Original Article

Factors Determining Cognitive Dysfunction in Cerebral Small Vessel Disease

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ABSTRACT

Introduction: Vascular dementia consists of cognitive and functional impairment due to cerebrovascular brain injury. With reference to small vessel disease (SVD), even though the radiological evidence of SVD is present in a large number of persons above the age of 80 years, less than one-third of the people progress to dementia. Hence, if those factors are identified, we may be able to formulate strategies to protect that percentage of patients who progress to dementia. In this study, we have analyzed some genetic and nongenetic factors in patients with and without a cognitive impairment in the presence of radiological SVD. **Patients and Methods:** Two hundred and ten patients who satisfied the criteria for the study were included. All medical comorbidities, demographic factors, substance abuse, etc., were documented and neuropsychological evaluation done. In addition, the genetic testing was done for the polymorphisms of TT, TC, and CC alleles of CYP11B2 based on the literature evidence of the association of CYP11B2 polymorphism and hypertension. **Results:** This prospective hospital-based study revealed a significant relationship among hypertension, hyperhomocysteinemia, and severity of white matter changes but other comorbidities did not correlate. No significant correlation was seen between cognitive dysfunction and severity of white matter changes or genotypes TT, TC, and CC. However, TC genotype was more common in male hypertensives. Even though hypertension and hyperhomocysteinemia were associated with leukoaraiosis, none of the factors studied trigger conversion of these radiological changes to clinical cognitive impairment. **Discussion and Conclusion:** Severity of cerebral white matter changes seems to correlate with hypertension and hyperhomocysteinemia, however, none of the co-morbidities studied including the three polymorphisms of CYP11B2, that is, TT, TC, and CC seem to determine the conversion of leukoaraiosis to dementia.

Key words: *Cognitive impairment, CYP11B2 gene polymorphism, leukoaraiosis*

INTRODUCTION

Dementia affects 7% of people above 65 years and 30% above 80 years.[1] Vascular dementia is a term used to describe a constellation of cognitive and functional impairment that can be viewed as a subset of the larger syndrome of vascular cognitive impairment associated

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with cerebrovascular brain injury. It is the second most common type of dementia and also contributes to cognitive dysfunction in degenerative dementias.[2] Vascular dementia is the complication of stroke, multi-infarct state, subcortical ischemic small vessel disease (SVD), microbleeds, and lacunes. Poststroke dementia is seen in 30% of stroke patients within a year of the event.[3] It is more as age advances and associated with strategic infarcts, subcortical infarcts, cortical infarcts, etc. Subcortical ischemia without dementia is seen even in relatively young people and increases with age. Etiology is not always clear and probably novel and unrecognized factors are likely to be operating. Depression, minor motor deficits, and urinary incontinence are not uncommon. Dementia is insidious and direct correlation with clinical features and imaging findings are less common. Lacunar infarcts are ischemic infarcts in the distribution of penetrating vessels and of size less than 15 mm. They are commonly located at basal ganglia, thalamus, internal capsule, corona radiata, and brainstem.[4] It accounts for 25% of all ischemic strokes. Asymptomatic ones are 5 times more common than symptomatic, and 5 times more common with increased risk of mild cognitive impairment, dementia, new strokes, and increased long-term risk of death.[5] Micro hemorrhages are defined as rounded foci of less than 5 mm in size that appear hypodense and are distinct from flow voids, hemosiderosis, or mineralization.[6] They are seen in cortico-subcortical junction and the prevalence is variable from the lowest in healthy persons, intermediate in ischemic strokes, and the highest in hemorrhagic strokes.[7] They seem to be related to underlying SVD. Monogenic causes well known with vascular dementias are cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy due to nonamyloid angiopathy and granular osmiophilic inclusion in vascular basal membranes in skin biopsy caused by mutation in NOTCH 3 gene which codes for a large transmembrane receptor. Autosomal dominant amyloid angiopathy is associated with bleeds.[6-10]

Cerebral SVD results from ischemia in the perforating arteries supplying the white matter and deep gray matter nuclei. Tissue elements get damaged based on their vulnerability in the following order neurons, oligodendrocyte, myelinated axon, astrocyte, and endothelial cells.[7] The pathology typically affects the frontal subcortical circuits and causes functional disability mostly with executive functions.

Genetic predisposition has been suggested for predisposition to SVD and its conversion to dementia. A number of genes involved in endothelial regulation have been implicated as risk factors for SVD, but many associations have been found to be inconsistent and not replicable. There is a paucity of data on the role of genetic factors in the etiopathogenesis of cognitive decline associated with SVD. A number of lines of evidence support a pathogenic role of endothelial activation and dysfunction in SVD.

