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Hyperhomocysteinemia and its effect on ageing and language functions – HEAL study

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Hyperhomocysteinemia or high levels (>15 µmol/L) of homocysteine (Hcy)in the blood has been suggested to affect the brain through vascular and neurodegenerative pathways and potentially impact cognition. The current study aims to explore the association of high homocysteine with cognition and brain volume changes in a cohort of middle and old agedr adults. The study recruited 1296 participants aged ≥ 45 years from Tata Longitudinal Study of Ageing (TLSA), an ongoing cohort study. The participants underwent detailed cognitive assessments using Addenbrooke's Cognitive Examination-III (ACE-III) and Computerized Assessment of Adult Information Processing (COGNITO) neuropsychological battery and MR imaging using a 3T scanner. The participants were classified based on the median homocysteine level (16.89 µmol/L) into low Hcy (≤ median) and high Hcy (> median) groups. When adjusted for age, gender, years of education, vitamin B12, folate and dyslipidaemia, Generalised Linear Model (GLM) found a significant association of high Hcy with vocabulary task [β (95% CI) – 1.354 (– 2.655, – 0.052); *p* = 0.041]. Significant associations was also obtained between cerebral white matter volume and high Hcy [β (95% CI) – 5617.182 (– 11062.762, – 173.602); *p* = 0.043]. The results suggest that people with high Hcy levels performed poorer in cognitive tasks related to language domain and had lesser cerebral white matter volume. This indicates that homocysteine might have a profound impact on brain structure as well as function.

Keywords Homocysteine, Hyperhomocysteinemia, Cognition, Risk factor, Ageing, Neuroimaging

With age, there is deterioration of physical health as well as cognitive functioning[1]. The rate of decline is determined by the presence of risk factors for cognitive impairment. These include hypertension, diabetes, obesity, smoking, alcohol consumption, education, social networking and traumatic brain injury, among others[2]. People with a combination of several of these risk factors have a faster decline than the rest. This can lead to significant disturbances in daily life functioning and further to dementia[3]. Since the contributory causes of dementia are not been established yet, it is necessary to identify risk factors that may predispose an individual to dementia. Many of the modifiable risk factors of dementia are cardiovascular risk factors too[4]. One of the important cardiovascular risk factors is hyperhomocysteinemia , which refers to the increased levels of homocysteine (Hcy) in blood[5,6]. Hyperhomocysteinemia is usually defined as serum Hcy levels above 15 µmol/L although several different cutoffs have been proposed to classify hyperhomocysteinemia in different populations[7–9]. Hcy levels between 5 µmol/L and 15 µmol/L are considered to be normal[7].

The amino acid, Hcy has serious implications on human health. It is mostly known for its role in atherosclerosis. Recent evidence also suggests the influence of Hcy on the central nervous system, in particular, the brain, affecting cognition through a wide range of potential mechanisms such as reduction in endothelial Nitric Oxide Synthase (eNOS) necessary to maintain healthy endothelium leading to endothelial damage and cerebrovascular disease[10]. Other mechanisms like oxidative stress and hypomethylation are also thought to play a role in cerebrovascular damage[11,12]. Hcy also causes the loss of blood brain barrier integrity[13]. Hcy may also affect cognition through neurodegenerative processes. Neurotoxicity is one such mechanism caused by N-Methyl D-Aspartate (NMDA) receptor agonist activity of homocysteic acid, a product of Hcy oxidation. In hyperhomocysteinemia, continuous excitation of NMDA receptors and calcium influx leads to excitotoxicity and neuronal death[14]. Lipid peroxidation and neural network suppression are other mechanisms by which hyperhomocysteinemia directly affects neurons[15,16].

These changes may lead to structural and functional changes in the brain and may manifest as mood and cognitive alterations[17–19]. Evidence suggests hyperhomocysteinemia to be associated with cognitive decline in

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several cognitive domains like memory, attention, visuospatial abilities, language and executive functioning[15,16]. Among them, the domains reported to be most commonly affected with hyperhomocysteinemia are episodic memory and language[20].

The universal cutoff used to define hyperhomocysteinemia is 15 µmol/L[21]. This cutoff has been used globally irrespective of the differences in population and age. This is not ideal as the Indian population have higher Hcy levels in general[22], leading to an overestimation of hyperhomocysteinemia burden and chances of missing a relationship of hyperhomocysteinemia with cognition. Thus, in the current study, we utilized our dementia-free cohort's median Hcy levels as the cutoff for classifying participants into low Hcy and high Hcy groups. One of the causes for higher Hcy levels in Indians is the high prevalence of vegetarianism in the country. Although vegetarian diet has lesser methionine content in addition to lesser vitamin B12 concentrations, vegetarians are seen to have high levels of Hcy in general. This is because, although non-vegetarian diet has high methionine levels, the difference between vegetarians and non-vegetarians is not major[23]. Also, moderately high levels of methionine do not pose risk of hyperhomocysteinemia when an individual has adequate concentrations of vitamin B12 and folate which are generally easily met in non-vegetarians[24].

