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Vascular Contributions in Alzheimer's Disease-Related Neuropathological Changes: First Autopsy Evidence from a South Asian Aging Population

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Abstract.
Background: Evidence from various consortia on vascular contributions has been inconsistent in determining the etiology of sporadic Alzheimer's disease (AD).
Objective: To investigate vascular risk factors and cerebrovascular pathologies associated in manifestation of AD-related neuropathological changes of an elderly population.
Methods: Postmortem brain samples from 76 elderly subjects (≥50 years) were used to study genetic polymorphisms, intracranial atherosclerosis of the circle of Willis (IASCW), and microscopic infarcts in deep white matters. From this cohort, 50 brains (≥60 years) were subjected to neuropathological diagnosis using immunohistopathological techniques.

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Results: Besides the association with age, the apolipoprotein E ε4 allele was significantly and strongly associated with Thal amyloid-β phases ≥1 [odds ratio (OR) = 6.76, 95% confidence interval (CI) 1.37–33.45] and inversely with Braak neurofibrillary tangle (NFT) stages ≥III (0.02, 0.0–0.47). Illiterates showed a significant positive association for Braak NFT stages ≥IV (14.62, 1.21–176.73) and a significant negative association for microscopic infarcts (0.15, 0.03–0.71) in deep white matters. With respect to cerebrovascular pathologies, cerebral small vessel lesions (white matter hyperintensities and cerebral amyloid angiopathy) showed a higher degree of associations among them and with AD-related neuropathological changes ($p < 0.05$) compared to large vessel pathology (IASCW), which showed a significant association only with Braak NFT stages ≥I ($p = 0.050$).

Conclusion: These findings suggest that besides age, education, and genetic factors, other vascular risk factors were not associated with AD-related neuropathological changes and urge prompt actions be taken against cerebral small vessel diseases since evidence for effective prevention is still lacking.

Keywords: Alzheimer's disease, apolipoprotein E, atherosclerosis, cerebral small vessel diseases, neuropathology

INTRODUCTION

Vascular-derived insults might initiate and/or contribute to neuronal degenerations [1]. Of note, midlife hypertension, diabetes mellitus, apolipoprotein E (*APOE*) ε4 isoforms, hypercholesterolemia, homocysteinemia, smoking, obesity, and, of course, age are vascular risk factors that predispose individuals to Alzheimer's disease (AD) [2–5]; however, interaction between vascular factors and amyloidosis or tauopathy still remains unresolved [3, 6]. Neuropathological examinations reveal that most cases of AD have mixed vascular pathology and small-vessel disease [6, 7]. In addition, brain hypoperfusion/hypoxia, silent infarcts, the presence of one or more infarcts, stroke episodes, and transient ischemic or hypoxic attacks all increase the risk of AD [8–10]; however, underlying mechanism in the pathogenetic processes of AD remains unclear. Cerebral hypoperfusion theory is one of the major theories in determining the etiology of sporadic AD and intracranial atherosclerosis of the circle of Willis (IASCW) has been associated with AD via mechanical obstruction and reduction in cerebral arterial inflow; caused by atherosclerotic lesions [11–14]. However, inconsistencies exist in other investigations [15–17], and the recent hypoperfusion studies developed via postmortem biochemical methods indicate that the main cause for AD is probably via non-structural vascular dysfunctions influenced strongly by amyloid-β (Aβ) rather than structural pathology [18, 19].

Asians, represent approximately 60% of the total world population [20], are identified as high-risk populations as several modifiable vascular risk factors such as intracranial atherosclerosis [21], hypertension [22], diabetes [23], and metabolic syndrome [24] are being increasingly recognized and may contribute to increased incidence of dementia in addition to their genetic predispositions, i.e., risk and protective effect of *APOE* ε4 and ε2 allelic frequencies, respectively [25, 26]. Within Asia, South Asia represents more than 40% of the total Asian and one-fourth (24.8%) of the total world population [20]. To the best of our knowledge, this is the first brain autopsy study undertaken in South Asia that was intended to explore the vascular risk factors and cerebrovascular pathologies associated with AD-related neuropathological changes, since identification of such factors and pathologies may offer new insights into diagnosis and/or early intervention in these populations.

