

Cytoskeletal Pathologies of Age-Related Diseases between Elderly Sri Lankan (Colombo) and Indian (Bangalore) Brain Samples

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Abstract: Within South Asia, Sri Lanka represents fastest aging with 13% of the population was aged over 60's in 2011, whereas in India it was 8%. Majority of the Sri Lankan population based genetic studies have confirmed their origin on Indian mainland. As there were inadequate data on aging cytoskeletal pathologies of these two nations with their close genetic affiliations, we performed a comparison on their elderly. Autopsy brain samples of 50 individuals from Colombo, Sri Lanka (mean age 72.1yrs \pm 7.8, mean \pm S.D.) and 42 individuals from Bangalore, India (mean age 65.9yrs \pm 9.3) were screened for neurodegenerative pathologies using immunohistochemical techniques. A total of 79 cases with incomplete clinical history (Colombo- 47 and Bangalore- 32) were subjected to statistical analysis and 13 cases, clinically diagnosed with dementia and/or Parkinsonism disorders were excluded. As per National Institute on Aging- Alzheimer's Association guidelines, between Colombo and Bangalore samples, Alzheimer's disease neuropathologic change for intermediate/ high level was 4.25% vs. 3.12% and low level was 19.15% vs. 15.62% respectively. Pathologies associated with Parkinsonism including brainstem predominant Lewy bodies- 6.4% and probable progressive supra nuclear palsy- 2.13% were found solely in Colombo samples. Alzheimer related pathologies were not different among elders, however, in Colombo males, neurofibrillary tangle grade was significantly higher in the region of hippocampus (odds ratio = 1.46, 95% confidence interval = 0.07-0.7) and at risk in midbrain substantia nigra (p = 0.075). Other age-related pathologies including spongiform changes (p < 0.05) and hippocampus cell loss in dentate gyrus region (p < 0.05) were also identified prominently in Colombo samples. Taken together, aging cytoskeletal pathologies are comparatively higher in elderly Sri Lankans and this might be due to their genetic, dietary and/ or environmental variations.

Keywords: AD neuropathologic change, cell loss, immunohistochemistry, parkinsonism, south asia, spongiform changes.

INTRODUCTION

Dementia is a growing public health challenge. Population aging is having a profound impact on dementia epidemic. Alzheimer's disease (AD) is the most common form of dementia and possibly contributes 60-70% of the cases [1]. Other major contributors include vascular dementia (VaD), Lewy body diseases (LBDs) and a group of diseases that contribute to frontotemporal dementia (FTD). The

boundaries between subtypes are indistinct, thus pure forms are quite rare. South Asia (23.2% of the world total population) is considered as one of the global burden of disease regions and about 4.5 million people lived with dementia in the year of 2010 [2, 3]. Among South Asian countries, Sri Lanka is continuously experiencing fastest ageing due to its rapid demographic transition (low fertility and higher life expectancy) and the proportion of Sri Lankan population aged over 60 years was 13% in 2011, whereas it was 8% in India, 7% in Bhutan, Bangladesh and Maldives, 6% in Nepal and Pakistan and 4% in Afghanistan [4]. Among them, low income countries are Afghanistan, Bangladesh and Nepal and lower-middle income countries are India, Sri Lanka, Pakistan and Bhutan and upper middle income country is Maldives [4]. About 58% of the people affected with demen-

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tia lived in low and middle income countries (LMICs) in 2010 [1] and this proportion is anticipated to rise to 63% in 2030 and 71% in 2050 [5].

Within South Asia, India is the largest populous country with over 1.2 billion people in the year of 2011, where elderly population aged over 60 years contributes around 99.3 million. Whereas, Sri Lanka remains around 21.0 million people in 2011, where elderly portion aged over 60 years is around 2.7 million [4]. Indian economy is one of the fastest growing economies of the world. Even though, due to its population explosions, demographic and socioeconomic parameters are still behind that of Sri Lanka and the statistics are as follows: Sri Lanka vs. India: life expectancy rate at birth and at 60 years - 75 years vs. 65 years and 20 years vs. 17 years in 2011; total fertility rate per woman - 2.3 vs. 2.6 in 2011; literacy rate among adults aged ≥ 15 years - 91% vs. 63% for a period of 2005 to 2011; living in urban areas - 15% vs. 31% in 2011; gross national income per capita (purchasing power parity int. \$) - 5520 vs. 3590 in 2011 and per capita government expenditure on health at average exchange rate (US\$) - 16 to 37 vs. 5 to 14 for a period of 2000 to 2011 [4]. Historically and culturally these two nations have been considerably close [6] and majority of the Sri Lankan population genetic studies have confirmed their origin on Indian mainland. However, such genetic studies looking at the immediate origin of Sinhalese (Sinhalese constitute 74.9% of the total Sri Lankan population) have been contradictory [7-10]. The most recent human genetic study [11] on Sri Lankan ethnic population based on mitochondrial DNA hyper variable segments for the genotyping, suggests that all island populations except some subgroups of the Vedda (indigenous people) form close genetic affiliations among themselves and with majority of the group from Indian subcontinent suggesting the origin on Indian motherland. However, no definite association of the Sinhalese with any specific ethnic or linguistic groups of Indian was identified; thus, their exact immediate origin on the motherland remains yet to be investigated.

Prevalence of dementia in Sri Lanka, reported from a semi-urban elderly Sinhala-speaking population (3.98%), is higher by comparing community based clinical studies from North India (2.7%) [12,13]. The prevalence of neuropathologically defined AD is higher than that reported in most previous studies based on clinical diagnosis alone. Epidemiological/ clinical studies investigating dementia prevalence face several barriers including lower literacy levels, lack of awareness on cognitive loss and memory problems and the dependent life style, where the activity of daily livings are highly limited to elderly people. Due to these reasons, results of clinical studies may not provide accurate estimates of prevalence and incidence of dementia. The discrepancy between the neuropathologic and clinical diagnoses of AD in elderly subjects, may affect the results of population-based studies [14], and thus, there is no alternative to neuropathology as the gold standard in dementia diagnostics. So far, neuropathologic changes related to AD and other dementia types have not been reported in the elderly Sri Lankans and there have been no direct comparative clinical and/ or autopsy studies between Sri Lankan and Indian elderly samples reported these pathologies. As there were inadequate

data on aging cytoskeletal pathologies of these two nations with their close cultural and genetic affiliations, we performed a direct comparison between their elderly samples.

