

Homocysteine Levels, White Matter Lesions Scores and Perceived Caregiver Stress in Alzheimer's Disease



Medical Science

KEYWORDS :

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ABSTRACT

Introduction : Alzheimer's disease (AD) is by far the most common type of dementia worldwide. Currently, the role of vascular factors has been increasingly appreciated in pathophysiology of AD. Serum homocysteine, and white matter lesions (WMLs) are markers of vascular risk. The present study was undertaken to explore association of serum homocysteine and WMLs in AD with Perceived Stress Scores (PSS).

Methodology : This is a case control study (n=65). The diagnosis of AD was made using National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer's disease related Disease Association criteria (NINCDS-ARDA). Blood samples were collected from the study subjects after overnight fasting. Serum separation was done immediately within half an hour of sampling and homocysteine levels were measured using enzyme cycling method.

Results : In AD group (n=35, M: F=28:7), mean age and duration of illness was 72±8.1 and 3.1±2.2 years respectively while in controls (n=30, M: F=24), mean age was: 68.6±8.6 years. The mean Mini Mental Status Score of AD group was 13.32±5.6. AD group showed high homocysteine levels and the odds ratio was higher in AD compared to controls (6.5; p-value<0.05, 95% confidence interval= 1.79 to 24.069). There was high intra-rater agreement (Cohen's Kappa=0.8) in between separately analyzed ratings of samples (n=10). The PSS of those with high homocysteine was significantly greater compared to those with normal homocysteine. Likewise, those with higher homocysteine had a greater White Matter Lesion Load (Odds Ratio=5.7, 1.1009 to 29.6594; p-value<0.05). ADL Scores were however not different between them (p-value=>0.05).

Conclusion : AD group had higher serum homocysteine compared to controls and also those with high homocysteine had more WMLs. PSS of those with raised homocysteine was higher compared to those with normal homocysteine (p-value<0.05).

Introduction

Alzheimer's disease (AD) is the most common type of dementia worldwide. Though considered to be a neurodegenerative in nature, role of vascular factors in AD pathophysiology is growing¹. Serum homocysteine is an established marker of vascular disease and has been studied in several diseases like diabetes, hypertension and stroke etc. A high serum level of homocysteine, known as hyperhomocystenemia is associated with vascular dysfunction such as altered angiogenesis and increased membrane permeability. Epidemiological studies have found associations between high homocysteine and AD¹⁻³. High homocysteine is associated with the hallmarks of dementia pathologies such as tau phosphorylation, A β aggregation, neurofibrillary tangle (NFT) formation, neuroinflammation, and neurodegeneration⁴. Previous reports also suggest homocysteine may promote AD like pathology by more than one mechanism, including cerebral microangiopathy, endothelial dysfunction, oxidative stress, neurotoxicity and apoptosis^{2,3}. Homocysteine has been associated with cognitive impairment and various psychiatric symptoms³. The progressive development of AD-related lesions, such as NFT, amyloid deposits and synaptic loss, and the occurrence of microvascular and small macrovascular pathology within the cerebral cortex are conspicuous neuropathologic features of brain aging. White Matter Lesions (WMLs) on Magnetic Resonance Imaging (MRI) are commonly found in AD and may contribute to cognitive impairment⁴. WMLs are attributed to degenerative changes of small vessels and are frequently associated with AD brains. The causative contribution of these lesions in causing or promoting AD pathology is beginning to be understood and the role of vascular factors in AD pathology is now being increasingly appreciated. In AD, the association has been appreciated since last decade or so^{5,6}.

It has been known for sometime that homocysteine may be a potentially modifiable risk factor for dementia. Serum homocysteine in AD been correlated with structural changes in the brain, with impaired cognition and behavioral and psychological disturbances of dementias among others². Total homocysteine levels are associated with silent brain infarcts and white matter lesions independent of each other and of other cardiovascular risk factors. Recent studies have shown that homocysteine levels are a sensitive marker of folate and vitamin B₁₂ levels and that increased blood homocysteine levels are correlated not only with cardiovascular disease but also with the severity of cognitive damage and dementia². It has been reported that hyperhomocystenemia is correlated with psychosis, depression, and other psychiatric disorders^{1,2}. Therefore, it was assumed that high serum homocysteine could be associated with perceived caregiver stress. In the present study, therefore the Perceived Stress Scores in the patients with AD having alteration in serum homocysteine were compared with those having normal serum homocysteine and study controls.

