Association between Serum Haptoglobin and the Pathogenesis of Alzheimer’s Disease

In-Uk Song, Young-Do Kim, Sung-Woo Chung and Hyun-Ji Cho

Abstract

Objective  Haptoglobin (Hpg) is known to have several functional properties, including antioxidant and anti-inflammatory activities. In addition, it has been shown that the pathogenesis of neurodegenerative disorders, such as Alzheimer’s disease (AD), involves inflammation as well as oxidative stress. However, evidence suggesting an association between the serum Hpg level and AD is lacking. Therefore, we conducted this study in order to investigate whether serum Hpg is associated with AD.

Methods  We compared the serum Hpg levels of 121 patients with newly diagnosed AD, 58 patients with Parkinson’s disease (PD) and 43 healthy controls. We also evaluated the relationship between the severity of cognitive impairment in patients with AD and the serum Hpg level.

Results  The mean serum Hpg level of the patients with AD was significantly higher than that of the healthy controls (p=0.042), although it was not significant different from that observed in the PD group (p=0.613). We also found a significant positive association between the serum Hpg level and the severity of cognitive impairment, as measured using several neuropsychological tests, in the patients with AD. The odds ratio (95% confidence interval) of the patients with AD grouped according to the Hpg level was 2.417 (95% confidence interval=1.134-5.149).

Conclusion  We observed a significantly higher mean serum Hpg level among the patients with AD compared to the healthy controls. These results support the hypothesis that oxidative stress and neuroinflammatory reactions play a role in the pathogenesis of AD.

Key words: Alzheimer’s disease, haptoglobin, inflammation, oxidative stress

Introduction

Alzheimer’s disease (AD) is one of the most disabling disorders of the elderly, the of which incidence is expected to increase dramatically worldwide in the near future. Therefore, a major concern among the scientific community is to identify putative biomarkers to assess the risk of developing AD. Considerable scientific evidence implicates inflammation in the pathophysiology of AD. For example, studies of brain tissue obtained from patients with AD consistently show signs of inflammation, as indicated by the presence of activated microglia, complement factors, cytokines and other inflammatory proteins (1). Elevated blood levels of inflammatory proteins, such as hs-C-reactive protein (CRP), have also been found in patients with AD (1, 2).

Haptoglobin (Hpg) is an acute-phase protein produced by the liver that functions to scavenge cell-free hemoglobin and its byproducts. Hpg exhibits several functional properties, including antioxidant and anti-inflammatory activities and the ability to participate in immune system regulation (3, 4). Furthermore, the serum Hpg levels have been shown to be increased in humans with sepsis (5), and the pathogenesis of neurodegenerative disorders, such as AD, has been reported to involve inflammation as well as oxidative stress (3). However, evidence of a direct association between the serum Hpg level and AD is lacking. In order to clearly evaluate the association between Hpg and AD, we analyzed changes in the Hpg levels in AD patients and compared this parameter between the AD patients, healthy control group and a
neurodegenerative disease control group comprised of subjects with de novo Parkinson’s disease (PD).

Materials and Methods

This study was approved by the local ethics committee, and each patient provided their written informed consent for participation. We prospectively screened all consecutive patients with complaints of memory impairment who presented to the Dementia and Memory Clinic of Incheon St. Mary’s Hospital between September 2012 and December 2013. The study included 121 patients newly diagnosed with AD, 58 patients with PD as a neurodegenerative disease control group and 43 normal subjects. All participants with AD were matched for gender, age and the level of education and underwent comprehensive cognitive evaluations using the Mini-Mental State Examination (MMSE), the extended version of the Clinical Dementia Scale (CDR) and the sum-of-box scores (SOB) of the CDR and the Global Deterioration Scale (GDS).

Consecutive patients with a normal cognitive function on general neuropsychological testing and similar ages to the AD subjects were chosen as healthy control subjects and subjects with PD without dementia. Patients with PD as a disease control group were diagnosed according to the United Kingdom Parkinson’s Disease Society Brain Bank Clinical Diagnosis Criteria for Parkinson’s disease. In addition, we only included patients with newly diagnosed de novo PD without a history of antiparkinsonian drug therapy, such as levodopa, dopaminergic agonists, catechol-O-methyltransferase (COMT) inhibitors, selegiline and amantadine. We excluded patients with cognitive impairment, secondary causes of parkinsonism, e.g. Wilson’s disease, neuroleptic drug use or psychiatric diseases that would, in the investigator’s judgment, interfere with the safe conduct of the study. PD patients with cerebrovascular disease or focal neurological signs of cerebral disease were also excluded from the study.