Elevated Hcy level implies a higher risk of cardiovascular diseases in general. Due to higher Hcy levels, brain health is also compromised. An increase in Hcy levels can be caused due to several factors including dietary factors (vegetarianism), lifestyle (smoking, alcohol consumption), metabolic (obesity, liver function) and genetic (Methylene Tetra Hydro Folate Reductase—MTHFR and Cystathionine Beta Synthase—CBS gene polymorphisms) factors. Despite being influenced by several parameters, it is an easily modifiable risk factor. Therefore, it is necessary to identify the prevalence of high Hcy levels in the Indian population and to assess its contribution to deviations from optimal brain functioning. The current study aims to find the association of cognition, grey matter and white matter volumes with high Hcy in an urban population of middle and old aged adults.

Methodology

Study participants

This cross-sectional study included participants from the ongoing Tata Longitudinal Study of Aging (TLSA), a cohort of adults aged 45 years and above, residing in Bengaluru, India[25]. The cohort comprising adults free of dementia, aims to identify midlife risk factors for dementia including smoking, obesity and diabetes. Studying such midlife risk factors is very valuable for the prevention and early detection of predementia stages[26]. The participants of this study undergo detailed clinical, cognitive and biochemical assessments annually. The baseline data from the cohort was included in the current analysis. The study has received approval from the Institutional Ethics Committee of the Centre for Brain Research, Indian Institute of Science (CBR/42/IEC/2022-23). Voluntary informed consent was obtained from all the participants recruited for the study. The study strictly adhered to the guidelines of the Declaration of Helsinki.

Clinical and cognitive assessments

The participants underwent a comprehensive clinical assessment and a structured interview through which information regarding their diet, physical activity, clinical history and lifestyle factors were collected. The presence of symptoms of depression was assessed using Geriatric Depression Scale (GDS-30). To test for anxiety, Generalised Anxiety Scale (GAD-7) scale was used.

Cognitive functions were assessed using Addenbrooke's Cognitive Examination III (ACE III). It is a 100-point test which evaluates performance in memory, attention, language, visuospatial abilities and fluency domains. The Indian-adapted version was used for the current study[27].

The COGNITO (Computerised Assessment of Adult Information Processing) neuropsychological battery adapted for the Indian population was used to assess cognitive functioning in various domains in detail. The tests of the COGNITO battery in the following domains include memory (Episodic memory immediate and delayed recall, name-face recognition, implicit memory), attention (reaction time, auditory attention, visual attention), language (reading and sentence comprehension, semantic association, phoneme comprehension, categorical fluency, letter fluency, vocabulary), executive functioning (Stroop test) and visuospatial abilities (visuospatial span, form matching, construction abilities)[28].

Biochemical tests

The participants' blood samples were collected by trained personnel in Ethylene Diamine Tetraacetic Acid (EDTA)—coated tubes. The serum was used to quantify several blood parameters including Hcy, vitamin B12 and folate. Chemiluminescence immunoassays were used to quantify Hcy, vitamin B12 and folate (Cobbas e 801 immunoassay analyser and VITROS ECiQ Immunodiagnostic Systems). Since there are no standard cutoffs for hyperhomocysteinemia specifically for the Indian population, the classification into low and high Hcy groups was made based on the median Hcy levels in our cohort. To determine the ApoE genotype, DNA was extracted from the blood samples. The DNA was PCR amplified using appropriate ApoE-specific primers and the amplified products were utilised to identify the ApoE genotype through Sanger sequencing. The participants were classified based on their ApoE status into ε4 non-carriers (ε2/ε2, ε2/ε3, ε3/ε3,) and ε4 carriers (ε2/ε4, ε3/ε4, ε4/ε4).

MR image acquisition and processing

All brain Magnetic Resonance Imaging (MRI) data were acquired using a 3 Tesla MR imaging system (Magnetom Prisma, Siemens). We used Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence to obtain high-resolution T1-weighted images for structural data. The Freesurfer software (v 7.2.0) was used for processing of the high resolution T1 weighted images and to measure grey matter and white matter volumes.