MATERIALS AND METHODS

Human brain samples were obtained at autopsy from 50 elderly Sri Lankans between May 2009 to March 2010 (age range 55-89yrs; mean age \pm S.D. = 72.1yrs \pm 7.8, male : female = 29:21, Sinhalese : Tamil = 45:5), at the Department of Judicial Medical Office, Colombo South Teaching Hospital following approval by the Institutional Scientific Ethics Committee to carry out the study, and with informed consent from the relatives. Representative brain samples were obtained from a semi urban population (Colombo South) with majority of Sinhalese people (90%). 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 and midbrain at the superior colliculus level. For the comparison, autopsy brain samples from 42 elderly Indians from a semi urban Bangalore population (age range 50-89yrs; mean age \pm S.D. = 65.9yrs \pm 9.3, male: female = 30:12) were obtained at the Human Brain Tissue Repository - National Institute of Mental Health and Neurosciences [HBTR-NIMHANS], Bangalore, India. In the total of 92 cases, there were 13 cases clinically diagnosed as dementia and/or Parkinsonism (3 samples from Colombo with Parkinson's disease (PD) and 10 samples from Bangalore: Parkinsonism- 7, AD- 2 and FTD- 1) and the remaining (47 Colombo samples and 32 Bangalore samples) had incomplete clinical history. Neuropathological screening for all the recruited samples was carried out in the host laboratory - NIMHANS, thus both Colombo and Bangalore samples were subjected to same methodological procedures and diagnostic criteria.

Following routine histological evaluation (with Haematoxylin and Eosin staining), the formalin fixed paraffin embedded brain sections were immunostained by standard immunoperoxidase technique following antigen retrieval by heat and DAB/H₂O₂ as the chromogen to visualize the immunolabelling (DAKO Envision Detection System). For this purpose, following three antibodies were used.

- (a) β amyloid- Monoclonal antibody (1:200 dilution) from NovacastraTM
- (b) Ubiquitin- Monoclonal antibody (1:150 dilution) from NovacastraTM
- (c) Phosphorylated tau- PHF-1 Monoclonal antibody (1:50 dilution) from USA (A gift)

The presence and extent of neurodegenerative pathologies were assessed in these stained sections blinded to the case histories under the direct supervision of two neuropathologists SKS and YTC. Final decisions were made jointly. The diagnostic criteria for "AD neuropathologic change" were based on National Institute on Aging-Alzheimer's Association guidelines for the neuropathological assessment of AD (NIA-AA) [15] a practical approach which included:

- Consortium to Establish a Registry for AD (CERAD) protocol for neuritic plaque (NP) scoring [16],

- Braak and Braak staging scheme for neurofibrillary degeneration [17, 18] which was adapted to four stages that improves inter-rater reliability (stage 0, stage I or II, stage III or IV and stage V or VI) [19] and

- A modified version of Thal phases of β amyloid ($A\beta$) plaque accumulation [20] which was adapted to four point scale (phase 0, phase 1 or 2, phase 3 and phase 4 or 5).

AD neuropathologic change was ranked by three parameters: $A\beta$ plaque score [20], Braak and Braak neurofibrillary tangle (NFT) stage [18], and CEARD NP score [16] to obtain ABC scores and then transformed into 4 levels: not, low, intermediate or high. Cerebral amyloid angiopathy (CAA) grades were not included in the ABC scores although amyloid buildup on the walls of the arteries was frequently observed along with cortical $A\beta$ plaques. Neuropathological criteria for LBDs including PD and dementia with Lewy bodies (DLB) were based on NIA-AA modifications to existing criteria which included Consortium on Dementia with Lewy Bodies 2005 (CDLB05) [21] and Braak's PD staging [22], and also based on the reconsidered staging procedure for the inclusion body pathology associated with sporadic PD [23].

Semi quantitative grading [24] of phosphorylated tau positive and $A\beta$ positive pathologies were performed in the regions of hippocampus along with parahippocampal gyrus, midbrain and in the neocortex as given below;

- 1) Semi-quantitative 0-3 scale (0- none, +- low, +- moderate, +++- high) for tau positive neuropil threads, neurons (neurons demonstrating tangle and pre-tangle pathology), NPs and white matter neuropil threads.
- 2) Semi-quantitative 0-3 scale (0- none, +- sparse, +- moderate, +++- frequent) for $A\beta$ positive senile plaques (SPs) (dystrophic neurites and an amyloid core) and diffuse plaques (DPs).

Actual burden of AD neuropathological hallmarks- tau positive NFTs, NPs and $A\beta$ positive SPs were counted in specific brain regions such as hippocampus, entorhinal cortex, superior frontal gyrus and midbrain based on Purohit *et al.* [25] study. For this purpose, a medium high power (20X) objective lens producing a visual field 0.785mm² (field diameter = 2.0mm) was used. Lesions were counted in medium high (200X magnification, Olympus U-CTR30-2 Trinocular objective tubes and 10X eye piece) power fields and then converted into average per 200X as follows: for superior frontal gyrus, areas with high NPs/SPs/NFTs were selected and visual counts were carried out in five non overlapping fields. For hippocampus, entorhinal cortex and midbrain, the areas with high NPs/SPs/NFTs were identified in each sub fields and then visual counts were carried out in the non over lapping fields (wherever possible five non overlapping fields were selected). CAA in leptomeningeal and cortical arteries was graded into 5 levels (grade 0, 1, 2, 3 and 4) based on Greenberg and Vonsattel [26] specifications and the average grade was reported for each case. The presence of other cerebrovascular changes including dilated perivascular spaces, spongiform changes of neuropil, and white matter lesion leukoariosis in frontal and temporal cortex regions and the hippocampus cell loss in Cornu Ammonis area 1 (CA1) and dentate gyrus (DG) regions were assessed

using H & E stained sections. Luxol fast blue and Eosin stained sections were used for the assessment of leukoariosis. Other than CAA, comorbidity of the above pathologies was reported as present or absent (none or rare). Due to the high variability of morphological findings and multifactorial pathogenesis of VaD, no generally accepted morphologic scheme for quantitating vascular brain injury and no validated neuropathological criteria for VaD have been established to date [27-29]. On the whole, the basis of VaD diagnosis is simply the presence of brain lesions related to vascular pathology and highly depends on neuropathologist's judgment.