MATERIALS AND METHODS

Sample collection

Consecutive human brain samples were obtained at postmortem examination from 76 older people between May 2009 and March 2010, in the Department of Judicial Medical Office, Colombo South Teaching Hospital. Ethics approval was obtained from the Institutional Scientific Ethics Committee to carry out the study, and informed consent was obtained from the kin to utilize the material for research. The age range was 50–89 years, mean age ± S.D was 67.3 ± 10 years, median age was 65.5 years, the male:female ration was 52:24, and the mean postmortem interval ± S.D was 17.3 ± 14.2 hours. Recruited cases represented a semi-urban population and the final autopsy diagnosis of these

subjects included cardiovascular diseases (45), coronary obstructive airway disease (2), suicide (6), accident (7), pneumonia (3), asphyxia (1), cancer (2), cirrhosis of the liver (1), septicemia/septic shock (3), protracted complicated illness (2), and unknown causes (4). As the samples were collected at post-mortem, detailed psychometric evaluation was not available, but the pathomorphological features were noted. An ante-mortem questionnaire was given to kin who were familiar with intellectual and motor functional status of the subjects before death. The purpose of this questionnaire was to obtain information on demographic data, past medical history, family history, health habits, and consumption pattern of the deceased. This information was held strictly confidential. All the recruited cases had incomplete clinical history except three cases that were clinically diagnosed as Parkinson's disease. Specific neuroanatomical regions were sampled for paraffin embedding and sectioning from both hemispheres: hippocampus along with parahippocampal gyrus, superior frontal gyrus, middle temporal gyrus, superior parietal lobule, midbrain at superior colliculus level, and deep white matters from corpus callosum-major inter-hemispheric white matter tract. Circle of Willis and cadaver blood/clotted blood were also collected at autopsy for the subsequent atherosclerotic and genetic polymorphism studies, respectively.

Screening for neurodegenerative pathologies using histopathological/immunohistochemical techniques

For this purpose, brain samples from 50 out of 76 elderly subjects aged ≥60 years (mean age 72.1 years ± 7.8, mean ± S.D., male:female = 29:21) were used. In the total of 59 cases at the age of ≥60 years, there were 9 cases with incomplete case histories and unavailability of some specific neuroanatomical regions were excluded from this screening. Following routine histological evaluation [hematoxylin and eosin (H&E) staining], brain sections (4 μm thick) were immunostained by standard immunoperoxidase technique following antigen retrieval by heat and DAB/H_2O_2 as the chromogen to visualize the immunolabelling (DAKO Envision Detection System). For this screening, following three antibodies namely (i) amyloid-β – monoclonal antibody (1:200 dilution) from Novacastra™, (ii) ubiquitin – monoclonal antibody (1:150 dilution) from Novacastra™, and (iii) phosphorylated tau– PHF–1 monoclonal antibody (1:50 dilution, a gift) were used. The diagnostic criteria for AD neuropathologic change and Lewy body diseases were based on National Institute on Aging-Alzheimer's Association guidelines-a practical approach (NIA-AA) [27]. Phosphorylated tau and Aβ positive pathologies were graded semi-quantitatively as given below.

1) Semi-quantitative 0–3 scale (0– none, +– low, ++– moderate, +++– high) for tau positive neurons (neurons demonstrating tangle and pre-tangle pathology), neuritic plaques (NPs) and neuropil threads.
2) Semi-quantitative 0–3 scale (0– none, +– sparse, ++– moderate, +++– frequent) for Aβ positive senile plaques (dystrophic neurites and an amyloid core) and diffuse plaques.

Actual burden of AD-related neuropathological changes [neurofibrillary tangles (NFTs) and senile plaques] were counted in specific brain regions such as hippocampus and parahippocampus, superior frontal gyrus, and midbrain based on the methods described by Purohit and colleagues [28].

Due to the high variability of morphological findings and multifactorial pathogenesis of vascular cognitive impairment/vascular dementia (VaD), no generally accepted morphologic scheme for quantitating vascular brain injury and no validated neuropathological criteria for VaD have been established to date [29, 30]. On the whole, the basis of VaD diagnosis is simply the presence of brain lesions related to vascular pathology and it highly depends on neuropathologist's judgment. IASCW was assessed macroscopically based on degree of stenosis of the each circle of Willis component artery [31] and gross visual inspection, and it was graded semi-quantitatively into four levels: none, mild, moderate, and severe. Cerebral amyloid angiopathy (CAA) in leptomeningeal and cortical arteries of the specific neuroanatomical regions was graded semi-quantitatively based on Greenberg and Vonsattel [32] specifications and the average CAA grade was reported for each case. To assess the microscopic infarcts, deep white matter sections (7 μm thick) from both hemispheres were stained with Luxol Fast Blue and Cresyl violet, and graded semi-quantitatively as absent (none or mild) or present (considerable extent at least in one hemisphere). Extent of white matter hyperintensities (WMHs) stained with Luzol fast blue and Eosin, and dilated perivascular spaces stained with H&E were identified in the regions of hippocampus and parahippocampus, middle temporal gyrus, and superior frontal gyrus and reported

semi-quantitatively as absent (none/rare) or present (minimum involvement at least in one region). Hippocampal cell loss in cornu ammonis area 1 (CA1) and dentate gyrus (DG) regions were also assessed semi-quantitatively as absent (none/rare) or present.

Finally, all elderly subjects were screened for polymorphisms in genes encoding for *APOE*, angiotensin converting enzyme (*ACE*), and methylenetetrahydrofolate reductase (*MTHFR C677T*) based on standard protocols using polymerized chain reaction based restriction fragment length polymorphism with cadaver blood deoxyribonucleic acids.