Statistical analysis

Kolmogorov-Smirnov test was used to check the type of distribution. Since the variables considered were found to be non-parametric, Mann-Whitney U test was used to compare the characteristics of the two groups. Chi-squared test was used to find the association between categorical variables. Spearman's correlation was used to assess the relationship between Hcy levels and cognitive performance. Further, Generalized Linear Model (GLM) was used to identify the association of high Hcy with various cognitive variables. Model 1 was unadjusted. Model 2 was adjusted for age, gender and years of education. Model 3 was adjusted for vitamin B12 and folate levels in addition to model 2. Model 4 was additionally adjusted for dyslipidaemia, which was considered as a binary variable assessed based both on history of dyslipidaemia medication as well as abnormal concentrations of various lipid parameters.

For all the MRI-obtained brain volumes, Total Intracranial Volume (TIV) was additionally used as a covariate in all models. All the analyses were performed using IBM Statistical Package for Social Sciences (SPSS) software version 28.0.1.1.

Results

A total of 1296 participants were included in the study. The mean age of the study group was 62.6 ± 9.62 years, 49.9% were males and 51.1% were females. When classified based on the global cutoff of 15 µmol/L, 512 participants (39.5%) belonged to normal group and 784 participants (60.5%) belonged to the hyperhomocysteinemia group. Since this cutoff may not be applicable for our population, we used median Hcy level (16.89 µmol/L) to classify the participants into high Hcy (> 16.89 µmol/L) and low Hcy (≤ 16.89 µmol/L) groups. Based on the above classification, it was found that 648 participants (50%) of the cohort had high Hcy levels. Chi-squared test revealed an association of high Hcy levels with gender [Male (64.5%) vs. Females (35.5%); $p < 0.001$], smoking status [Low Hcy (2.8%) vs. high Hcy (4.8%); $p = 0.043$] and alcohol consumption status [Low Hcy (16.5%) vs. high Hcy (33.5%); $p = 0.004$]. Using a Mann-Whitney U test, it was found that the high Hcy group had significantly higher body weight ($p = 0.003$)and lower GDS ($p = 0.009$) and GAD scale ($p = 0.027$) scores (Table 1). The frequency of ApoE ε4 allele was not associated with Hcy levels ($p = 0.466$).

Demographic characteristics	Total	Low Hcy	High Hcy	*p*-value
Age (years)	63.00 (55.25, 69.00)	62.00 (55.00, 69.00)	63.00 (56.00, 70.00)	0.153
Gender				
Male	649 (50.10%)	231 (35.60%)	418 (64.50%)	
Female	647 (49.90%)	417 (64.40%)	230 (35.50%)	<0.001*
Education (years)	15.00 (13.00, 17.00)	15.00 (13.00, 17.00)	15.00 (13.00, 17.00)	0.788
Smoking				
Never smoked	780 (60.80%)	407 (63.70%)	373 (58.00%)	
Currently abstinent	453 (35.30%)	214 (33.50%)	239 (37.2%)	
Currently smoking	49 (3.80%)	18 (2.80%)	31 (4.80%)	0.043*
Alcohol consumption				
Never consumed	638 (49.80%)	347 (54.40%)	291 (45.30%)	
Currently abstinent	401 (31.30%)	186 (29.20%)	215 (33.50%)	
Current consumption	241 (18.80%)	105 (16.50%)	136 (21.20%)	0.004*
GDS	1.00 (0.00, 4.00)	2.00 (0.00, 5.00)	1.00 (0.00, 4.00)	0.009*
GAD	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.027*
HMSE	31.00 (30.00, 31.00)	31.00 (30.00, 31.00)	31.00 (30.00, 31.00)	0.481
Height (cm)	160.50 (154.00, 168.00)	158.00 (153.00, 165.00)	163.00 (156.50, 170.00)	<0.001*
Weight (kg)	69.00 (61.70, 78.50)	68.00 (60.58, 76.70)	70.30 (63.00, 79.00)	0.003*
BMI (kg/m^2)	26.70 (24.09, 29.90)	26.85 (24.16, 30.00)	26.45 (23.95, 29.64)	0.126
Homocysteine (µmol/L)	16.89 (12.78, 22.60)	12.78 (10.52, 14.70)	22.60 (19.34, 27.35)	<0.001*
CDR				
0	1147 (90.30%)	570 (89.90%)	577 (90.70%)	
≥0.5	123 (9.70%)	64 (10.10%)	59 (9.30%)	0.622
ApoE ε4 status				
Non-carrier	889 (84.90%)	439 (84.10%)	450 (85.70%)	
Carrier	158 (15.10%)	83 (15.90%)	75 (14.30%)	0.466

Table 1. Characteristics of the whole study population, high Hcy and low Hcy groups. Continuous variables are described as median (interquartile range) while categorical variables are indicated as frequency (percentage). Mann-Whitney-U test was used for comparing continuous variables, Chi-squared test was used for comparing categorical variables. *GDS* Geriatric depression scale, *GAD* Generalised anxiety disorder scale, *HMSE* Hindi mental state examination, *BMI* Body mass index, *CDR* Clinical dementia rating, *ApoE* Apolipoprotein E; *$p<0.05$.