Hypertension is the most important risk factor for SVD. The renin angiotensin aldosterone system (RAAS) is a homeostatic system that affects blood pressure regulation, vasoconstriction, thrombosis, and vessel wall damage and could contribute to SVD. Our aim is to study the associations of gene of the RAAS system (aldosterone synthase CYP11B2 T-344C), to determine its contribution in the development of cognitive decline in magnetic resonance imaging (MRI)-confirmed cerebral SVD. Knowledge in this area may point to future directions of research on processes responsible for the vascular lesions, especially in younger patients.

PATIENTS AND METHODS

A hospital-based prospective study was carried out in the Departments of Neurology and Clinical Psychology. Study period: May 2013-December 2013. From the point of view of ethical issues informed consent was taken for genetic analysis. Inclusion criteria was radiologically confirmed patients with cerebral SVD. A written informed consent was obtained from all participants.

Exclusion criteria included patients with a diagnosis of degenerative dementia, mixed dementia, patients with primary psychiatric disorders, cortical infarction of any size, and a potential source of cardiac embolism, (Adams *et al*. 1993) and large vessel cerebrovascular disease defined as carotid or vertebral artery stenosis >50%.

All participants underwent a standardized clinical assessment. Demographic data including details regarding the risk factors for stroke was recorded. After detailed clinical examination, the clinical data and blood pressure were recorded. Modified Addenbrooke's cognitive assessment scale was applied to all the patients. Hindi mental status examination (HMSE), Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) for the vascular cognitive decline and Hachinski Ischemic Score were carried out.

A volume of 5 ml of venous blood was collected after an overnight fast from the median cubital vein for genetic analysis.

Study design

MRI scans were assessed as follows: Axial T2-weighted images were evaluated. The Fazekas scale was used to score leukoaraiosis as this scale has been shown to reflect the pathological severity of cerebral SVD in a postmortem validation study (Fazekas *et al*., 1993) [Figure 1].

An additional category was included to allow differentiation of more severe cases of leukoaraiosis. Leukoaraiosis was rated as: 1 = absent or mild (equivalent to Fazekas periventricular score <2); 2 = moderate (Fazekas scale 3); 3 = severe (more than half of the hemispheric white matter involved). In addition to the score, the number of lacunar infarcts as follows: 1 = <2 lesions; 2 = 3 ± 5 lesions; 3 = >5 lesions. Separate scores were generated for small (<5 mm maximal diameter) and large (6 ± 14 mm maximal diameter) focal lesions.Cerebral SVD patients were sub-typed according to their scan appearances. Isolated lacunar infarction was defined as at least one focal lesion and a leukoaraiosis score of 1 (absent or mild). Ischemic leukoaraiosis was defined as at least one focal lesion and a leukoaraiosis score of 2 ± 3.

DNA isolation

Blood samples were processed for DNA isolation using phenol-chloroform method.

Segments of CYP11B2 gene were amplified by polymerase chain reaction (PCR) in 20 µl reaction containing 1X reaction buffer, 25 mmol $MgCl_2$, 0.25 mmol/L each deoxynucleotides triphosphates, 10 pmol of each primer, and 0.3 units of Taq polymerase.

PCR reaction time includes initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, primer annealing at 59.1°C for 30 s, and 72°C extension for 40 s. Genotyping of CYP11B2 344 T/C was done using primer GAGATTCCTCACATGGAACCA (forward) and AAGTCCTGCTGGTCTGAGGAT (reverse).

Then 5 µl PCR product (307 bp) was digested using 5 units of restriction endonuclease HaeIII in the supplied buffer for 2 hours. Digested product was analyzed on 2.5% agarose gel. 344T allele lacks an HaeIII site (GGCC) present in −344C allele, so homozygous 344T allele are detected by two fragments of 224 bp and 83 bp bands, homozygous 344C allele are detected by three fragments of 153 bp, 83 bp, and 71 bp bands, and heterozygous TC allele are detected by four fragments of 224 bp, 153 bp, 83 bp, and 71 bp bands [Figures 2 and 3].

Genotyping

The following polymorphisms were tested - Aldosterone synthase *CYP*11B2 T-344C.

Statistical analysis

Alteration in aldosterone synthase was compared with patients with and without a cognitive decline in the presence of varying grades of radiological SVD.