The control subjects reported no history or symptoms of memory impairment and exhibited no symptoms or risk factors for cognitive impairment based on their self- or family-reported medical history and a detailed neurological examination performed by a neurologist. They also displayed no signs of cognitive dysfunction, as assessed on a dementia screening questionnaire and general dementia scale. All patients included in the study underwent brain magnetic resonance imaging (MRI) to rule out cognitive impairment caused by cerebrovascular disease or space occupying lesions, such as tumors. In order to reduce the effects of confounding of risk factors for AD and factors affecting the Hpg level, we excluded subjects with hypertension, cardiovascular disease, diabetes mellitus, hypercholesterolemia, a history of cigarette smoking and current infection.

All patients with AD met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria for AD as well as the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD (6). Patients diagnosed with probable AD had no focal neurological signs or symptoms according to their history or on a physical examination, nor radiological evidence of cerebrovascular disease. Additionally, none of the patients with AD fulfilled the criteria for mixed dementia or vascular dementia, according to the National Institute of Neurological Disorders and Stroke and the Association Internationale la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) criteria (7).

Venous blood samples were collected from all subjects in order to measure the serum Hpg level. The blood samples were separated via centrifugation at 3,000 rpm for 10 minutes immediately after collection. The separated serum was stored at -80°C until the laboratory evaluation. The laboratory data were collected by an examiner blinded to the clinical details and patient information.

The statistical analysis was performed using the SPSS software package, version 18.0. The results are expressed as the mean ± standard deviation. The analysis of variance (ANOVA) with a post hoc analysis, covariance analysis and independent T-test was used to compare continuous variables, and a Pearson’s Chi-squared analysis was used to compare categorical variables. We also used a receiver operating characteristic (ROC) curve analysis of the Hpg level specific to the AD group. In addition, the area under the ROC curve (AUC), cut-off value determined according to the maximum Youden index (sensitivity +1-specificity), sensitivity and specificity were calculated. In order to evaluate the strength of the association between the serum Hpg level, categorized as ≤121 mg/dL or >121 mg/dL according to the cut-off value calculated based on the above-mentioned method, and AD, we determined the odds ratio using binary logistic regression analyses. Statistical significance was assumed at the 5% error level.

Results

The demographic characteristics of the subjects are summarized in Table 1. There were no significant differences in age or gender distribution between the patients with AD and the controls. There were also no significant differences in the mean serum Hpg level between the AD and PD groups (p=0.613). However, the mean serum Hpg level was significantly higher in the patients with AD than in the controls (122.83±70.92 vs. 99.21±45.45, respectively; p=0.042) (Fig. 1). The mean MMSE score was 18.03±5.43 in the patients with AD, 28.17±1.53 in the patients with PD and 28.95±10.8 in the control subjects (p<0.001). The analysis of covariance of the education level of the subjects showed the same results as those of the ANOVA; namely, there was a significant difference between the AD patients and the healthy controls.

A ROC curve analysis conducted to assess the usefulness of the Hpg level for detecting AD demonstrated an AUC of
Table 1. Baseline Characteristics of the Study Group

|                | AD    | PD    | HC    | p value |
|----------------|-------|-------|-------|---------|
| Number of subjects | 121   | 58    | 43    | -       |
| Gender, male     | 40    | 26    | 18    | >0.05   |
| Age (year)       | 75.83±7.49 | 75.51±7.47 | 73.20±8.49 | 0.058   |
| Education duration (year)* | 4.93±4.72 | 11.78±3.42 | 12.33±0.58 | <0.001  |
| Haptoglobin (mg/dL)** | 122.83±70.92 | 118.32±46.11 | 99.21±45.45 | 0.043   |
| MMSE*            | 18.03±5.43 | 28.17±1.53 | 28.95±10.8 | <0.001  |
| CDR              | -     | -     | -     | -       |
| SOB              | -     | -     | -     | -       |
| GDS              | -     | -     | -     | -       |

AD: Alzheimer’s disease, PD: Parkinson’s disease, HC: Healthy control group, MMSE: Mini-mental State Examination, CDR: Clinical Dementia Scale, SOB: the Sum of the Box score of the CDR, GDS: Global Deterioration Scale

Values was expressed as mean ± standard deviation

Gender was analyzed by Pearson chi-square test

p value was calculated by independent T-test

* Post-hoc analysis: AD < PD = HC

** Post-hoc analysis: AD = PD > HC

Figure 1. Comparison of the mean serum haptoglobin (mg/dL) level between the patients with Alzheimer’s disease and Parkinson’s disease and the healthy controls. The box plot shows the median value (thick line), with the 25th and 75th percentiles. p-value between Alzheimer’s disease (AD) and Parkinson’s diseases (PD) = 0.613, p-value between AD and healthy control (HC) = 0.042, p-value between Parkinson’s disease and healthy control = 0.041.