Spearman correlation analysis identified significant negative correlation of Hcy levels with the scores in ACE III fluency domain (r=-0.097; $p = 0.001$), reading and sentence comprehension (r = − 0.069; $p = 0.022$), episodic memory immediate recall (r = − 0.134; $p = 0.001$), episodic memory delayed recall (r = − 0.099; $p = 0.004$), categorical fluency (r = − 0.149; $p < 0.001$) and letter fluency (r = − 0.088; $p = 0.016$) (Table 2, Figure 1).

Cognitive parameters	Correlation coefficient (r)	*p*-value
ACE III total	− 0.027	0.334
ACE III attention	0.044	0.112
ACE III memory	0.030	0.290
ACE III fluency	− 0.097	0.001*
ACE III language	− 0.020	0.479
ACE III visuospatial	0.031	0.275
Reaction time	− 0.009	0.762
Reading and sentence comprehension	− 0.069	0.022*
Auditory attention	− 0.033	0.272
Visual attention	− 0.012	0.682
Stroop test	− 0.027	0.381
Episodic memory immediate recall	− 0.134	<0.001*
Episodic memory delayed recall	− 0.099	0.004*
Visuospatial span	0.030	0.351
Form matching	− 0.044	0.163
Word comprehension	− 0.027	0.423
Semantic associations	− 0.041	0.194
Fluid reasoning	− 0.024	0.815
Name-face recognition	− 0.042	0.218
Categorical fluency	− 0.149	<0.001*
Letter fluency	− 0.088	0.016*
Vocabulary	0.057	0.143
Construction ability	− 0.077	0.109
Implicit memory	− 0.044	0.262

Table 2. Correlation between Homocysteine levels and cognitive test scores. Spearman's correlation was used. *ACE III* Addenbrooke's cognitive examination III; *p<0.05.