Material & Methods

This is a case control study (n=65; cases with AD=35, Controls=30). The study was conducted in the Out Patient Department (OPD) of the Neurology Department, Psychiatry and Medicine Department of a Tertiary Care Hospital in Northern India. An informed consent was taken from all study participants. The caregivers of cases and controls were interviewed for evaluation of caregiver stress. The scoring was done as per Perceived Stress Scale (detailed below). The study was approved by the Institutional Ethics Committee.

Recruitment & Patient Evaluation

Subjects were selected randomly from the General Outpatient

Department of Neurology and were asked to attend a specialized memory clinic for detailed evaluation. After the initial General Physical, Neurological and Neuropsychological Examination, the diagnosis of AD was made using National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer's disease related Disease Association criteria (NINCDS-ARDA). The Criteria has high sensitivity and specificity and the same has been demonstrated in Indian patients as well².

Controls

A total of 30 subjects in the controls group were taken. There were a total of 16 male and 14 females caregivers who were interviewed regarding the Perceived Stress Scale Score (PSS) assessment. Controls were free from the vascular risk factors known to contribute to rise in homocysteine e.g. diabetes, and hypertension.

Scales

Mini Mental State Examination (MMSE)^{7,8} was done in all patients, and all the patients underwent MRI of the Brain. The Fazekas WML Score⁹ was used to rate the patients' WML load. Perceived Stress was studied using Perceived Stress Scale¹⁰. The scales used in the current study have been used in Indian patients earlier².

The Perceived Stress Scale¹⁰ is the most widely used psychological instrument for measuring the subjective perception of stress. It measures the degree to which situations in one's life are appraised as stressful. The questions are of a general nature and are relatively free of content specific to any sub-population group. Hence, it is appropriate for use in caregivers. The questions in the PSS are 10 in number, Likert type and ask about feelings and thoughts during the last month. The PSS has a high internal consistency of 0.85 (Cronbach co-efficient) and test-retest reliability. The Blessed ADL Scale¹¹ was described by Blessed, Tomlinson and Roth in 1965 and consisted of 11 questions. It is divided into two parts: In the first part, 10 activities are covered, the scores are rated as 0, 0.5 and 1 and in the second part there are four possible points to select, from 0 to 4. This scale is helpful in the evaluation of the degree of disability and in the planning of social support.

Biochemical protocol

A total of 5-ml blood samples were collected¹² from the antecubital veins of the selected study subjects after an overnight fasting using vacutainer with clot activator gel (BD Biosciences). Samples were immediately kept on ice and serum was separated within half an hour of blood collection. The serum was aliquoted and stored at -80°C till analysis. Serum homocysteine levels were measured using enzymatic cycling method (Dialab) on fully automated analyzer (Olympus AU-400). The homocysteine assay has analytical sensitivity of 0.4 µmol/L, analytical range 3- 50 µmol/L with intra and inter assay coefficient of variation (CV) 4.61% and 5.98% respectively. Reference range for serum homocysteine was taken to be 5-15 µmol/L. Hyperhomocystenemia was defined as homocysteine value >15 µmol/L.

Magnetic Resonance Imaging and visual ratings scales

Magnetic Resonance Imaging (MRI) of the brain was done in both cases and controls. Images were acquired using Siemens Megnatom Symphony 1.5T scanner, and were acquired in T1, T2, FLAIR sequences in axial, coronal and sagittal planes. The imaging protocol comprised of a coronal T1-weighted FLASH (Fast Low Angle Shot) with slice thickness 5mm, repetition time 400 ms, echo time 9.5 ms, flip angle 25°, and matrix of 160 x 256. In T2 weighted images, TR was 50400 and TE was 111 ms. In FLAIR, TR was 900 and TE was 153. Slice thickness was 5mm.

A visual assessment of White Matter Lesions was done using the Fazekas Rating Scale⁹ (Figure-1).