STATISTICAL ANALYSIS

To compare elderly samples, a total of 79 cases (Colombo- 47 and Bangalore- 32) with incomplete clinical history were subjected to statistical analysis and excluded 13 clinically diagnosed cases with dementia and/ or Parkinsonism disorders. Analysis was performed with statistical software SPSS version 16.0. Hierarchical multiple regression adjusted for age and sex was used to determine the odds ratios (OR) with a 95% confidence interval (CI) for Colombo and Bangalore samples for different AD related neuropathological scores in different brain regions. Binary logistic regression adjusted for age and sex, was used to determine the OR with a 95% CI for the presence of co-morbid pathologies between elderly samples. Degree of association among these age-related pathologies was determined using Fisher's exact test (2X2 contingency table) and Kendall's tau b correlation coefficient as the sample size is small and the variables are categorical.

RESULTS

Our study included 92 brains of deceased persons aged 50 to 89yrs (Colombo50 and Bangalore- 42) and their mean age at death is almost equivalent to their standard population life expectancy. In the cases with incomplete clinical history (79 cases), about 87% (41/47) of the Colombo and 62.5% (20/32) of the Bangalore sample brains showed some degree of Alzheimer related pathology at death ($p = 0.230$, $OR = 2.15$, $95\% CI = 0.6-7.5$, reference category: Bangalore samples). Moderate to severe neurofibrillary degenerations *i.e.* extended to limbic and neocortical regions (Braak stage III to stage VI) were observed in 34% of the Colombo and 18.75% of the Bangalore samples. Thal $A\beta$ phases for SPs in neocortical and allocortical regions (phase 1-2) were observed in 21.3% of the Colombo and 15.6% of the Bangalore sample. Thal $A\beta$ phase extended to midbrain nuclei was found in single cases in both samples. Tau positive NPs in neocortical regions were comparatively lower than $A\beta$ positive SPs in both groups and it was 10.63% in Colombo sample and 6.25% in Bangalore sample. Between Colombo and Bangalore samples, AD neuropathologic change for "intermediate/high" level was 4.25% and 3.12% respectively. For individuals with incomplete clinical history, AD neuropathologic change for higher levels is considered as greater likelihood for cognitive impairment [15] and found in one Bangalore sample. AD neuropathologic change for low levels, although they are considered as inadequate explanation for cognitive impairment or dementia [15] were 19.15% in Colombo samples and 15.62% in Bangalore samples. Sam-

ple summary consist of AD, CAA and LBD neuropathological changes are given in (Table 1).

Neuropathological findings on 13 clinically diagnosed cases are given in (Table 2) and the majority showed clinicopathological correlations. A variety of neurodegenerative pathologies including AD, PD, progressive supra nuclear palsy (PSP), diffuse Lewy body disease (DLBD), frontotemporal lobar degeneration taupathy and CAA were observed in those brains and are illustrated in (Fig. 1).

Moreover, we have summarized the AD related neuropathological scores of our study and the scores obtained for elderly Mumbai and New York samples from Purohit *et al* autopsy study [25] in (Table 3). This table facilitates the comparison on AD related neuropathologic scores among LMICs and between LMICs and high income country (HIC).

Mean counts of both, NFTs and SPs (count/mm²) among different age groups were plotted against specific neuro-anatomical regions: entorhinal cortex, hippocampus, superior frontal gyrus and midbrain in (Fig. 2) and (Fig. 3) to show their involvement during aging. Overall pattern of their involvement follows the Braak and Braak staging scheme for NFTs and Thal A β phases for SPs accumulation.

Between the males of both population, mean NFT grade was significantly different (mean 1.05, standard error of mean (SEM) = 0.13 vs. 0.34, SEM = 0.07, $p = 0.019^*$, odds ratio (OR) = 1.46, 95% CI = 0.07-0.7) in hippocampus and at risk (mean 0.81, SEM = 0.09 vs. 0.25, SEM = 0.13, $p = 0.075$, OR = 1.39, 95% CI = -0.03-0.7) in midbrain nigral neurons with hierarchical multiple regression adjusted for age, and it was prominent in Colombo samples. Apart from above findings, AD related neuropathological scores (grades and counts) between elderly samples, between sex and between the females of both population were not different in any brain region. (Fig. 4) shows the mean grades of AD related pathologies between Colombo and Bangalore samples and it was not significantly different among them. Tau positive pathologies were comparatively higher in Colombo samples, whereas A β positive pathologies were prominent in Bangalore samples.

Furthermore, A β positive CAA pathology in cortical and leptomeningeal arteries was found in 19.15% of the Colombo and 9.37% of the Bangalore samples. Brainstem predominant Lewy bodies were observed in 6.4% of the Colombo samples and possibly at stage 3 [23]. Meanwhile, none of the Bangalore samples showed Lewy body pathology at death. Pathology associated with PSP (tufted astrocytes and globose tangles in the midbrain) was observed in one Colombo sample (2.13%) and due to its incomplete clinical history; it was diagnosed as probable PSP. Other age-related pathologies and their extent between elderly samples are given in percentage in the following (Table 4) with p values. Among them, spongiform changes ($p = 0.000$, OR = 8.75, 95% CI = 2.8-27.6) and granular cell loss in DG region ($p = 0.009$, OR = 5.65, 95% CI = 1.5-20.6) were found prominently in Colombo samples.