Statistical analysis

For this purpose, statistical software SPSS version 16.0 was used. Due to the small number of samples, each dependent variable was individually tested for each independent variable/factor, controlling for age and sex. Binary logistic regression was used to find the odds ratio (OR) for each factor with a 95% confidence interval (CI). For this purpose, dependent variables were dichotomized primarily based on number of cases that have presented it and then by its severity/topographical progression.

I. CERAD (Consortium to Establish a Registry for Alzheimer's disease) NP scores were divided into none versus CERAD scores A to C (presence of tau positive NPs) as few number of cases presented it (6/50);
II. Thal Aβ phases were divided into none versus phases $\geq$1 (12/50), and phases $\leq$1 versus phases $\geq$2 (8/50) as few number of cases presented it (12/50) and the neocortical Aβ plaques are considered as the best correlate for cognitive impairment in AD;
III. Braak staging was divided into none versus stages I–VI (42/50, presence of tau positive neurofibrillary pathology), stages 0–II (32/50, none to low) versus stages III–VI (18/50, moderate to severe), and stages 0–III (43/50, none to moderate) versus stages IV–VI (7/50, moderate to severe) as it was detected more frequently than tau and Aβ positive plaques, and based on its topographical progression.

Factors including, age, sex, vascular genetic risk factors (*APOE, ACE,* and *MTHFR*), physiological factors (diabetes, high blood pressure or hypertension, high cholesterol, ischemic heart disease, and stroke) and lifestyle factors (level of education, smoking and alcohol consumption) were tested in this analysis. Hierarchical multiple regression adjusted for age and sex was used to find the relationship between continuous dependent variables (mean NFTs counts, total senile plaque counts, total CAA score, and total stenosis score) and dichotomized factors (i.e., presence of *APOE* ε4 allele versus *APOE* ε3 and ε2 alleles, presence of *MTHFR CT/TT* genotype versus *CC* genotype, etc.). Degree of association between AD-related neuropathological stages and cerebrovascular pathologies were assessed using Fisher's exact test (2 × 2 contingency table) and Kendall's tau b correlation coefficient as the sample size is small and the variables are categorical.

RESULTS

Our study consisted of 76 elderly brains aged between 50 and 89 years (mean age 67.3 years ± 10.0; male:female = 52:24) and out of that, 50 elderly brains aged $\geq$60 years were used for neuropathological diagnosis. As per NIA-AA [27] guidelines, in the cases with incomplete clinical history (47 cases out of 50), AD neuropathologic change for intermediate level was 4.25% (2/47) and low level was 19.15% (9/47). Parkinsonism associated pathologies were identified in 8.51% (4/47) of the cases: brainstem predominant Lewy bodies, hallmarks of Parkinson's disease at 6.38% (3/47) and probable progressive supranuclear palsy at 2.13% (1/47). Since the lack of high specificity/high correlation among the studied cerebrovascular pathologies and the non-availability of validated criteria for neuropathological diagnosis of VaD, we could not identify any cases confined to VaD. Within the clinically diagnosed cases (3 cases out of 50), case one presented mixed dementia pathologies including definite progressive supranuclear palsy, intermediate AD neuropathologic change, and brainstem predominant Lewy bodies; case two presented vascular Parkinsonism due to ischemic stroke; and case three presented idiopathic Parkinson's disease at Braak stage III.

Population demographics including allelic frequency of *APOE, ACE,* and *MTHFR C677T* genes, AD-related neuropathological changes and comorbid pathologies are summarized in Table 1. Factors that have demonstrated considerable associations with AD-related neuropathological stages and cerebrovascular pathologies are located in Table 2 (only factors with p values $\leq$0.2 are presented). Age is the strongest risk factor for dementia whether it results from vascular or neurodegenerative origin or both, and in our

Table 1
Population demographics on allelic frequency of *APOE*, *ACE*, and *MTHFR C677T* genes, AD-related neuropathological changes and comorbid pathologies