605, with a cut-off value of 121 mg/dL (sensitivity, 48.3%; specificity, 72.1%) (Fig. 2). In regard to the associations between the serum Hpg level and the cognitive impairment scores in the AD patients, there were statistically significant associations between the Hpg levels and the MMSE, CDR, SOB and GDS scores (Table 2). In other words, the serum Hpg level was found to be related to the severity of cognitive impairment in the patients with AD. In addition, a binary logistic regression analysis revealed an odds ratio (95% confidence interval) of patients with AD grouped according to the Hpg level of 2.417 (95% confidence interval=1.134-5.149).

Discussion

Hpg is the major hemoglobin-binding protein as well as an acute-phase protein, the expression of which is increased during inflammation (8). The primary function of Hpg is to bind extracorpuscular free hemoglobin in order to mitigate the oxidative and inflammatory effects of hemoglobin (9). Previous studies have found increased serum Hpg levels in patients with AD, various inflammatory conditions, traumatic brain injury and Guillain-Barré syndrome (3, 10). In addition, inflammation has been implicated in the development of AD; for example, the levels of C-reactive protein, a
nonspecific marker of inflammation, are elevated in the brain and serum in patients with AD (2, 11). In addition, although the etiology of AD remains unclear, oxidative stress is strongly implicated as a primary pathogenic event in such cases (12). For example, several studies have demonstrated increased levels of oxidative stress markers, including those of protein oxidation, lipid peroxidation and DNA oxidation, in the brain in patients with AD compared to healthy subjects (12-14). Furthermore, additional data support the hypothesis that A-beta plays a crucial role in the pathogenesis and progression of AD, possibly triggering the oxidative stress-mediated damage that ultimately results in neuronal death (12).

In the present study, we found that the patients with AD had a significantly higher mean serum Hpg level than the healthy controls, consistent with the findings of previous reports of the anti-inflammatory and anti-oxidative functions of Hpg (8, 9). Unlike other inflammatory biomarkers, such as hs-CRP, Hpg has an anti-inflammatory effect. Therefore, we hypothesize that the higher Hpg levels observed in our patients with AD were due to an immune response to the brain’s inflammatory reaction. Although accumulating evidence suggests a key role for oxidative stress in the pathogenesis of AD, the mechanisms connecting oxidative damage to the onset and progression of AD remain controversial (15, 16). One published study suggested that oxidative stress results from the perturbation of the natural balance between pro- and anti-oxidant systems, resulting in damage to biomolecules, loss of functionality and cell death (16). Our finding of increased Hpg levels in AD patients is consistent with this hypothesis. Furthermore, our results showing an association between the Hpg level and the severity of cognitive impairment may reflect the strength of the immune system’s anti-inflammatory response to neuroinflammation in the setting of AD.

Despite the higher serum Hpg levels observed in the patients with AD compared to the controls in the present study, we cannot firmly conclude that an elevated serum Hpg level is associated with the risk of developing AD due to the low AUC value. Additionally, we cannot declare the Hpg level to be a risk factor for AD because this study was not longitudinal study. Therefore, we can only cautiously suggest that the results of the present study indicate this parameter to be a potential risk factor, as our findings did not show chronological clinical changes in AD. Another limitation of the present study is that we cannot be certain about the accuracy of our clinical diagnoses due to the lack of neuropathologic confirmation (all patients remain alive). However, we attempted to reduce the impact of these confounders by including such patients, all of whom fulfilled two sets of diagnostic criteria and exhibited brain radiological findings, as assessed by an experienced neurologist and radiologist.