Degree of associations among these age-related pathologies are given in (Table 5) and the majority showed a significant relationship with positive correlations.

DISCUSSION

While there is voluminous literature in the West, there is glaring paucity of data on age associated pathomorphological changes in the brain in Sri Lankan population. This is the first autopsy study in Sri Lanka (Colombo) and the first direct comparative study between Sri Lankan and Indian (Bangalore) elderly samples that investigated the extent of age related cytoskeletal pathologies. In overall, 87% (41/47) of the Colombo sample and 62.5% (20/32) of the Bangalore sample show some degree of cytoskeletal pathology at death but it was not significantly different ($p = 0.23$). Neuropathological diagnosis indicated that AD related pathologies obtained from a semi urban Colombo samples are remarkably similar to that in the brains obtained from a semi urban Bangalore samples. Meanwhile, presence of spongiform changes and granular cell loss in the DG region are higher ($p < 0.05$) in Colombo samples after controlling age and sex. Moreover, pathologies associated with Parkinsonism including PD- 6.4% (3/47) and probable PSP- 2.13% (1/47) were found only in Colombo samples.

To date, there is only one study reported the dementia prevalence from Sri Lanka [12]. This study was performed in a semi urban Sinhala speaking elderly population (>65yrs) based on clinical diagnosis (Diagnostic and Statistical Manual of Mental Disorders IV: DSM-IV). All kinds of dementia were 3.98% (95% CI = 2.6-5.7) including AD 2.85%, VaD 0.6% and the remaining due to other causes of dementia. Dementia prevalence in India (>65yrs) based on clinical diagnosis (DSM-III, DSM-IV criteria) are as follows: all dementia 2.7% (95% CI = 1.4- 4.0), AD 1.3%, vascular dementia 1.1% and the remaining due to other causes [13]. Dementia prevalence in Bangladesh was reported very recently and it is 3.6% based on clinical diagnosis (>60yrs) [30]. Besides the reports from Sri Lanka, India, and Bangladesh, prevalence of dementia has not been extensively studied in other South Asian countries. Dementia prevalence in some other Asian human races is as follows: Chinese 3.1% (>65yrs) [31], Koreans 6.3% (>65yrs) [32], Thai people 3.4% (>60yrs) [33], Singaporeans 6.5% (≥ 65 yrs) [34] and Israelis 26.4% (>65yrs) [35]. Dementia prevalence in Japan based on recent systemic review has been increased from 2.9% to 12.5% [36]. According to Prince *et al.* [2] systematic review on global burden of disease regions, in the year 2010, prevalence of dementia aged >60yrs was as follows: Asia 3.9% (Central Asia 4.6%, East Asia 3.2%, South Asia 3.6% and Southeast Asia 4.8%), Australia 6.4%, Europe 6.2%, The Americas 6.5% and Africa 2.6%. These estimates are entirely based on clinical criteria and the prevalence rates seem to be high in developed regions.

In our study, we found that the AD neuropathologic change for intermediate/ high level is 4.25% (2/47) in Colombo sample and 3.12% (1/32) in Bangalore sample and it is not different between elders ($p = 0.798$, after age and sex adjustment). Our study was not a prospective clinicopathological correlative study. However, it appears that in both populations, percentage of AD neuropathologic change for higher levels is greater than that reported in literatures based on clinical diagnosis alone (4.25% vs. 2.85% in Sri Lanka and 3.12% vs. 1.3% in India). Therefore, such prospective clinico-pathological study in Sri Lanka could provide better

Table 1. Sample summary on AD, CAA and LBD neuropathological changes.

Sample Size	Braak NFTstage	CERAD NP score	Thal A β phase	NIA-AA (criteria)	CAA grade	LBD stage
Sri Lanka (47 cases)	none 17.02%	none 89.4%	none 76.6%	not 76.6%	none 80.85%	none- 93.6%
	stage I-II 48.94%	stage A 2.13%	phase 1-2 21.28%	intermediate/high 4.25%	grade 1 17.02%	brainstem predominant- 6.4%
	stage III-IV 31.91%	stage B 4.25%	phase 3 2.13%		grade 2 2.13%	limbic (transitional)- 0%
	stage V-VI 2.13%	stage C 4.25%	phase 4-5 0%	low 19.15%	grade 3 0%	neocortical (diffuse)- 0%
			grade 4 0%		amygdala-predominant- N/A	
India (32 cases)	none 37.5%	none 93.7%	none 81.25%	not 81.25%	none 90.62%	none- 100%
	stage I-II 43.8%	stage A 3.12%	phase 1-2 15.65%	intermediate/high 3.12%	grade 1 3.12%	brainstem predominant- 0%
	stage III-IV 15.62%	stage B 0%	phase 3 0%		grade 2 0%	limbic (transitional)- 0%
	stage V-VI 3.12%	stage C 3.12%	phase 4-5 3.12%	low 15.62%	grade 3 6.25%	neocortical (diffuse)- 0%
				grade 4 0%	amygdala predominant- N/A	

NFT: neurofibrillary tangle, CERAD NP score: Consortium to Establish a Registry for AD (CERAD) protocol for neuritic plaque score, A β : β amyloid, NIA-AA: National Institute on Aging- Alzheimer's Association, AD: Alzheimer's disease, CAA: cerebral amyloid angiopathy, LBD: Lewy body disease, N/A: not available.

Table 2. Neuropathological findings of 13 clinically diagnosed cases with dementia and/ or Parkinsonism disorders.