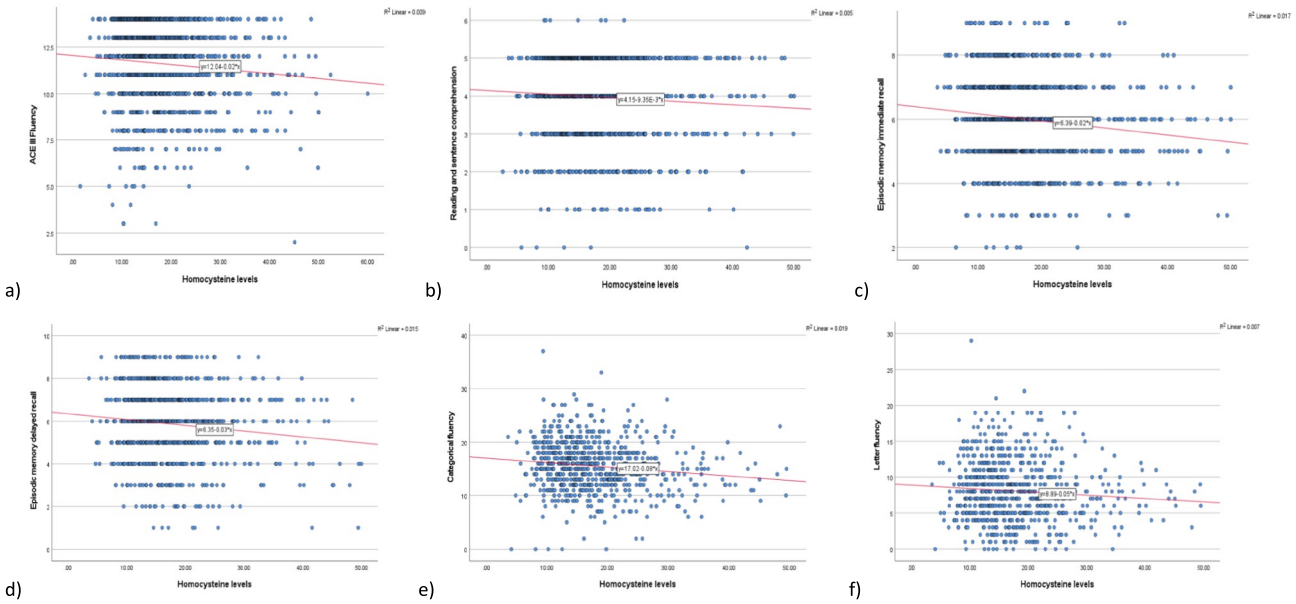

Figure 1. Scatter plots showing relationship of Homocysteine levels with (**a**) ACE III Fluency; (**b**) Reading and sentence comprehension; (**c**) Episodic memory immediate recall; (**d**) Episodic memory delayed recall; (**e**) Categorical fluency; (**f**) Letter fluency ACE III—Addenbrooke's Cognitive Examination III.

GLM analysis was performed with the low Hcygroup as the reference group and cognition as the dependent variable. In the unadjusted model 1, high Hcy was found to be associated with scores in fluency subgroup of ACE III [β (95% CI) − 0.347 (− 0.580, − 0.114); $p = 0.004$], episodic memory immediate recall [β (95% CI) − 0.310 (− 0.474, − 0.145); $p<0.001$], episodic memory delayed recall [β (95% CI) − 0.382 (− 0.616, − 0.148); $p = 0.001$], reading and sentence comprehension [β (95% CI) − 0.197 (− 0.327, − 0.067); $p = 0.003$], categorical fluency [β (95% CI) − 1.175 (− 1.841, − 0.508); $p < 0.001$] and letter fluency [β (95% CI) − 0.757 (− 1.358, − 0.157); $p = 0.013$] tests.

After adjusting for age, gender and years of education, model 2 found a significant association of high Hcy with reading and sentence comprehension task scores [β (95% CI) − 0.162 (− 0.296, − 0.029); $p = 0.034$]. Model 3 identified a significant association of high Hcy with vocabulary task [β (95% CI) − 1.386 (− 2.685, − 0.088); $p = 0.036$]. Model 4, additionally adjusted for dyslipidaemia, found an association of high Hcy with vocabulary task [β (95% CI) − 1.354 (− 2.655, − 0.052); $p = 0.041$] (Table 3).

A GLM adjusted for TIV, age, gender, years of education, vitamin B12 and folate levels found significant associations of high Hcy with total cerebral white matter volume [β (95% CI) − 5617.182 (− 11062.762, − 173.602); $p = 0.043$]. When additionally adjusted for dyslipidaemia, no significant association was found between high Hcy and total white matter and grey matter.

Discussion

Among the study population, 60.5% were classified as hyperhomocysteinemia based on the global cutoffs. This alarming number highlights the importance of studying Hcy as a cardiovascular and cognitive risk factor. It also emphasises the necessity for developing cutoffs that are relevant to the Indian population. The study also identified higher median levels (16.89 µmol/L) of Hcy when compared with other cohorts[29,30]. Studies from Indian population have also described different cutoffs for hyperhomocysteinemia. A study by Bhargava et al.[31], 2023 on people in the age range of 22–75 years showed a lower cutoff (13.5 µmol/L). Another Indian study by Iqbal et al.[32], 2021 studied dementia patients and used a very high cutoff of 22 µmol/L to classify hyperhomocysteinemia. The present study used a median split to compare cognition and brain volumes of people belonging to the low and high Hcy level groups. Although there was an even gender distribution in the population included in the analysis, majority of the participants in the high Hcy group were males (64.5%). Previous studies have also reported higher levels of Hcy in males owing to hormonal factors, increased muscle mass and higher incidence of vitamin B12 deficiency[33]. The high Hcy group also had higher body weight and frequencies of smoking and