Cognitive assessment

The HMSE, a test devised for people who are illiterate, is a tool developed by the Indo-US Cross-National Dementia Epidemiology Study, the total score if all items are answered is 31. Addenbrooke's Cognitive Examination Revised version (ACE-R): The ACE-R1 is a brief cognitive test that assesses five cognitive domains, namely attention/orientation, memory, verbal fluency, language, and visuospatial abilities. The total score is 100, higher scores indicate better cognitive functioning. Administration of the ACE-R takes, on average, 15 min. Cut-off <88 gives 94% sensitivity and 89% specificity for dementia. Cut-off <82 gives 84% sensitivity and 100% specificity for dementia.

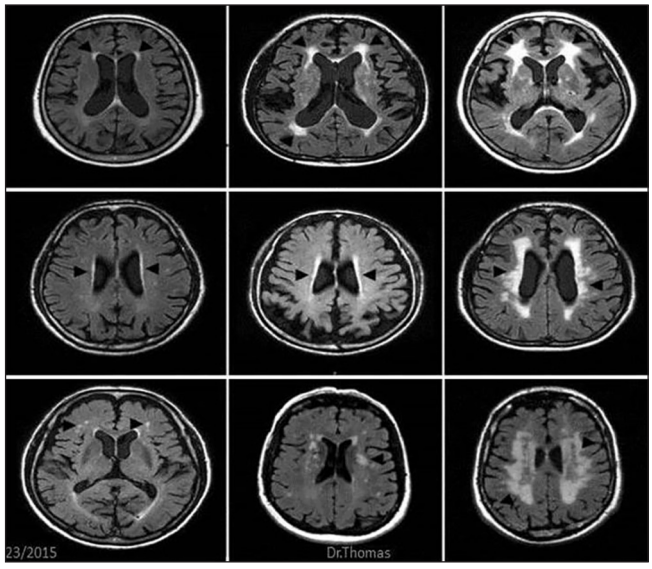

Figure 1: Magnetic resonance imaging showing white matter changes Grade 1, 2, and 3 of Fazekas

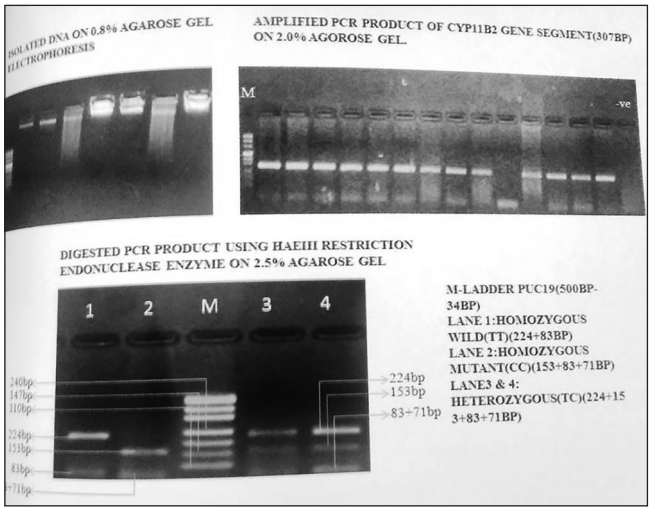

Figure 2: Isolated DNA with amplified polymerase chain reaction product of CYP11B2 segment

RESULTS

Two hundred and ten patients were included in the study. The mean age of SVD patients were 60 years, (range: 48-95 years). A maximum number of patients belonged to the age group of 60-69 years followed by age group of 70 years and more. Approximately, two-thirds of the patients were men (n = 132; 62.9%) and one-thirds were women (n = 78, 37.1%). Maximum numbers of patients were in age group of 60-69 years. Age was a significant contributor to the incidence of SVD, but there was no statistically significant relationship between age and Fazekas grading. There was no statistically significant relationship between gender and Fazekas grading. P = 0.679. The majority of the patients were from the rural area, with low income of 1000 and 3000 Indian rupees/month. Predominant patients had the primary education (77 patients) [Table 1a and b].

A history of smoking was present in 58 patients (27.6%). The majority of the patients were nonsmokers (152 patients). There was no statistically significant relationship between smoking and Fazekas grading.

The history of hypertension was present in 106 patients (50.5%). Nonhypertensives constitute 104 in number. There was a statistically significant relationship between hypertension and Fazekas grading. P = 0.007. The history of diabetes was present in 82 patients (39%). There was no statistically significant relationship between diabetes and Fazekas grading P = 0.145.