		Percentage (%)
Allelic frequency	*APOE* ε3 allele	79.86(115/144)
($n=72$; missing cases – 04)	*APOE* ε4 allele	14.58(21/144)
	APOE ε2 allele	5.55(8/144)
	ACE D allele	52.08(75/144)
	ACE I allele	47.91(69/144)
	MTHFR C allele	93.75(135/144)
	MTHFR T allele	6.25(9/144)
AD-related neuropathological changes	Braak NFT stages I–VI	84.0(42/50)
($n=50$)	Braak NFT stages III–VI	36.0(18/50)
	Braak NFT stages IV–VI	14.0(7/50)
	Thal Aβ phases ≥1	24.0(12/50)
	Thal Aβ phases ≥2	16.0(8/50)
	CERAD NP scores A–C	12.0(6/50)
CAA ($n=50$)	grade 0	80.0(40/50)
	grades 1–4	20.0(10/50)
WMHs ($n=50$)	none/rare	68.0(34/50)
	present	32.0(16/50)
Dilated perivascular spaces	none/rare	12.0(6/50)
($n=50$)	present	88.0(44/50)
Lewy bodies ($n=50$)	absent	90.0(45/50)
	present	10.0(5/50)
Hippocampal cell loss in CA1 region ($n=50$)	none/rare	48.0(24/50)
	present	52.0(26/50)
Hippocampal cell loss in DG region ($n=50$)	none/rare	50.0(25/50)
	present	50.0(25/50)
IASCW($n=73$; missing cases – 03)	no	9.59(7/73)
	mild	36.99(27/73)
	moderate	36.99(27/73)
	severe	16.44(12/73)
Microscopic infarcts	none/mild	32.0(24/75)
($n=75$; missing case – 01)	present	68.0(51/75)

n, sample size; *APOE*, apolipoprotein E; *ACE*, angiotensin converting enzyme; *MTHFR*, methylenetetrahydrofolate reductase; AD, Alzheimer's disease; NFT, neurofibrillary tangles; Aβ, amyloid- β; CERAD, Consortium to Establish a Registry for Alzheimer's disease; NP, neuritic plaque; CAA, cerebral amyloid angiopathy; WMHs, white matter hyperintensities; CA1, cornu ammonis area 1; DG, dentate gyrus; IASCW, intracranial atherosclerosis of the circle of Willis.

study, it demonstrated significant associations with Braak NFT stages, CAA, IASCW. and hippocampal cell loss in CA1 region. Besides age, illiteracy, *APOE* ε4 allele. and *MTHFR T* allele also showed significant associations with tau and/or Aβ positive pathologies. IASCW was significantly and strongly associated with *APOE*ε3/ε4 genotype and history of hypertension, and inversely associated with *APOE* ε3/ε2 genotype. Increased risk of microscopic infarcts was significantly and strongly associated with history of diabetes and inversely associated with illiteracy. Hierarchical multiple regression analysis did not show any significant associations between factors and mean NFTs counts or total senile plaque counts in specific neuroanatomical region or in all regions except age and mean NFTs counts ($p<0.000$). Total stenosis score was significantly and strongly associated ($p<0.000$) with history of hypertension, whereas total CAA score was not associated with any factors.

Co-morbid cerebrovascular and neurodegenerative pathologies that are commonly detected in the elderly Sri Lankan brains (Fig. 1), and the degree of association between AD-related neuropathological changes and cerebrovascular pathologies are summarized in Table 3. A higher degree of positive association was observed between AD-related neuropathological changes and both the presence of WMHs and CAA. The presence of CAA and WMHs also showed a higher degree of positive association among them. Whereas IASCW was associated only with Braak NFT stages ≥I ($p=0.050$). The number of cases that have presented cell loss in the DG and CA1 regions of the hippocampus (Table 1) was almost equal but they did not show any significant associations (Fisher's exact test, $p=0.77$) among them. Hippocampus cell loss in

Table 2
Factors associated with AD-related neuropathological changes and cerebrovascular pathologies

Dichotomized variables	Factors	*p* value	Odds Ratio	95% CI
AD-related neuropathological changes (n = 50)				
Braak NFT stages				
stage 0 versus stage I–VI	age	0.005*	1.30	1.08–1.57
	illiteracy	0.184	6.45	0.41–99.93
stage 0–II versus III–VI	age	0.001**	1.22	1.08–1.37
	APOE ε4 allele	0.015*	0.02	0.00–0.47
	illiteracy	0.166	4.37	0.54–35.14
stage 0–III versus IV–VI	age	0.009**	1.22	1.05–1.42
	illiteracy	0.035*	14.62	1.21–176.73
	APOE ε3/ε4 genotype	0.037*	0.05	0.00–0.83
	ACE DD genotype	0.160	0.14	0.01–2.15
Thal Aβ phases				
phase 0 versus phase ≥1	*APOE* ε4 allele	0.019*	6.76	1.37–33.45
	sex	0.063	4.90	0.92–26.14
	hypertension	0.120	0.28	0.06–1.39
phase 0 versus phase ≥2	*APOE* ε3/ε4 genotype	0.038*	7.02	1.11–44.35
	APOE ε4 allele	0.034*	6.10	1.15–32.42
CAA grades (n = 50)				
grade 0 versus grade 1–4	age	0.047*	1.12	1.00–1.23
	MTHFR T allele	0.036*	10.58	1.16–96.22
	ACE DD genotype	0.122	3.93	0.69–22.33
	hypertension	0.186	0.32	0.06–1.73
WMHs (n = 50)				
absent versus present	age	0.052	1.09	1.00–1.18
	APOE ε3/ε4 genotype	0.096	3.87	0.79–19.03
IASCW (n = 76)				
none versus ≥mild	age	0.046*	1.10	1.00–1.22
	*APOE*ε3/ε2 genotype	0.028*	0.03	0.00–0.68
≤mild versus ≥moderate	hypertension	0.000**	15.06	3.27–69.31
	age	0.057	1.05	1.00–1.10
	diabetes	0.176	2.31	0.69–7.75
	high cholesterol	0.172	3.55	0.58–21.93
	ischemic heart disease	0.190	2.25	0.70–7.58
≤moderate versus severe	*APOE*ε3/ε4 genotype	0.038*	4.48	1.09–18.44
	hypertension	0.050*	5.68	1.00–32.18
	sex	0.143	0.39	0.11–1.38
Microscopic infarcts (n = 76)				
none/mild versus present	diabetes	0.028*	10.80	1.29–90.60
	illiteracy	0.017*	0.15	0.03–0.71
	ischemic heart disease	0.084	3.58	0.84–15.16
	alcohol consumption	0.109	6.24	0.66–58.65
	hypertension	0.174	2.53	0.66–9.62