	Model 1		Model 2		Model 3		Model 4	
Cognitive parameters	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value	Beta (95% CI)	*p*-value
ACE III total	0.021 (− 0.821, 0.862)	0.962	− 0.047 (− 0.848, 0.754)	0.908	0.171 (− 0.890, 1.232)	0.752	0.159 (− 1.215, 0.898)	0.768
ACE III attention	0.068 (− 0.108, 0.244)	0.447	− 0.072 (− 0.246, 0.102)	0.416	− 0.145 (− 0.386, 0.096)	0.237	− 0.148 (− 0.093, 0.388)	0.228
ACE III memory	0.158 (− 0.172, 0.488)	0.348	0.150 (− 0.176, 0.475)	0.368	0.419 (− 0.043, 0.880)	0.075	0.415 (− 0.876, 0.045)	0.077
ACE III fluency	− 0.347 (− 0.580, − 0.114)	0.004*	− 0.199 (− 0.426, 0.029)	0.087	− 0.081 (− 0.401, 0.239)	0.621	− 0.083 (− 0.236, 0.403)	0.609
ACE III language	0.042 (− 0.159, 0.242)	0.685	0.085 (− 0.119, 0.289)	0.413	0.031 (− 0.238, 0.301)	0.819	0.028 (− 0.295, 0.240)	0.839
ACE III visuospatial	0.100 (− 0.107,0.307)	0.343	− 0.011 (− 0.213, 0.191)	0.917	− 0.053 (− 0.330, 0.224)	0.708	− 0.053 (− 0.224, 0.330)	0.709
Reaction time	− 0.239 (− 5.247, 4.769)	0.925	2.138 (− 2.981, 7.257)	0.413	− 1.831 (− 8.461, 4.798)	0.588	− 1.831 (− 4.798, 0.293)	0.588
Reading and sentence comprehension	− 0.197 (− 0.327, − 0.067)	0.003*	− 0.162 (− 0.296, − 0.029)	0.017*	− 0.055 (− 0.233, 0.122)	0.541	− 0.056 (− 0.121, 0.233)	0.536
Auditory attention	− 0.003 (− 0.145, 0.139)	0.969	− 0.007 (− 0.154, 0.139)	0.921	− 0.025 (− 0.220, 0.171)	0.805	− 0.025 (− 0.170, 0.221)	0.800
Visual attention	0.051 (− 0.167, 0.268)	0.648	− 0.033 (− 0.248, 0.182)	0.764	0.058 (− 0.225, 0.340)	0.689	0.057 (− 0.339,0.225)	0.693
Stroop test	0.190 (− 0.179, 0.559)	0.313	0.112 (− 0.261, 0.484)	0.557	0.202 (− 0.297, 0.702)	0.428	0.198 (− 0.697, 0.301)	0.437
Episodic memory immediate recall	− 0.310 (− 0.474, − 0.145)	<0.001*	− 0.100 (− 0.264, 0.064)	0.233	− 0.098 (− 0.310, 0.115)	0.368	− 0.097 (− 0.115, 0.309)	0.370
Episodic memory delayed recall	− 0.382 (− 0.616, − 0.148)	0.001*	− 0.065 (− 0.298, 0.168)	0.584	− 0.033 (− 0.339, 0.273)	0.833	− 0.027 (− 0.279, 0.031)	0.861
Visuospatial span	0.091 (− 0.063, 0.245)	0.247	0.084 (− 0.075, 0.243)	0.299	0.003 (− 0.209, 0.215)	0.978	0.005 (− 0.217, 0.207)	0.964
Form matching	− 0.072 (− 0.268, 0.124)	0.472	− 0.044 (− 0.244, 0.156)	0.665	0.105 (− 0.157, 0.355)	0.433	0.102 (− 0.363, 0.159)	0.443
Word comprehension	− 0.055 (− 0.184, 0.074)	0.404	− 0.055 (− 0.186, 0.077)	0.417	− 0.065 (− 0.198, 0.069)	0.341	− 0.064 (− 0.069, 0.198)	0.344
Semantic associations	− 0.013 (− 0.103, 0.076)	0.770	− 0.016 (− 0.108, 0.075)	0.729	0.032 (− 0.089, 0.153)	0.606	0.029 (− 0.149, 0.091)	0.638
Name-face recognition	− 0.254 (− 0.528, 0.020)	0.070	− 0.046 (− 0.313, 0.221)	0.734	0.028 (− 0.324, 0.381)	0.876	0.034 (− 0.387, 0.318)	0.848
Categorical fluency	− 1.175 (− 1.841, − 0.508)	0.001*	0.026 (− 0.616, 0.667)	0.938	− 0.126 (− 0.977, 0.726)	0.772	− 0.117 (− 0.735, 0.969)	0.788
Letter fluency	− 0.757 (− 1.358, − 0.157)	0.013*	− 0.388 (− 1.000, 0.224)	0.214	0.156 (− 0.653, 0.965)	0.705	0.168 (− 0.977, 0.641)	0.684
Vocabulary	0.625 (− 0.584, 1.834)	0.311	0.796 (− 0.454, 2.046)	0.212	− 1.386 (− 2.685, − 0.088)	0.036*	− 1.354 (0.052, 2.655)	0.041*
Construction ability	− 0.705 (− 2.217, 0.807)	0.361	− 0.310 (− 1.878, 1.258)	0.698	− 0.080 (− 2.124, 1.964)	0.939	− 0.139 (− 2.186, 1.908)	0.894
Implicit memory	− 0.026 (− 0.147, 0.096)	0.681	0.027 (− 0.101, 0.155)	0.681	0.159 (− 0.018, 0.336)	0.078	0.167 (− 0.010, 0.343)	0.064