Sample	Age at death	Gender	Clinical Diagnosis	Neuropathological diagnosis
Sri Lanka (3 cases)	(1) 74	Male	PD	Mixed dementia (definite PSP, AD neuropathologic change- intermediate and brainstem predominant Lewy bodies)
	(2) 73	Female	PD	Brainstem predominant Lewy bodies Idiopathic PD, stage III
	(3) 73	Male	PD	No Lewy bodies Vascular Parkinsonism due to ischemic stroke
India (10cases)	(4) 89	Male	Parkinsonism	No lesions
	(5) 86	Male	AD	AD neuropathologic change- intermediate Severe CAA
	(6) 76	Male	Parkinsonism	Brainstem predominant Lewy bodies Idiopathic PD, stage III
	(7) 75	Male	Parkinsonism	Mixed dementia (neocortical Lewy bodies- diffuse Lewy body disease and AD neuropathologic change- intermediate)
	(8) 72	Male	AD	AD neuropathologic change: high
	(9) 62	Female	Parkinsonism	Brainstem predominant Lewy bodies and multiple system atrophy
	(10) 61	Male	Parkinsonism	Brainstem predominant Lewy bodies Idiopathic PD, stage III
	(11) 60	Male	Parkinsonism	Brainstem predominant Lewy bodies and Creutzfeldt-Jakob disease
	(12) 52	Male	Parkinsonism	Brainstem predominant Lewy bodies Idiopathic PD, stage III
	(13) 50	Male	FTD	Frontotemporal lobar degeneration - taupathy

PD: Parkinson's disease, AD: Alzheimer's disease, PSP: progressive supra nuclear palsy, FTD: Frontotemporal dementia

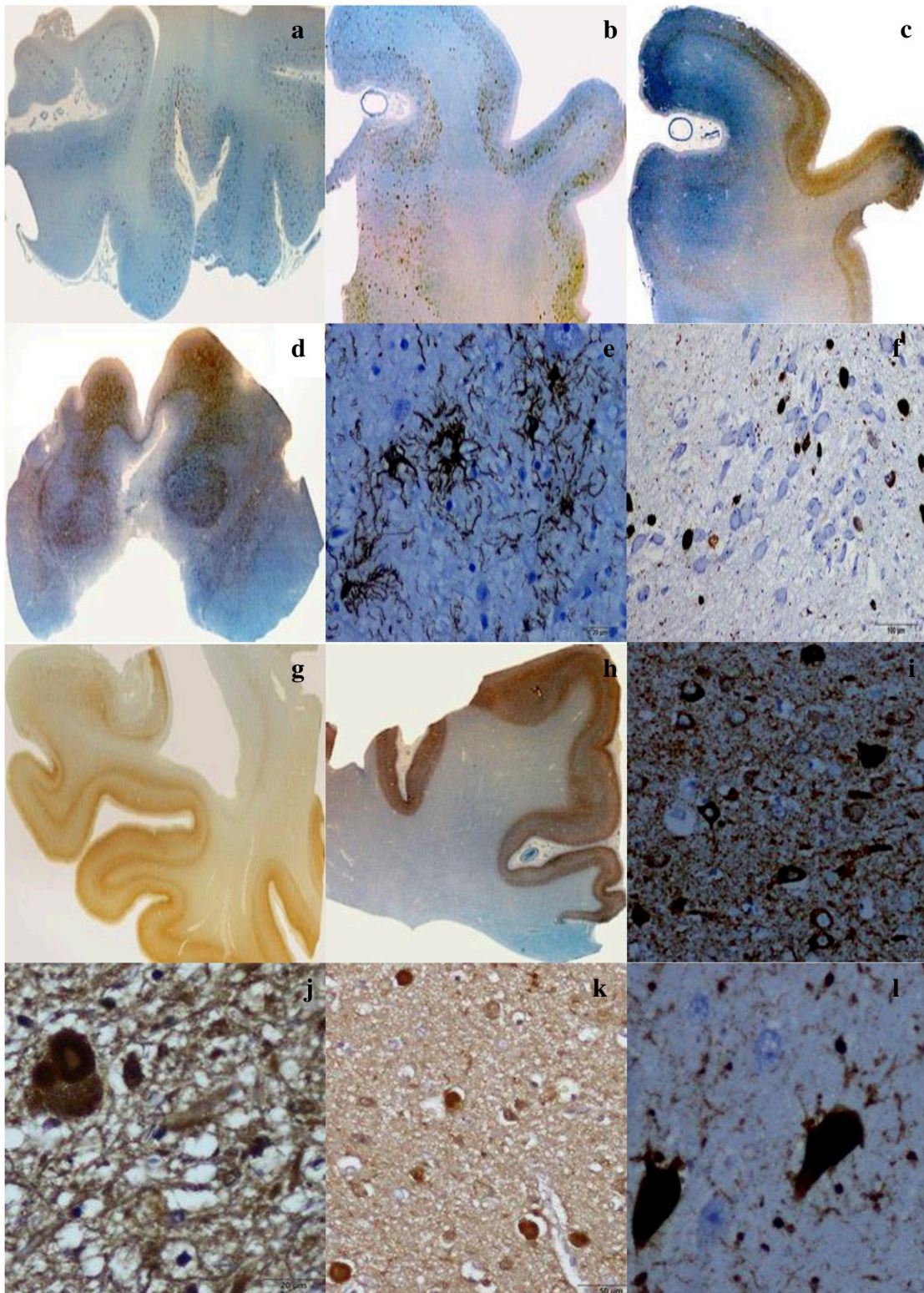


Fig. (1). Neurodegenerative pathologies: a,b&c- Alzheimer's disease (a) NPs in hippocampus whole mount, (b) β amyloid (+) ve SPs in frontal cortex, (c) tau (+) ve neurofibrillary pathology in the frontal cortex; d,e&f- Progressive supra nuclear palsy (d) tau(+) ve lesions in mid-brain whole mount, (e) tufted astrocytes at 400X (scale bar- 20 μ m), (f) globes tangles at 100X (scale bar- 100 μ m); g,h&i- Fronto temporal dementia- frontotemporal lobar degeneration taupathy (g) tau(+) ve inclusion bodies in the hippocampus whole mount, (h) frontal cortex whole mount (i) at 200X (scale bar- 50 μ m) in hippocampal CA1 region; j-Brainstem predominant Lewy bodies in substantia nigra of a Parkinson disease case at 400X (scale bar- 20 μ m), k- Neocortical Lewy bodies in the frontal cortex of a diffuse Lewy body disease case at 200X (scale bar- 50 μ m) and l-argyrophilic grains at 400X (scale bar- 20 μ m) in hippocampalCA1 region.