Factors, which were given the *p* values ≤0.2 are only presented. Significant levels are at $^{**}p < 0.01$ and $^{*}p < 0.05$ controlled for age and sex. Reference categories: female sex; *APOE* ε3/ε3 genotype; *APOE* ε3 and ε2 allele; *ACE II* and *ID* genotype; *MTHFR C* allele; history of diabetes- no, history of hypertension- no, history of high cholesterol- no, history of ischemic heart disease- no, alcohol consumption- no, education- ≥primary. n, sample size; *APOE*, apolipoprotein E; *ACE*, angiotensin converting enzyme; *MTHFR*, methylenetetrahydrofolate reductase: AD, Alzheimer's disease: NFT, neurofibrillary tangles: Aβ, amyloid- β; CAA, cerebral amyloid angiopathy; WMHs, white matter hyperintensities; IASCW, intracranial atherosclerosis of the circle of Willis.

the CA1 region was significantly and strongly related to age ($p = 0.001$, odds ratio = 1.2, 95% CI 1.07 to 1.33), whereas cell loss in the DG region was not related to any factors.

DISCUSSION

This is the first brain autopsy study from a South Asian aging population that intended to investigate the vascular risk factors and cerebrovascular pathologies associated with AD-related neuropathological changes. It demonstrates that increasing age, *APOE* ε4 allele, *MTHFR T* allele, and illiteracy are associated strongly with AD-related tau and/or Aβ positive pathologies. It also demonstrates that presence of WMHs and CAA could be considered as potential coexistent cerebrovascular lesions underlying cerebral hypoperfusion mechanism in sporadic AD rather