Table 3. Association between Hcy-based groups and cognition using generalised linear models. Model 1—Unadjusted; Model 2—Adjusted for age, gender, years of education; Model 3—Model 2 + Vitamin B12 and folate; Model 4—Model 3 + Dyslipidaemia. *ACE III* Addenbrooke's Cognitive Examination III; *$p<0.05$.

alcohol consumption. This can be attributed to the higher proportion of males in the high Hcy group. This can also suggest that smoking and alcohol consumption are associated with an increase in Hcy levels as evidenced previously in literature[34,35].

The high Hcy group was found to have lesser GDS and GAD scores when compared with the normal group. However, previous studies suggest high Hcy to be associated with both depression and anxiety[36,37]. Although we see an unexpected relationship in the current study, it is to be noted that the mean GDS and GAD scores in both groups were in the healthy range. The relationship identified could also be due to the gender differences in Hcy levels, depression and anxiety. Both depression and anxiety are known to be more prevalent in females when compared with males[38,39]. Since the high Hcy group had a higher proportion of males, the average GDS and GAD scores might have been better.

The unadjusted model identified significant relationships of high Hcy with tests of memory and language domains. On adjustment with covariates, only scores in tests of the language domain remained significantly associated with high Hcy levels. Several previous studies suggest Hcy to be associated with cognitive impairment[16,40,41]. However, the effect on language domain has not been reported widely. A study by West et al.[42], 2011 found that high Hcy levels were associated with poorer scores in tests of executive functioning/language. The set of tests included fluency and vocabulary, which were also significantly associated with high Hcy in our study. Another study by Lin et al.[43], 2020 reported a marked decrease in language and executive functions in people with advanced structural and functional atherosclerosis during a 10-year period. Since Hcy can cause atherosclerosis, this study supports the finding of language domain affected in people with high Hcy levels.

Vitamin B12 and folate are involved in the pathways metabolising Hcy to methionine. Deficiency of these vitamins results in hyperhomocysteinemia[44]. The deficiency of vitamin B12 or folate can also have independent direct effects on cognition[45]. To nullify that effect, vitamin B12 and folate levels were additionally used as covariates in GLM. Even in this model, a significant association between vocabulary test and high Hcy was observed. This suggests that there exists a significant relationship of functioning in language domain with high Hcy, independent of vitamin B12 and folate. It is also important to note that vitamin B12 and folate malabsorption can also result in hyperhomocysteinemia. In such cases, supplementation with vitamin B12 and folate may not be effective in reducing the Hcy levels. There have been studies suggesting a significant positive relationship between dyslipidaemia and Hcy levels[46,47]. Dyslipidaemia has also been found to have an important role in causing cognitive decline[48]. A significant association of high Hcy with vocabulary test performance was observed after adjusting for dyslipidaemia too. This implies that high Hcy level has an independent effect on cognitive functioning and dyslipidaemia does not have a role in Hcy-associated cognitive impairment.

From the results, it is seen that people having Hcy levels less than median had better cognitive performance which probably indicates that the high Hcy group can have the abnormal hyperhomocysteinemia phenotype, while the low Hcy group can contain both normal and mildly elevated Hcy levels based on the global cutoff. Then the relationship of Hcy levels and cognitive performance was assessed without taking any cutoffs for classification. Hcy levels were also found to be negatively correlated in tests of language, fluency and memory. Previous studies also report a significant negative correlation of Hcy levels with cognitive performance[49,50].

The study found smaller total cerebral white matter volume in people in the high Hcy group. A previous study by Feng et al.[51], 2013 also found that elevated Hcy levels corresponded to reduced white matter volumes in the brain. In patients with hyperhomocysteinemia, treatment using CerefolinNAC, a combination used for people with mild cognitive impairment and hyperhomocysteinemia, was found to reduce the rate of white matter atrophy[52]. Hcy is seen to affect the white matter by causing endothelial dysfunction which leads to insufficient blood supply to the white matter. Further, it causes inflammatory changes in the white matter microstructure which leads to damage of myelin and subsequently white matter as a whole[53]. This suggests the importance of the effect of Hcy in affecting white matter health. It is also known that lower total cerebral volume corresponds to poorer cognitive functioning and depending on the specific region affected, corresponding cognitive domains are also impacted[54,55]. But when adjusted for dyslipidaemia in addition, no association was found between high Hcy and cerebral white matter volume. This suggests a coexisting role of dyslipidaemia along with high Hcy in affecting the white matter structures in the brain.