Table 3. Comparison of AD related neuropathological scores between LMICs, and between LMICs and HIC.

	Colombo Sri Lanka (SE)	Bangalore India (SE)	Mumbai India (SE)	New York USA (SE)
	Reference [25]			
Mean age ± S.D. in years	72.1 ± 7.8	65.9 ± 9.3	71.1 ± 8.3	71.1 ± 9.3
NFT in Hippocampus (grade)	0.91 (0.11)	0.75 (0.08)	1.24 (0.18)	1.16 (0.18)
NFT in Hippocampus (count/mm2)	4.36 (1.16)	1.30 (0.42)	4.41 (0.87)	3.78 (0.78)
NFT in EC (grade)	1.20 (0.14)	1.11 (0.11)	1.39 (0.20)	1.32 (0.16)
NFT in EC (count/mm2)	4.58 (0.82)	3.27 (0.80)	4.06 (0.71)	3.81 (0.63)
SP in neocortex (count/mm2)	0.44 (0.17)	0.95 (0.60)	1.15 (0.31)	0.59 (0.18)
Braak stage for NFT	1.91 (0.2)	1.19 (0.2)	1.01 (0.14)	1.11 (0.12)
SP, age adjusted CERAD score	0.45 (0.13)	0.41 (0.16)	0.80 (0.17)	0.39 (0.11)

LMICs: low and middle income countries, HIC: high income country, USA: United States of America, S.E.: standard error, S.D.: standard deviation, NFT: neurofibrillary tangle, EC: entorhinal cortex, SP: senile plaque.

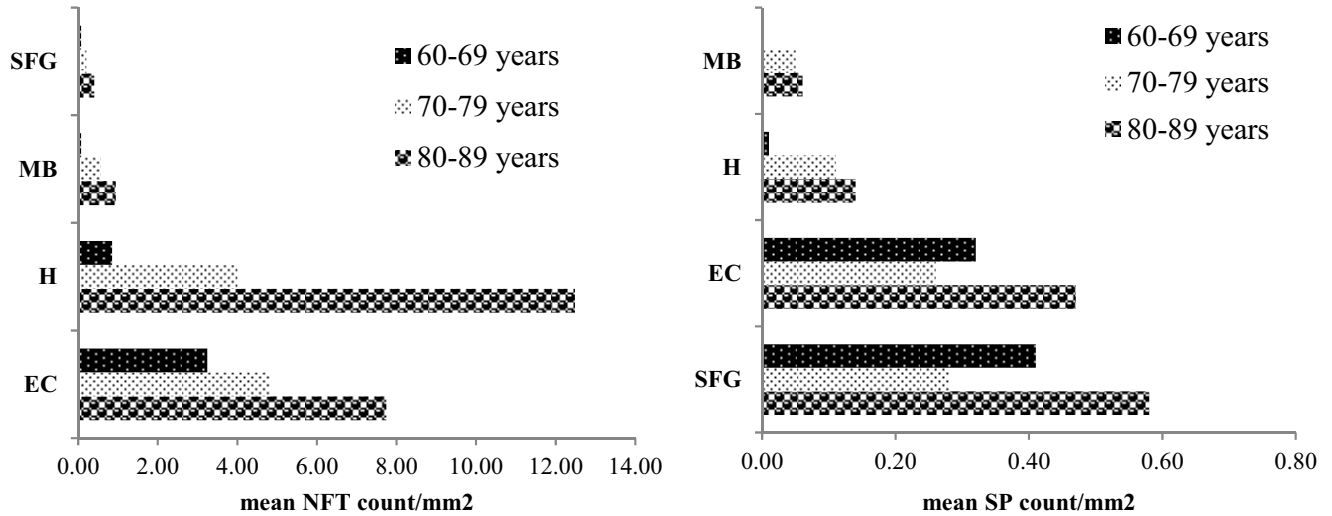


Fig. (2). Colombo samples: Mean NFT and SP counts were plotted against different neuroanatomical regions (EC- entorhinal cortex, H- hippocampus, MB- midbrain, SFG- superior frontal gyrus, NFT- neurofibrillary tangle, SP- senile plaque. Excluded clinically diagnosed cases and aged <60 years).

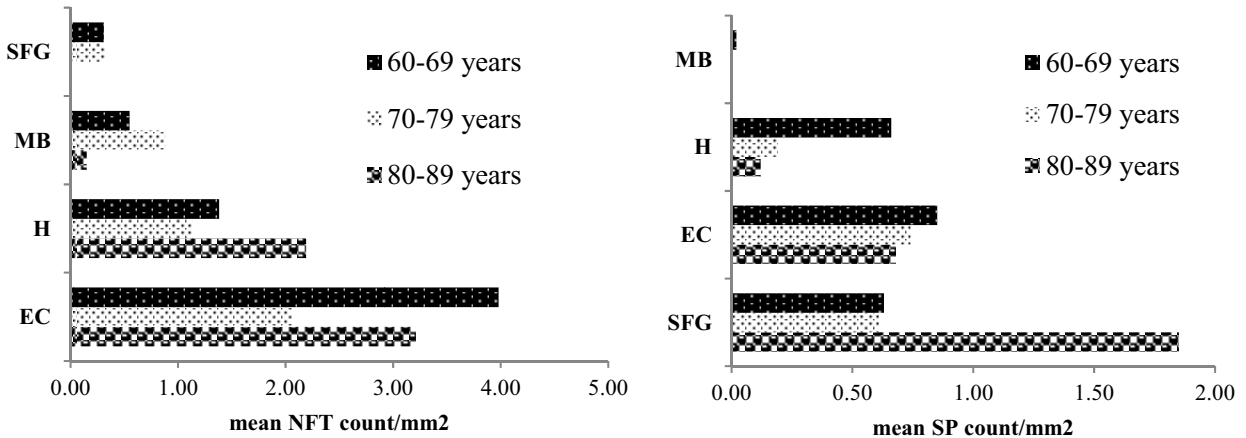


Fig. (3). Bangalore samples: Mean NFT and SP counts were plotted against different neuroanatomical regions (EC- entorhinal cortex, H- hippocampus, MB- midbrain, SFG- superior frontal gyrus, NFT- neurofibrillary tangle, SP- senile plaque. Excluded clinically diagnosed cases and aged <60 years).