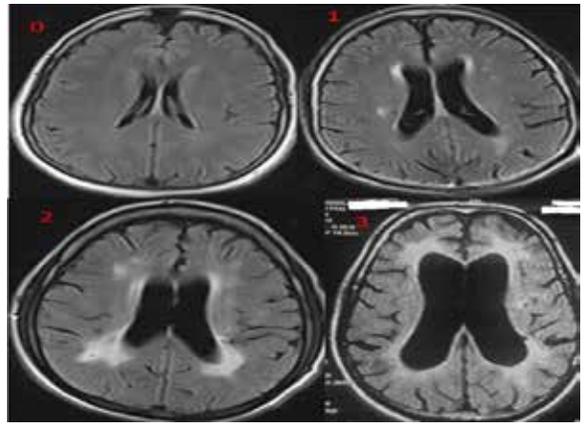


Figure-1: Fazekas Scoring Scale

Rating was done by two observers (SD & VD), both having an experience of 4 years and 15 years respectively. Ratings were reviewed by other two (KSA & UCG) having experience of more than 25 years each in Neurology and Neuroradiology respectively. An inter-rater agreement was calculated for both the cases and controls for all the three visual rating scales. Fazekas scale is clinically used to rate white matter lesions⁶ both, in the periventricular and deep white matter regions of the brain. T2 Turbo Spin Echo and T2 FLAIR sequences were used for Fazekas Scoring.

Statistics

The latest version of SPSS[®] (SPSS Inc., Chicago, IL) was used for data analysis. Normality of data was checked using Q-Q plot. Correlation and Regression were performed. Two sided p-value <0.05 was used to test level of significance. One way Analysis of Variances (ANOVA) was used to see the difference between three groups of scores of PSS. A Chi-square analysis was done to calculate the Chi-square statistics.

Results

In AD group (n=35, M: F=29:7), mean age and duration of illness was 72±8.1 and 3.1±2.2 years respectively while in controls (n=30, M: F=24), mean age was 68.6±8.6 years. The other summary measures are given in the table-1. The mean MMSE score of AD group was 13.32±5.6. AD group showed high homocysteine levels and the odds ratio was higher in AD compared to controls (6.5; p-value<0.05, 95% confidence interval= 1.79 to 24.069). There was high intra-rater agreement (Cohen's Kappa=0.8) in between separately analyzed ratings of samples (n=10) by the same single rater. The mean serum homocysteine levels in the cases was 21.29±6 µmol/L and that of the controls was 12.27±4.9 µmol/L respectively (p-value=0.0001). There was a positive correlation between the serum homocysteine and MMSE (Pearson Correlation Coefficient r =0.75). The figure-2 given below shows the relationship between serum homocysteine (x) and MMSE (y)

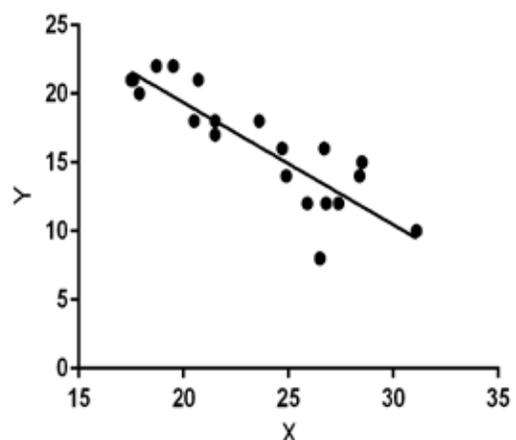


Figure-2: Correlation between serum homocysteine (x) and MMSE (y) [Pearson Correlation Coefficient=0.75].

A total of 21 patients with a diagnosis of AD had a higher serum homocysteine out of a sample of 35 (60%). Only 2 (0.06%) out of the control group had high serum homocysteine compared to 21 out of 35 (Odds ratio=25; 95% confidence interval= 5.4122 to 115.4788, p value=<0.0001). Correlation (Figure-3) between serum homocysteine (x) and perceived stress score (y) was modest [Pearson correlation coefficient=0.23]. Likewise, there was a weak correlation between serum homocysteine and Fazekas Lesion Score [Pearson correlation coefficient=0.11]. Chi-square test comparing the WML load frequent in those with high serum homocysteine compared to those with controls showed a statistically significant difference (Chi-square statistics=5.05, p-value<0.05). Those with higher homocysteine had a greater White Matter Lesion Load (Odds Ratio=5.7, 1.1009 to 29.6594; p-value<0.05).