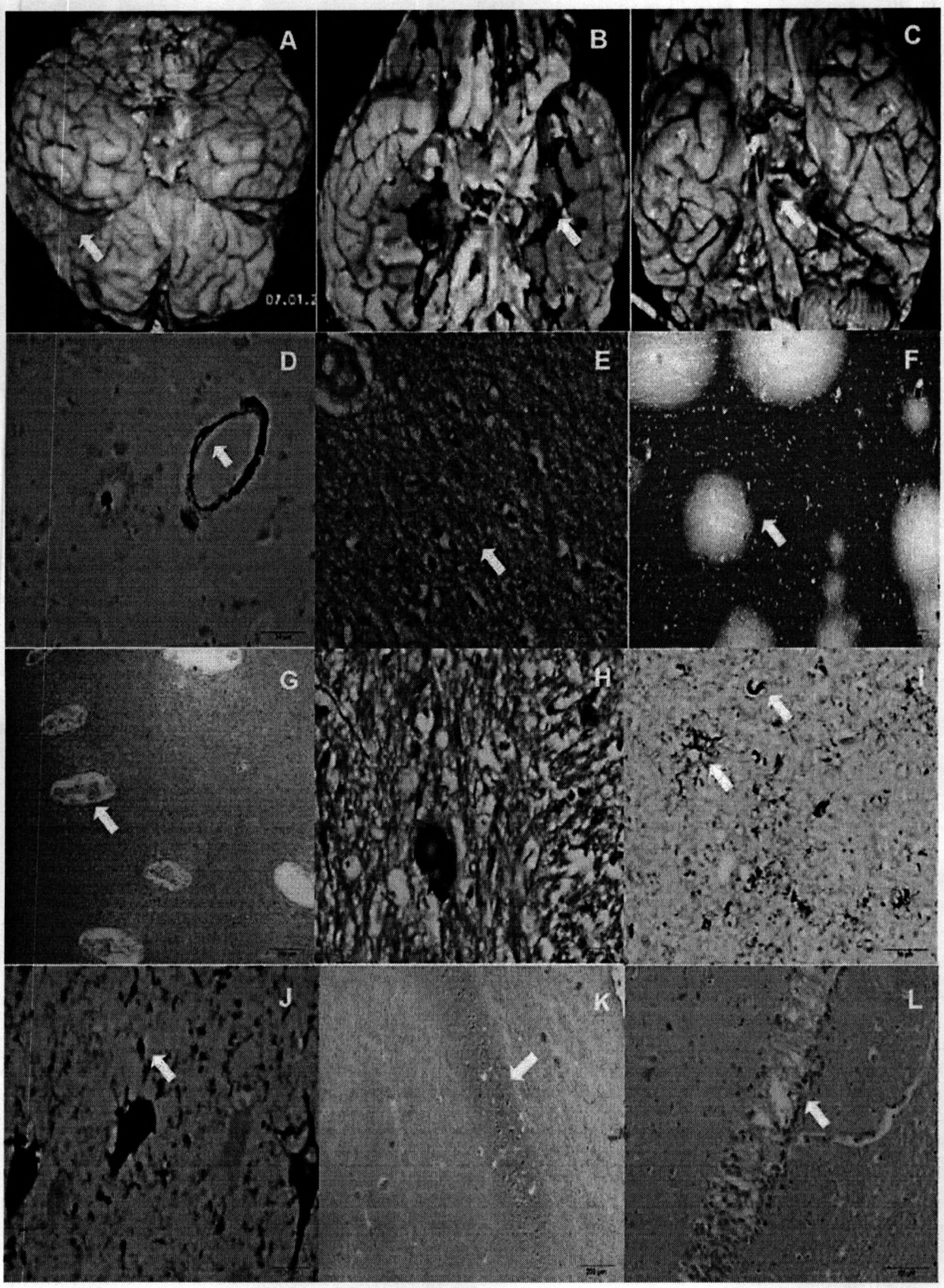

Fig. 1. Co-morbid cerebrovascular and neurodegenerative pathologies (indicated by arrows): ischemic stroke attack at the left hemisphere (A), hypertensive intra cerebral hemorrhage (B), severe intracranial atherosclerosis of the circle of Willis (C), amyloid-β positive cerebral amyloid angiopathy (bar = 50 μm) (D), white matter hyperintensities in superior frontal gyrus with Luzol fast blue and Eosin stains (bar = 50 μm) (E), microscopic infarcts in deep white matters with Luzol fast blue and Cresyl violet stains (bar = 200 μm) (F), dilated perivascular spaces in superior frontal gyrus (bar = 200 μm) (G), ubiquitin positive Lewy bodies in substantia nigra (bar = 20 μm) (H), tau positive tufted astrocytes and coiled bodies in midbrain (bar = 20 μm) (I), tau positive argyrophilic grains in hippocampus (bar = 20 μm) (J) cell loss in hippocampus CA1 region (bar = 200 μm) (K), cell loss in hippocampus dentate gyrus region (bar = 200 μm) (L) (G, K and L with H & E staining).

Table 3
Degree of association between AD-related neuropathological changes and cerebrovascular pathologies

AD-related neuropathological changes	Cerebrovascular pathologies	p value (Fisher's exact test)	Kendall's tau b correlation
Thal Aβ phase 1–5	CAA 1–4	0.001**	0.539
Thal Aβ phase ≥2	,,	0.041*	0.327
CERAD NP score A–C	,,	0.011*	0.431
Braak NFT stage IV–VI	,,	0.616	0.086
Braak NFT stage III–VI	,,	0.138	0.250
Thal Aβ phase 1–5	WMHs (present)	0.040*	0.312
Thal Aβ phase ≥2	,,	0.094	0.281
CERAD NP score A–C	,,	0.011*	0.404
Braak NFT stage IV–VI	,,	0.030*	0.338
Braak NFT stage III–VI	,,	0.009**	0.407
Braak NFT stage I–VI	,,	0.245	0.190
Thal Aβ phase ≥2	IASCW ≥mild	0.421	–0.118
Braak NFT stage III–VI	,,	0.288	0.195
Braak NFT stage I –VI	,,	0.050*	0.382
Thal Aβ phase 1–5	IASCW ≥moderate	0.316	–0.178
Thal Aβ phase ≥2	,,	0.060	–0.287
CERAD NP score A–C	,,	0.381	–0.180
Braak NFT stage IV–VI	,,	0.683	0.118
Braak NFT stage III–VI	,,	0.377	0.147
Braak NFT stage I –VI	,,	0.443	0.118
Thal Aβ phase 1–5	IASCW ≥severe	0.173	–0.252
Thal Aβ phase ≥2	,,	0.322	–0.195
CERAD NP score A–C	,,	0.571	–0.165
Braak NFT stage IV–VI	,,	0.581	–0.180
Braak NFT stage III–VI	,,	0.693	–0.108
Braak NFT stage I –VI	,,	0.581	0.180
Thal Aβ phase 1–5	microscopic infarcts (present)	0.416	0.164
Braak NFT stage IV–VI	,,	0.616	–0.086
Braak NFT stage III–VI	,,	0.463	–0.146
Thal Aβ phase 1–5	dilated perivascular spaces (present)	0.024*	–0.369
Thal Aβ phase ≥2	,,	0.019*	–0.356
CERAD NP score A–C	,,	0.146	–0.242
Braak NFT stage I–VI	,,	0.242	0.175
CAA 1–4	WMHs (present)	0.001**	0.511
,,	IASCW ≥moderate	0.482	0.132
,,	IASCW ≥severe	0.180	–0.224
,,	microscopic infarcts (present)	0.397	–0.125
,,	dilated perivascular spaces (present)	0.327	0.185