Our study did not observe any difference in grey matter volume differences among the high Hcy and the normal groups. This is in accordance with previous literature suggesting that grey matter is comparatively unaffected with elevated Hcy levels[56]. A study by Ford et al.[57], 2012 also found that the effect of Hcy on grey matter was lost after adjusting for demographic factors and comorbidities. This implies that although there are reports of reduced grey matter volume in people with elevated Hcy levels, the effect is majorly explained by other factors and no causal relationship exists between the two.

The finding that vocabulary task performance corresponds to high Hcy levels and reduced white matter volume early in the course of cognitive impairment in middle and old aged adults has important implications in early diagnosis. A simple vocabulary test can be advocated to be used as a bedside screening tool to identify early signs of cognitive impairment. Further studies on this may enable using a combination of Hcy levels and vocabulary test scores to predict cognitive decline, very early in the trajectory.

The study results have important implications for public health. Since the burden of hyperhomocysteinemia is higher in Indians, maintaining optimal Hcy levels becomes very necessary. It is also well known that the Hcy levels in the plasma increase with age. The prevalence of cognitive impairment, in itself, increases with age. Thus, increased hyperhomocysteinemia burden in the elderly population adds to the risk of developing dementia. Owing to these reasons, maintaining healthy levels of Hcy in the blood becomes very important to preserve cognitive functioning in the elderly.

To maintain optimum levels of Hcy, nationwide fortification of cereals with vitamin B12 or folate can be an important strategy. At an individual's level, regularly checking vitamin B12 and folate levels and managing

deficiency using supplements must be advised. This can reduce the risk of cognitive decline and dementia and also greatly improve the quality of life of the elderly.

The major strengths of the study include a large sample size and the utilization of detailed cognitive assessments. Given the scarcity of studies on the association between Hcy and cognitive functioning in the Indian context, the current study assesses the relationship in a large population. The COGNITO neuropsychological battery used in the study measures the performance in various cognitive domains. This enabled the identification of specific domains, particularly language and memory, affected due to high Hcy levels. Another strength of the study is the availability of comprehensive clinical, biochemical and neuroimaging data. In our study, we have also adjusted for several confounding factors thereby identifying the independent effect of Hcy on cognitive functioning.

Some of the limitations of the study are listed. Due to the cross-sectional nature of the study, a causal relationship between high Hcy levels and cognitive impairment and smaller brain volumes could not be analysed. The genetic causes of high Hcy levels, namely MTHFR and CBS gene polymorphisms, could not be explored because of the unavailability of data. Another limitation is the absence of normative cutoffs for hyperhomocysteinemia, specific to the Indian population, due to which the median Hcy concentration was used as the cutoff. We also need to explore the reasons that might have contributed to the lower GDS and GAD scores in the high Hcy group.

Conclusion

The present study identified significant associations of high Hcy levels with cognitive functioning, especially in the language domain, after adjusting for confounding variables. It also identified smaller cerebral white matter volumes in the brain in the high Hcy group. This suggests that structural brain changes can potentially mediate the effect of high Hcy on cognitive impairment. The study highlights the importance of considering Hcy as a risk factor for cognitive impairment and dementia. In the elderly population who are already vulnerable to cognitive decline and dementia, it is necessary to monitor the Hcy levels on a regular basis and maintain healthy levels to reduce the risk of cognitive decline in addition to the risk of cardiovascular diseases.

Data availability

The datasets analysed during the current study are not publicly available as the study is a longitudinal cohort study and is currently ongoing, the data is still being collected and curated and being monitored by the Institutional Ethics Committee (IEC) and Technical Advisory Committee (TAC). Therefore, it is not made public at this point of time. Data request can be directed to the corresponding author Dr. Thomas Gregor Issac who is the PI of TLSA study and data will be shared if approved by the IEC and TAC.

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Author contributions

S.G.—Design of the work, interpretation of data, drafted the work; M.S.—Analysis, interpretation of data, drafted the work; S.S.—Analysis, interpretation of data; A.S.—Drafted the work, substantially revised; L.D.—Analysis, substantially reviewed; T.G.I.—Conception, design of work, interpretation of data, drafted the work, substantially revised.

Competing interests

The authors declare no competing interests.

Additional information

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