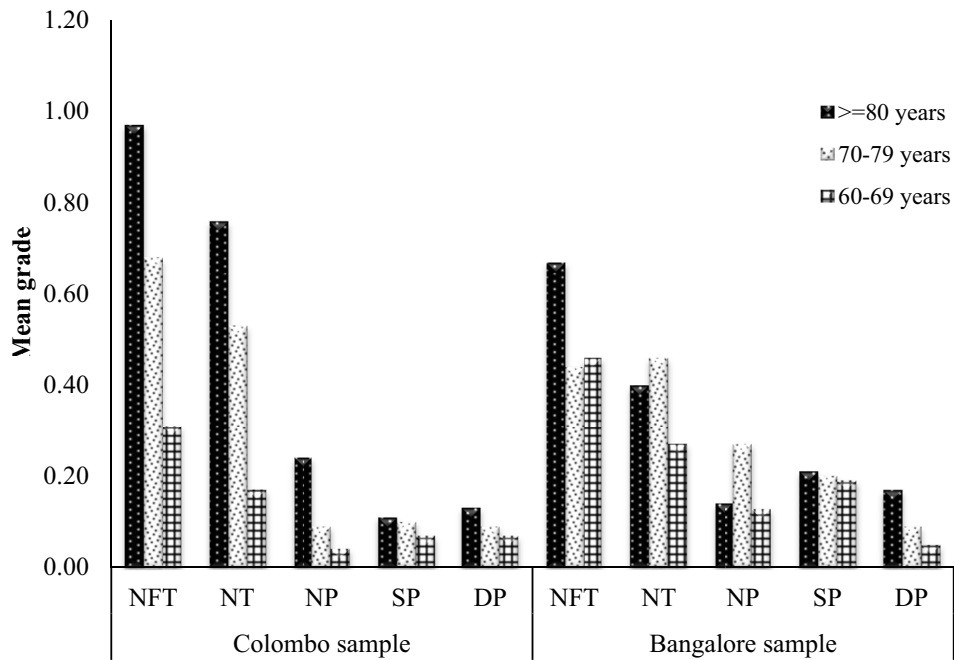


Fig. (4). Mean grades of both tau and beta amyloid positive pathologies were plotted against elderly Colombo and Bangalore samples (NFT- neurofibrillary tangle, NT- neuropil thread, NP- neuritic plaque, SP- senile plaque and DP- diffuse plaque)

Table 4. Co-morbid age-related pathologies between Colombo and Bangalore samples.

Pathology	Presence in %		P value (age, sex adjusted)	Odds ratio (95% CI)
	Colombo samples	Bangalore samples		
Cerebral amyloid angiopathy	19.1%	9.4%	0.732	1.30 (0.3-5.9)
Lewy bodies	6.4%	0.0%	-	-
Spongiform changes	80.9%	29.0%	0.000* *	8.75 (2.8-27.6)
Dilated perivascular spaces	87.2%	68.8%	0.073‡	3.19 (0.9-11.3)
Leukoaraiosis	32.6%	31.0%	0.706	0.80 (0.3-2.5)
Cell loss in CA1 region	53.2%	17.2%	0.162	2.55 (0.7-9.5)
Cell loss in DG region	51.1%	13.8%	0.009* *	5.65 (1.5-20.6)

Dichotomized variables (present or absent) with binary logistic regression adjusted for age and sex (reference category- Bangalore samples, ** p value significant at <0.01 and ‡ at risk at <0.1) CI: confidence interval, Coronu Ammonis area 1: CA1, DG: dentate gyrus

Table 5. Degree of association among the age-related pathologies: 2X2 contingency table.

Pathology (Dichotomized variables)		P value (Fisher's exact test)	Kendall's tau b correlation	Strength
AD intermediate/ high	CAA grade 2/3	0.003	0.654	strong
Thal Aβ phase 1-5	CAA 1-4	0.000	0.465	moderate
Thal Aβ phase ≥2	CERAD NP score A-C	0.000	0.808	very strong
Braak NFT stage IV-VI	CERAD NP score A-C	0.000	0.448	moderate
Braak NFT stage IV-VI	Thal Aβ phase ≥2	0.028	0.298	weak

(Table 5) contd...

Pathology (Dichotomized variables)		P value (Fisher's exact test)	Kendall's tau b cor- relation	Strength
Braak NFT stage IV-VI	Leukoaraiosis	0.006	0.317	weak
CERAD NP score A-C	Leukoaraiosis	0.005	0.325	weak
CAA 1-4	Leukoaraiosis	0.014	0.324	weak
Lewy bodies	Leukoaraiosis	0.030	0.298	weak
Braak NFT stage III-VI	cell loss in CA1	0.036	0.270	weak
Spongiform changes	cell loss in CA1	0.005	0.302	weak
Thal A β phase 1-5	dilated perivascular space	0.015	-0.273	weak

P value is calculated using Fisher's exact test and presented only the significant p values at <0.05

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.

indications of the true extent of dementia burden, help plan for future health care needs and offer neurobiological perspectives that underlie lifestyle and environmental risk/ protective factors for AD affecting these cultures [25]. It is suggested in the NIA-AA guidelines, that for individual with cognitive impairment at the time of tissue was obtained, intermediate or high level of AD neuropathologic change should be considered as adequate explanation of cognitive impairment or dementia and should be reported with a final diagnosis of AD. Unfortunately, clinical diagnosis of dementia or AD in these cases had failed in obtaining ante-mortem detection. This may be due to identification of dementia as a clinical entity is not common in the population from which subjects were obtained or the prevailing notion among the people that the memory problems and cognitive loss are natural phenomena during aging and are not due to a disease which might require an intervention [25].