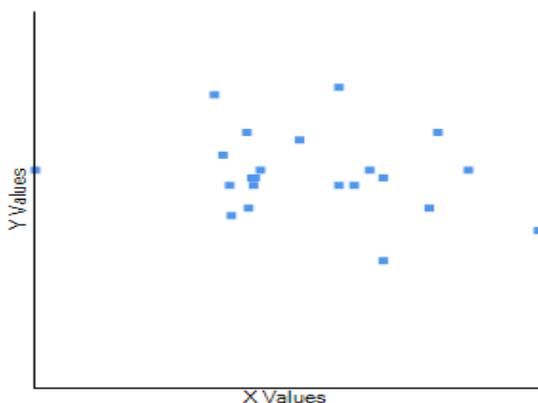


Figure-3: Correlation between serum homocysteine (x) and perceived stress score (y) [Pearson Correlation Coefficient=0.23].

Perceived Stress Score of those with high homocysteine was significantly greater compared to those with normal homocysteine (18.23±1.7 vs 8.9± 2.81;p-value=<0.005). The PSS score in the controls was 8.9± 2.81. Using one way Analysis of Variance (ANOVA), a significant difference was present among the groups (p-value <0.05). Using Tukey's Honestly Significant Difference test, it was found; All three groups were significantly different from each other (p-value <0.05).

A statistically significant difference in the means of Fazekas Lesion Load Score was found between the AD and control group. PSS score was also significantly different in AD group compared to controls. Similarly, AD and control group differed in ADL scores as well (p-value=<0.05).

Table-1: Summary Measures cases and controls in the present study

	Cases (n=35)	Controls (n=30)
Age	72±8.1	68.6±8.6 years.
Duration of Illness	3.6±0.2 years	Healthy
MMSE	13.32±5.6	28±1.5
Fazekas Lesion Load	0.6±0.8	0.07±0.25
Perceived Stress Scale Score (PSS)	23.46±5.6	5.6±2.3
Activity of Daily Living Score (ADL)	7.82±2.3	0.00
Serum homocysteine levels	21.2989±6 mmoles/Liter	12.2737±4.9 moles/Liter

*Significant difference was present between the two groups (p-value=<0,05).

Likewise, those with higher homocysteine had a greater white matter lesion load. ADL Score was marginally significant between those with high homocysteine compared to those who had normal serum homocysteine. Between those with AD, had a highly significant difference compared to controls.

Alzheimer's disease; though traditionally considered to be neurodegenerative in etiology, the role of vascular factors in AD pathophysiology is growing²⁻⁴. Serum homocysteine has emerged as one of the important marker of vascular risk¹². For example, a high circulating concentration of the amino acid homocysteine is an independent risk factor for stroke. AD commonly co-occurs with stroke. Epidemiological studies found associations between hyperhomocysteinaemia and both histologically confirmed AD and disease progression and revealed that dementia in AD was associated with evidence of brain infarcts on autopsy. Thus, hyperhomocysteinaemia and AD could be linked by stroke or microvascular disease. However, given known relations between B-group-vitamin deficiency and both hyperhomocysteinaemia and neurological dysfunction, direct causal mechanisms are also plausible⁴. A recent prospective study colleagues showed hyperhomocysteinaemia to be a strong, independent risk factor for dementia and AD⁵. The high homocysteine have also been observed in oldest of the old in general population and also those who are cognitively impaired^{6, 12-13}. In light of these data and the growing parallel interest in serum homocysteine as an emerging vascular risk factor there has been considerable hope that vitamin therapy with folate, B12 and B6, could lower plasma homocysteine levels, and could significantly reduce the risk of stroke and dementia^{7,12-13}.

Higher homocysteine has the potential to affect brain structures¹⁴⁻¹⁵. Individuals with higher plasma levels of homocysteine had lower gray matter thickness in bilateral frontal, parietal, occipital, and right temporal regions and lower gray matter volumes in left frontal, parietal, temporal, and occipital regions, after controlling for diagnosis, age, and sex and after correcting for multiple comparisons⁸⁻⁹. It appears that there is a critical level of brain shrinkage, possibly mediated by elevated homocysteine, which when reached, results in cognitive decline, especially in episodic memory performance¹⁰. The effect of WMLs the MR imaging or vascular atherosclerosis on cognitive function is not fully understood in AD¹¹⁻¹². Likewise, it has been postulated that white matter lesions and beta-amyloid have a possible connection¹³⁻¹⁴.