Significant levels are at $^{*}p \leq 0.05$ and at $^{**}p \leq 0.01$. P values equal to 0 and 1.0 are removed from this table. Correlation strength: very weak 0.00–0.19, weak 0.20–0.39, moderate 0.40–0.59, strong 0.60–0.79 and very strong 0.80–1.00. AD, Alzheimer's disease; NFT, neurofibrillary tangles; Aβ, amyloid- β; CAA, cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's disease; NP, neuritic plaque; WMHs, white matter hyperintensities; IASCW, intracranial atherosclerosis of the circle of Willis.

than large vessel pathology- IASCW. It is also noted in this study that other vascular risk factors such as hypertension and diabetes are associated with degenerative cerebrovascular pathologies rather than degenerative Alzheimer or Lewy body pathology.

To date, only one clinical study has reported dementia prevalence in elderly Sri Lankans [33], and it was 3.98% (age ≥65 years) including AD at 2.85%, VaD at 0.6%, and the remaining due to other causes. In contrast, our neuropathological findings in elderly Sri Lankans showed a higher prevalence of Parkinsonism associated pathologies (8.51%) followed by AD (4.25%). In low and middle income countries (LMICs), dementia prevalence based on clinical diagnosis is often underestimated [34–36], whereas neuropathological evidences from such populations [28, 37] indicate that actual burden of AD-related pathologies are approximately equivalent between LMICs and high income countries. Therefore, documentation of substantial heterogeneity in dementia prevalence among different counties/ethnicities needs to be investigated on the basis of genetic, environmental, and cultural factors, and the preventive approaches in reducing the burden of dementia [35].

Contribution of vascular risk factors

Genetic factors: APOE, ACE, and MTHFR C677T

The ε4 allele of *APOE* has been found to be the most consistent risk factor in late-onset AD, notably via Aβ protein, whereas associations obtained between *APOE* ε4 allele and NFTs are inconsistent [38–42]. In our study, decedents with 1 or 2 *APOE* ε4 allele demonstrated a significant positive association with Thal Aβ phases ≥1 and in contrast, a significant negative association with Braak NFT stages ≥III controlling for age and sex. While the former is in line with worldwide evidences, the latter differs from the literature. This finding might be related to *APOE* ε4 allele dispersal among the decedents, which shows an age-associated variation in *APOE* ε4 allele frequency that is presumed to be due to survival effect [43]. The majority of the recruited cases died in their 70 s [52% (26/50)] which reflects the average life expectancy of the Sri Lankans, followed by their 60 s [32% (16/50)] and 80 s [16% (8/50)], and therefore a higher number of *APOE* ε4 alleles was detected in those aged in their 70 s (6/13), followed by ≥60 year olds (5/13) and ≥80 year olds (2/13). In addition to that, our findings also suggest that there is an age associated increase in risk of NFTs, an *APOE* ε4 allele associated increase in risk of Aβ plaques, and the survival probabilities of *APOE* ε4 allele carriers could possibly be a one of the reasons for the discordance between Braak and CERAD scores which have been discussed in population based studies [44, 45]. Frequency of the *APOE* ε4 allele is an important genetic risk factor for explaining ethnic differences [25] and the ε4 allele frequency seems to be higher in elderly Sri Lankans compared to the general frequency in Asians (14.6 versus 9.0%) [46]. Therefore, *APOE* genotypes and their survival probabilities in different ethnic populations could possibly be a one of reasons for the differences observed in AD prevalence and needs to be confirmed through large scale patho-genetic studies, across a large range of ethnicities.

Elderly subjects with the *APOE* ε3/ε4 genotype also demonstrate a strong association with severe IASCW as opposed to the *APOE* ε3/ε2 genotype, which showed a protective effect against the presence of IASCW [47]. In addition to *APOE*, we also noted a significant association between *MTHFR T* allele and sporadic CAA. Plasma total homocysteine has considered as a major vascular risk factor [2] and *MTHFR* is the central enzyme involved in homocystenine metabolism particularly *TT* genotype or *T* allele is associated with high homocysteine levels. Hyperhomocysteinemia is suggested to increase intima-media thickness which in turn damages the micro-vasculature, leading to neuronal damage or death [48]. However, *MTHFR* polymorphism (*C677T*) as a risk factor for CAA remains unclear and needs further studies in the future that may offer new therapeutic implications, e.g., folic acid supplementation. Effects obtained with *ACE* polymorphisms were not statistically significant either for AD-related changes or cerebrovascular pathologies.

