with that of ox-LDL (r = 0.441, P < 0.05) (Figure 5), the serum levels of SOD and MDA is not relevant with HCY (r = -0.110, P > 0.05; r = 0.108, P > 0.05) (Figure 6, 7). Therefore, elevated plasma homocysteine enhanced oxidative stress by lipid peroxidation in AD with hyperlipidemia.

![Figure 5 correlation analysis between ox-LDL and HCY.](image)

![Figure 6 correlation analysis between SOD and HCY.](image)

![Figure 7 correlation analysis between MDA and HCY.](image)

4. Discussion

Abnormality of lipid metabolism plays an important role in the development of arteriosclerosis and AD. Elevated plasma homocysteine levels have been associated with poor cognition and dementia. The findings of the current study suggest a strong association between lipid peroxidation and elevated plasma homocysteine, the risk of AD. Abnormal lipid metabolism, lipid peroxidation plays an important role in the formation and progression of AD. HCY involved in behavioral symptoms of AD, hyperlipidemia contributes to AD by elevated plasma homocysteine. Correlation analysis between ox-LDL, SOD, MDA and HCY showed that elevated plasma homocysteine enhanced oxidative stress by lipid peroxidation in AD with hyperlipidemia.

Free radical (inducing lipid peroxidation, protein peroxidation, DNA peroxidation and oxidative stress) is one of central elements of age-related diseases [21, 22]. MDA and SOD may indirectly respond to the metabolism level of free radical. MDA, the product of lipid peroxidation, is one of greatest toxic action. Its content responds to speed and intensity of lipid peroxidation and it indirectly responds to the damage degree of free radical. SOD, enhancing clearance of free radical and preventing cellular damage caused by reactive oxygen species (ROS) [23, 24], plays an important role in neuroprotection for age-related diseases. Evidence has shown that senescent changes are primarily caused by ROS, followed by the accumulation of macromolecular oxidative damage generated by ROS [25, 26]. The amount of oxidative modified molecules increases as a function of aging in virtually all species that have been examined. As a neurodegenerative disease associated with aging [27, 28], AD is characterized by neuronal loss, neurofibrillary tangles, and senile plaques. Although the cause of neuronal death in AD is not clear, the pivotal role of Aβ, as an essential factor in neurodegeneration of AD, has been supported by many more studies [29, 30]. Strong evidence has shown that free radicals and oxidative stress induced by Aβ play an important role in neurodegeneration of AD [31, 32].

Low-density lipoprotein (LDL) exists within the brain and is highly vulnerable to oxidative modifications [33]. Once formed, ox-LDL is capable of eliciting cytotoxicity, differentiation, and inflammation in nonneuronal cells. Although ox-LDL has been studied primarily for its role in the development of atherosclerosis, recent studies have identified a possible role for it in neurological disorders associated with oxidative stress. Polymorphism of the oxidized low-density lipoprotein receptor-1 gene has been reported to be associated with late-onset AD [34, 35]. A significant correlation was also found between levels of CSF antichymotrypsin (ACT), IL-6, MCP-1, oxLDL and the ratio of CSF to serum albumin, which is used as a measure of the blood-brain barrier function. The inflammatory markers in the plasma and CSF of patients with AD and provide good evidence that levels of ox-LDL in plasma and CSF might be candidates as biomarkers for monitoring the inflammatory process in AD [12, 36, 37]. These data demonstrate that ox-LDL induces neuronal death in AD.
In the present study, we measured serum level of SOD, MDA, ox-LDL and lipid metabolism factors in AD and non-AD controls. The results demonstrated that the serum level of ox-LDL in AH, A, H groups is significantly higher than that of in normal group; that of ox-LDL in AH, H groups is significantly higher than that of A group; there was no significant difference between A group and H group. The serum level of MDA in AH group is significantly higher than that of in normal group; the serum level of SOD in AH group is significantly lower than that of in normal group; the serum level of SOD and MDA in A, H groups compared with N group has no significant change: SOD and MDA levels had no differences among A groups and H group. Taken together, these data demonstrate that SOD, MDA, ox-LDL and abnormal lipid metabolism participate in pathogenesis of AD, and abnormal lipid metabolism can increase oxidative stress and induces expression of ox-LDL in AD.

In the present study, linear correlation analysis demonstrated that MMSE score for AD patients has negative direction with serum ox-LDL level, serum level of ox-LDL in AH group is higher than A. MMSE score for AD patients has negative direction with serum MDA level. MMSE score for AD patients has the same direction with serum SOD level, serum level of MDA, SOD in AH group is not higher than A. The ox-LDL level in AD is positive correlation with the serum level MDA. The ox-LDL level in AD is negative correlation with the serum level SOD. The hypothesis was made that oxidative stress plays an important role in AD by the function of linking agent.

Homocysteine, a sulfur-containing amino acid, is a metabolite of the essential amino acid methionine. High blood levels of homocysteine result in far-reaching biochemical and life-threatening consequences. Homocysteine exists at a critical biochemical intersection in the methionine cycle between S-adenosylmethionine, the ubiquitous methyl donor, and vitamins B12 and folic acid. Indirect and direct vascular damage can be caused by homocysteine, a putative atherothrombotic risk factor [18, 38, 39]. Homocysteine has been associated with vascular disease, particularly in subjects with significant carotid stenosis. Increasing evidence for a connection between homocysteine metabolism and cognitive function is surfacing, and this includes from mild cognitive decline (age-related memory loss) to vascular dementia and AD [40]. Elevated plasma homocysteine levels are associated with carotid atherosclerosis and an increased risk of stroke. Atherosclerosis and stroke, in turn, increase the risk of clinical AD [41, 42]. Increased concentrations of homocysteic acid, an N-methyl-D-aspartate receptor agonist and a metabolite of homocysteine, may result in excitotoxic damage to neurons [43, 44]. Homocysteine promotes coppermediated and Aβ-mediated toxic effects in neuronal cell cultures and induces apoptosis in hippocampal neurons in rats. Elevated plasma homocysteine levels have been associated with poor cognition and dementia [11, 17, 45, 46]. In the present study, the results demonstrated that HCY involved in behavioral symptoms of AD. At the same level of serum level of HCY value, MMSE score for AD group without hyperlipidemia was significantly lower than non-AD patients with hyperlipidemia. Therefore, hyperlipidemia contributes to Alzheimer’s disease by elevated plasma homocysteine. To further explore the mechanism of that hyperlipidemia contributes to the pathogenesis of AD, correlation analysis between ox-LDL, SOD, MDA and HCY was done in this study. The results showed that the serum level of HCY in AD patients is positive correlation with that of ox-LDL, the serum level of SOD MDA is not relevant with HCY. Therefore, elevated plasma homocysteine enhanced oxidative stress by lipid peroxidation in Alzheimer’s disease with hyperlipidemia.

The relation between elevated plasma homocysteine levels and dementia must be evaluated in other cohort studies. If such studies confirm our findings, proof of a causal association between plasma homocysteine and lipid peroxidation in the process of AD will require further elucidation of the pathophysiology mechanisms and direct evidence from controlled clinical trials in humans that interventions that reduce plasma homocysteine levels can reduce the risk of clinical dementia and AD. What’s more, should plasma lipoproteins be proven to play a role in the pathogenesis of AD, their modification with statins or antioxidants may offer therapeutic benefit.

Conflict of interest: None declared.

References