Brain images of patients with AD on MRI show WMLs, which are attributed to degenerative changes of small vessels^{2,7-9}. These lesions are supposed to be among the factors supporting the diagnosis of probable AD¹²; however their correlation with the severity of dementia requires further studies. WMLs are prevalent in nondemented aging and in AD, and their presence influences cognitive impairment in the AD^{2,9}. Individuals with AD may be more vulnerable to the cognitive effect of WMLs than nondemented aging individuals with similar WML burden. In another Asian study from Iran, the average serum homocysteine level in patients was higher than in the control group. More of men, which have higher homocysteine in some of the reported studies, could be the reason responsible for the same. Moreover, their sample size was also small limiting the generalizability.

Elevated serum homocysteine concentrations¹⁶⁻²¹ and low serum folate concentrations are independent predictors of the development of dementia and AD. Both systemic circulatory changes and alterations directly within the brain vasculature have been suggested to contribute to both the onset and progression of neurological conditions such as AD²²⁻²⁷. It is now well established

that vascular risk factors²⁸⁻²⁹ including hypertension, diabetes, obesity, atherosclerosis, metabolic syndrome, and stroke significantly increase one's risk of later developing AD²². That is why, the role of vascular factors in AD pathophysiology is growing²³⁻²⁴. The same has been seen in patients with Mild Cognitive Impairment³⁰ and Parkinson's disease-Mild Cognitive Impairment (PD-MCI)³¹. The relevance of serum homocysteine in these subgroup of patients is however not known²⁵ though several studies have reported an increase in homocysteine in AD patients, this association is naturally, not unique²⁵⁻²⁶. In AD however, it is related at a genetic level²⁷. Hyperhomocysteinemia is supposed to be one of the modifiable risk factors that, if treated, may delay the onset of AD²⁸⁻³⁶.

Major strengths of the study are: it presents the carefully selected patients with AD in an institutional study showing high homocysteine in AD compared to controls. The study has correlated WML, and perceived stress with homocysteine. The current study reports a correlation between MMSE and serum homocysteine. Likewise, a significant difference in the means of Fazekas Lesion Load Score was found between the AD and control group. PSS score was also significantly different in AD group compared to controls, and also in those patients with high serum homocysteine compared to those who have normal serum homocysteine. Similarly, AD and control group differed in ADL scores as well. A high Perceived Stress found in our study perhaps reflects the difficulties the AD patients and their caregivers. The statistical difference between them may indicate the potential contribution of serum homocysteine. We had earlier demonstrated higher caregiver burden in patients of AD with BPSD. Other group demonstrated the BPSD to be more frequent if high homocysteine was present. There seems to be a synergy between these two studies. Homocysteine may be causing be involved at a pathophysiological level. Elevated homocysteine for example, have been found to contribute to increased AD pa-

thology, particularly neurofibrillary tangles burden. This effect seems to be more pronounced in the presence of cerebrovascular pathology²⁹. In a recent study³⁰, greater homocysteine level and WMLs were associated with processing speed impairment and greater Vascular Index score was associated with memory impairment. Likewise, serum homocysteine has been correlated with degree of cognitive impairment; and this was confirmed in our study too³⁷. Similarly, there was a significantly higher WML load in patients with AD having high serum homocysteine compared to those whose levels were within normal range. In a large recent community-based sample (n=1163), white matter lesion load was inversely associated with cognitive performance, independent of brain atrophy³⁸. So WMLs contribute to cognitive decline and hence the association between cognitive decline, serum homocysteine and caregiver stress becomes interesting. WMLs have been correlated with measures of brain atrophy including hippocampal atrophy³⁹. So it looks plausible that caregiver stress may be directly the end result of homocysteine induced aggravation of pathophysiology of AD cases. We did not see a significant correlation between the ADL and serum homocysteine. That may be because, it has been correlated with some measures in AD, but ADL are significantly impaired late in the course of disease. There are some limitations as well. Our sample size is not very large and the study involves subjects in the hospital setting. Moreover, the predominately male population may be limiting the generalization of the study in AD.

Conclusion

AD group had higher serum homocysteine compared to controls and also those with high homocysteine had higher perceived stress score compared to those with normal serum homocysteine. Also, those with higher homocysteine had more WMLs and a weak correlation between WML and serum homocysteine was present.

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