In conclusion, in this study, we observed significantly higher serum Hpg levels in the patients with AD and PD compared to that observed in the controls, with no significant differences between the AD and PD groups. These results support the hypothesis that oxidative stress and neuroinflammation play important roles in the pathogenesis of AD. Furthermore, the findings of significantly higher serum Hpg levels observed in the AD and PD patients are in agreement with the previous hypothesis that the pathogenesis of neurodegenerative disorders involves inflammation as well as oxidative stress. To the best of our knowledge, this is the first published study to evaluate the relationship between the serum Hpg level and AD.

The authors state that they have no Conflict of Interest (COI).

Table 2. Relation between Haptoglobin and Variables in AD

| Correlation coefficients | p value |
|--------------------------|---------|
| Age                      | 0.184   | 0.044  |
| MMSE                     | -0.301  | 0.001  |
| CDR                      | 0.229   | 0.012  |
| SOB                      | 0.233   | 0.01   |
| GDS                      | 0.067   | 0.619  |
| Education                | -0.195  | 0.055  |

Values are Spearman’s rank order correlation coefficients and p value.

AD: Alzheimer’s disease
HC: Healthy control group,
MMSE: Mini-mental State Examination,
CDR: Clinical Dementia Scale,
SOB: the Sum of the Box score of the CDR,
GDS: Global Deterioration Scale

Figure 2. Receiver operating characteristic curves of the serum haptoglobin level for predicting Alzheimer’s disease. The area under the ROC curve (AUC) is 0.605. The best cut-off value is 121 mg/dL (sensitivity, 48.3%; specificity, 72.1%).
References

1. Yarchoan M, Louneva N, Xie SX, et al. Association of plasma C-reactive protein levels with the diagnosis of Alzheimer’s disease. J Neurol Sci 333: 9-12, 2013.
2. Kravitz BA, Corrada MM, Kawas CH. Elevated C-reactive protein levels are associated with prevalent dementia in the oldest-old. Alzheimers Dement 5: 318-323, 2009.
3. Chang KH, Tseng MY, Ro LS, et al. Analyses of haptoglobin level in the cerebrospinal fluid and serum of patients with neuro-myelitis optica and multiple sclerosis. Clin Chim Acta 417: 26-30, 2013.
4. Arredouani MS, Kasran A, Vanoirbeek JA, et al. Haptoglobin dampens endotoxin-induced inflammatory effects both in vitro and in vivo. Immunology 114: 263-271, 2005.
5. Janz DR, Bastarache JA, Sills G, et al. Association between haptoglobin, hemopexin and mortality in adults with sepsis. Crit Care 17: R272, 2013.
6. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer’s disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer’s Disease. Neurology 34: 939-944, 1984.
7. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 43: 250-260, 1993.
8. Lee CW, Cheng TM, Lin CP, et al. Plasma haptoglobin concentrations are elevated in patients with coronary artery disease. PLoS One 8: e76817, 2013.
9. Bowman BH, Kurosky A. Haptoglobin: the evolutionary product of duplication, unequal crossing over, and point mutation. Adv Hum Genet 12: 189-261, 453-454, 1982.
10. Pantoni L, Inzitari D. Abnormal penetration of haptoglobin through the blood-brain-barrier (BBB) into the cerebro-spinal fluid (CSF) in Alzheimer’s disease patients. Acta Neurol Scand 91: 225, 1995.
11. Song IU, Kim JS, Chung SW, et al. Is there an association between the level of high-sensitivity C-reactive protein and idiopathic Parkinson’s disease? A comparison of Parkinson’s disease patients, disease controls and healthy individuals. Eur Neurol 62: 99-104, 2009.
12. Coccio A, Di Domenico F, Coccia R, et al. Decreased expression and increased oxidation of plasma haptoglobin in Alzheimer disease: Insights from redox proteomics. Free Radic Biol Med 53: 1868-1876, 2012.
13. Butterfield DA, Drake J, Pocernich C, et al. Evidence of oxidative damage in Alzheimer’s disease brain: central role for amyloid beta-peptide. Trends Mol Med 7: 548-554, 2001.
14. Mangialasche F, Polidori MC, Monastero R, et al. Biomarkers of oxidative and nitrosative damage in Alzheimer’s disease and mild cognitive impairment. Ageing Res Rev 8: 285-305, 2009.
15. Di Domenico F, Coccia R, Butterfield DA, et al. Circulating biomarkers of protein oxidation for Alzheimer disease: expectations within limits. Biochim Biophys Acta 1814: 1785-1795, 2011.
16. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272: 20313-20316, 1997.

© 2015 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imonline/index.html