Physiological factors: Diabetes and hypertension

We obtained strong associations between history of hypertension and moderate and severe IASCW, and between history of diabetes and microscopic infarcts in deep white matter. However, associations obtained for AD-related neuropathological changes were not statistically significant. Our findings are in line with previous studies [49, 50] and suggest that metabolic dysfunctions (diabetes and hypertension) could contribute to dementia via vascular degenerations rather than AD-related changes.

Lifestyle factors: Education, smoking, and alcohol consumption

Accumulating evidence in population-based studies has emphasized the association between education and reduced risk of dementia, and the real effect is explained via two hypotheses: "brain reserve" and "brain battering" [51, 52]. A previous dementia prevalence study in elderly Sri Lankans [33] has also demonstrated that illiteracy is strongly associated with clinical dementia in addition to greater age and female gender. However, there have been no autopsy verification studies representing LMICs that confirm the association between education and reduced risk of dementia pathology. In this study, we found a strong association between illiteracy and both increased risk of neurofibrillary degenerations and reduced risk of microscopic infarcts. Increased risk of neurofibrillary degenerations in illiterates is consistent with the "brain reserve hypothesis", whereas reduced risk of microscopic infarcts may be ascribed to their low level of exposure to cerebrovascular risk factors such as smoking, hypertension, diabetes, and obesity [53, 54]. Moreover, effects seen with smoking or alcohol consumption were not found to be statistically important. As far as smoking/alcohol consumption is concerned, it could be that there is a small effect

that cannot be detected with statistical significance in a relatively small sample, but it may also be attributable to cultural factors that may attenuate the overall effects given that Sri Lankan women are generally fairly conservative in their smoking/alcohol consumption habits.

Contribution of cerebrovascular pathologies

Apart from vascular risk factors, contribution of cerebrovascular pathology to the burden of sporadic AD has received increasing interest in recent decades. However, autopsy evidence which rely on IASCW, a major cause for cerebral hypoperfusion, are found to be inconsistent [15–17] and the recent studies indicate a strong relationship between white matter disease (MRI-based) and AD type pathology (Braak score, CERAD score, and a composite AD pathology score) that would explain in part the relationship between cerebral small vessel disease and cognition [55, 56]. There are several studies that have attributed the relationship between white matter disease and cognition to coexisting atherosclerosis [11–13]; however, it is not the case in all neuropathological studies [55]. Our findings are congruent with those of Moghekar et al. [55] and to a certain extent with both Roher et al. [11] and Luoto et al. [15]. Histopathologically assessed WMHs showed a higher degree of association with all AD-related neuropathological changes (Braak NFT, CERAD NP and Thal Aβ scores), whereas CAA is related only with plaques (CERAD NP and Thal Aβ scores) and IASCW is associated only with tangles (Braak NFT score). Our study also demonstrates a strong association between WMHs and CAA, which supports the previous studies that have suggested WMHs as a feature of sporadic CAA [57–59]. However, IASCW was not associated with any of the cerebrovascular pathologies studied in contrast to studies that have ascribed white matter disease to coexisting atherosclerosis [11–13]. Further, as per Luoto et al. [15], it is also arguable that a significant association noted between Braak NFT stage (I-VI) and IASCW (≥mild) grades of our cohort may be attributed to the effect of age and not due to a pathologically direct relationship as other grades of Braak NFT score or IASCW did not show any significant associations.

While this is the first autopsy verification study of an aging population from South Asia, we acknowledge there are limitations to this study such as sample size, incomplete clinical history of the recruited samples lacking objective psychometry and dementia scores, reliability of the case histories obtained through kin, ubiquitin immunohistochemistry for labeling Lewy bodies instead of α-synuclein, histopathological assessments of WMHs instead of radiological examinations, semi-quantitative assessments of certain comorbid pathologies lacking exact quantifications and the non-availability of some brain regions as specified under minimum tissue requirements by NIA-AA guidelines.

Concisely, our findings suggest that besides age, education, and genetic factors (*APOE* and *MTHFR*), other vascular risk factors were not associated with AD-related neuropathological changes. It further confirms the strong contribution of cerebral small vessel diseases in AD-related changes, contributes to the uncertainty surrounding the true effect of IASCW/large vessel disease on AD-related changes, and urge prompt action be taken against cerebral small vessel diseases possibly via therapeutic agents that contains both anti-amyloidogenic and anti-atherosclerotic properties as a preventative strategy for subsequent neurodegenerative disease.

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