The history of hypercholesterolemia was present in 51 patients (24.3%). There was no statistically significant relationship between dyslipidemia and Fazekas grading P = 0.646.

Homocysteinemia was present in 57 patients (27.1%). There was a statistically significant relationship between homocysteinemia and Fazekas grading P = 0.039.

Patients were divided after using the cutoff ≤88 and more than 88 in Addenbrooke's score. Each group included 105 patients. Patients were also divided after using the cutoff ≤81 and more than 81. The first group included 103 patients. The second group included 107 patients.

Patients were divided after using the cutoff ≤23 and more than 23 in HMSE scores. The first group included 105 patients. The second group included 105 patients. There was no statistically significant relationship between HMSE, Addenbrooke's, and Fazekas grading.

Frequency and percentages of various genotypes are TT (87) 41.4%, TC (92) 43.8%, and CC (31) 14.8%. Three polymorphisms TT, TC, and CC were analyzed with respect to the Fazekas scale. There was no statistically significant association between genotypes and Fazekas scoring P = 0.641. Three polymorphisms TT, TC, and CC were analyzed with respect to the Addenbrooke's scale and HMSE. There was no statistically significant association between genotypes and cognitive scoring, and C allele was studied with respect to the radiological scores (Fazekas) and cognitive scoring. There was no statistically significant association among T, C allele with Fazekas, and cognitive scoring [Figures 4 and 5 and Tables 2-8].

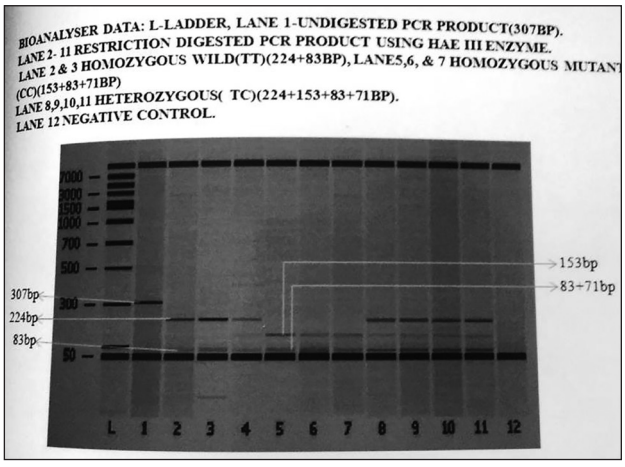

Figure 3: Bioanalyzer data showing undigested polymerase chain reaction product, restriction digested polymerase chain reaction product, homozygous wild (TT), homozygous mutant (CC), heterozygous (TC) of cases, and lane 12 showing the negative control

DISCUSSION

This study included a fairly large cohort of persons with radiological cerebral SVD. They were thoroughly evaluated clinically, neuropsychologically, and the findings were correlated with comorbidities as well as three gene polymorphisms of Aldosterone synthase. The results revealed the significant correlation of leukoaraiosis with hypertension and

Table 1a: Results

Baseline charecteristics	210 Patients
Male: female	132:78
Age	55-86 Yrs
Diabetes mellitus	82
Cholesterol	51
Smoking	58
Homocysteine	57
Hypertension	106
Obesity	32
Inactive lifestyle	57

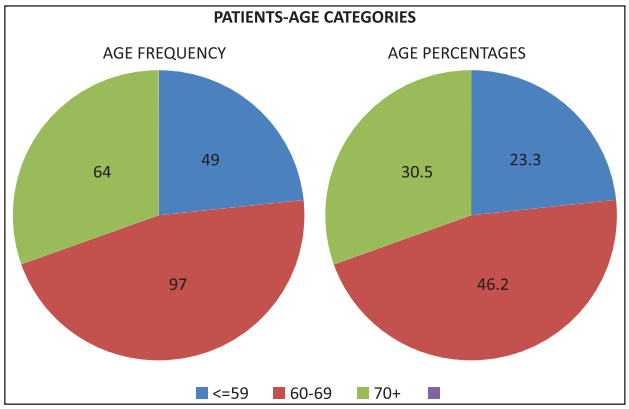

Figure 4: Demographic details

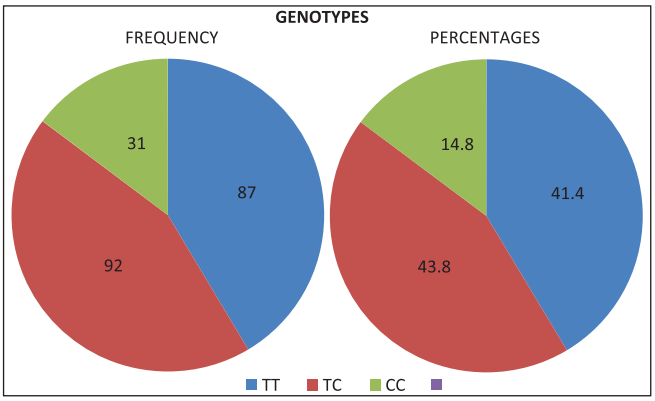

Figure 5: Genotypes, frequency and percentages

Table 1b: Demographic details

Baseline characteristics	Parameter	Frequency	Percentages
Background	Rural:semiurban:urban	98:74:38	46.7:35.2:18.1
Income (Monthly in rupees)	<1000:1000-3000,3000-5000:>5000	70:79:43:18	33.3:37.6:20.5:8.6
Education	Illiterate: primary: college: graduate: post graduate	6:77:67:54:9	3:36.7:34.6:31.44.3

Table 2: Assessment of risk factors with radiological scores (FAZEKAS)

Risk factors	Fazekas grade mild: Moderate: Severe	*P* value
Age	15:51:144	*P*=0.316
Sex (female:male)	4,19,55:11,32,89	*P*=0.679
Smoking	1:13:44	*P*=0.138
Hypertension	8:16:82	*P*=0.007
Diabetes	6:14:62	*P*=0.145
Cholesterol	5:11:35	*P*=0.646
Obesity	2:12:18	*P*=0.166
Homocysteinemia	0:13:44	*P*=0.039

Table 3: Cognitive scores and Fazekas number

Cognitive score	Fazekas grade mild:moderate:severe	*P* value
<=87	4:28:73	*P*=0.151
<=82	4:27:72	*P*=0.186
<=23	4:28:73	*P*=0.151

There was no statistically significant relationship between addenbrookes scoring and Fazekas grading. $P = 0.151$. There was no statistically significant relationship between MMSE and Fazekas grading. $P = 0.151$

Table 4: Genotypes

Genotypes	Frequency	Percentages
TT	87	41.4
TC	92	43.8
CC	31	14.8
Total	210	100

hyperhomocysteinemia, but no factor was identified among the genotypes and other comorbidities as a determinant of progressive cognitive dysfunction in these patients. We could not however include normal controls, the investigators were not blinded to radiological changes, patients with leukoaraiosis and lacunes were not separately grouped.

CONCLUSION

There is a statistically significant relationship between hypertension and homocysteinemia with the severity of white matter changes in MRI imaging. There was no statistically significant relationship among diabetes, hypercholesterolemia, smoking with the severity of white matter changes in MRI imaging and between cognitive scores and severity of white matter changes in MRI imaging. There is no statistically significant association between genotypes TT, TC, and CC and white matter changes in MRI imaging as well as cognitive dysfunction. TC genotype was more associated with male hypertensives. Periventricular white matter changes are more in hypertensives and in patients with hyperhomocysteinemia, but none of the parameters studied seem to trigger the conversion of these radiological changes to clinical cognitive impairment. Hence, the control of hypertension and hyperhomocysteinemia seems to be the single major factor in preventing cerebral SVD and the associated complications.

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Conflicts of interest
There are no conflicts of interest.

Table 5: T and C allele

	Frequency	Percentages
Other	31	14.8
T	179	85.2
Total	210	100
Other	88	41.9
C	122	58.1
Total	210	100

Table 6: *CYP*11B2 T-344C polymorphism with radiological scores (FAZEKAS) frequency

*CYP*11B2 T-344C	Mild	Moderate	Severe	Total
CC	1	7	23	31
TC	9	24	59	92
TT	5	20	62	87
Total	15	51	144	210

There were no statistically significant association between Genotypes TT, TC, CC and FAZEKAS scoring. $P = 0.641$

Table 7: Results

Cognitive assesment	210 Patients
Cognitive decline: Normal cognition	
Addenbruchs <=87:>88	105:105
Addenbruchs <=81:>82	103:107
Mmse (mini mental status examination)	
<=23:>24	105:105

Table 8: *CYP*11B2 T-344C polymorphism with cognitive scores frequency

*CYP*11B2 T-344C	<=87	Normal	Total
CC	17	14	31
TC	38	54	92
TT	48	39	87
Total	103	107	210
***CYP*11B2 T-344C**	**<=81**	**Normal**	**Total**
CC	17	14	31
TC	38	54	92
TT	48	39	87
Total	103	107	210

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