An older autopsy study, conducted by our co-authors Yasha and colleagues [37] on AD related pathology of the 53 Bangalore samples collected over 10 years indicated that the incidence of both SPs and NFTs together were found to increase with age from 21% in seventh decade to 33% in eighth decade and 54% in ninth decade and the increasing incidence of NFTs being statistically significant. Above study was entirely based on silver stains for the assessments of neuropathological scores. In our study, recently collected samples from same Bangalore population show that incidence of both SPs and NFTs together were found to increase with age from 20.8% in sixth decade to 37.5% in seventh decade and 60% in eighth decade and the higher percentage of this report may be a reflection of more sensitive pick up using immunohistochemistry and may also represent the vulnerability of current population to such pathologies which start at their early periods as indicated. Yasha and her colleagues [37] also suggested from their findings that AD related lesions were similar to that reported in the high income countries (HICs), although they didn't conduct a direct comparison. Meanwhile, a recent autopsy report [25] from India, which compared the profile of AD related pathology in an ageing semi urban Mumbai population with similar samples from New York, USA indicated that the burden of AD related pathology was approximately equivalent in Mumbai and New York samples, which is at variance with expected

lower AD-related lesion burden based on the clinical/ epidemiological studies suggesting lower prevalence of AD in India. To compare LMICs and HIC, we have summarized the findings of our study with AD related neuropathological scores of Mumbai and New York samples obtained from Purohit *et al* [25] study in (Table 3). Overall summary of these neuropathological scores shows that AD related neuropathological changes in all four elderly populations are almost similar to each other and suggests that income status, in other words, living standard didn't cause any vital difference in AD occurrence.

Various clinical and epidemiological studies have highlighted the functional consequences of the phenotype- genotype relationship of gene apolipoprotein E (APOE) and its affiliation with diverse pathological conditions and cognitive traits [38-40]. APOE $\epsilon 4$ allele is the strongest genetic risk factor for AD and VaD and its frequency in Colombo elderly samples is 0.146 (article under preparation). In addition to APOE, methylenetetrahydrofolate reductase (MTHFR) T allele has also been discussed in AD [41], PD [42] and other dementias [43] and its frequency in elderly Colombo samples is 0.062, whereas in previous Sri Lankan population based study, it was 0.049 [44]. Although we didn't carry out a simultaneous genotyping in elderly Bangalore samples, according to the community/ population based findings from India, frequency of APOE $\epsilon 4$ allele is 0.101 [45], 0.073 [46] and 0.068 ± 0.030 [47], and frequency of MTHFR T allele is 0.18 [48], 0.10 [49] and 0.03 [50]. Overall allelic frequencies in Sri Lankan and Indian community/ population based genetic studies are comparable to each other; however frequency of APOE $\epsilon 4$ allele seems to be high in Colombo samples (0.146 vs. 0.068 ± 0.030 [47]).

On the other hand, observational studies suggest that lower risk of dementia in some developing countries can be attributed to the type of diet [51]. Accumulating evidence is highlighting that oxidative stress via generating free radicals plays a pivotal role in neurodegenerative diseases and that can be reduced by diets that are rich in antioxidants such as fruits, vegetables and tea [52-55]. Tea is the major beverage in Sri Lanka and the total content of antioxidants in both green tea (190.0 mg/g) and black tea (186.6 mg/g) are highest in Sri Lankan products compared to other manufacturers of the different countries [56]. India and Sri Lanka are the

two largest black tea exporters in the world and their annual per capita tea consumption is 0.64-0.66Kg and 0.9-1.29Kg [57,58], respectively. In our study, tau positive pathologies are more frequently detected in the brains of elders [74.68% (59/79)], considering that amyloid plaques are comparatively low [26.58% (21/79)]. However, between elderly samples, Bangalore brains show more amyloid plaques than the brains obtained from Colombo (Fig. 4). In summary, these findings open a biologic means to investigate in-depth scientific and large scale observational studies in relation to neuroprotective role of tea in future.

Age is by far the largest factor for these diseases, however, besides age, regional variations including genes, environment, infectious agent, chemicals, neurotoxins, diet, vascular disease and its risk factors, and the gene-environment interactions are all suggested to be the risk factors in dementia occurrence [1, 59]. Therefore, differences observed in Colombo samples including pathologies associated with Parkinsonism and cerebrovascular changes need to be investigated in detail with respect to regional variations. Apart from those differences, AD neuro-pathologic changes are almost equal in both Colombo and Bangalore elderly samples and surprisingly, the neuropathological scores among LMICs and HIC are remarkably similar based on Purohit *et al* findings [25].

Neuropathology of the AD extends beyond amyloid plaques and NFTs and the review of various consortia data shows more than 30% of the AD cases exhibit cerebrovascular pathology [60]. Certain vascular pathologies such as CAA, microvascular degeneration, and periventricular white matter lesions are evident in almost all the cases of AD and whether these vascular lesions are coincidental or causal in the pathogenetic processes of AD need further studies [60]. Recent NIA-AA guidelines [15] emphasize a structured approach for the diagnosis of co-morbid pathologies in addition to AD neuropathologic change. However, in our study, we reported the co-morbid pathologies as present or absent (Table 5) with their strength of association. The presence of white matter lesion-leukoaraiosis is significantly associated with AD and other neurodegenerative pathologies and shown in the (Table 5). Similar findings have been discussed in previous studies, where more than half of the AD cases exhibit diffuse/ non-focal white matter lesions, with the loss of myelin-stained fibres not proportional to the expected level due to cortical neuronal degeneration [61, 62]. In addition to leukoaraiosis, CAA is also associated with AD and the progression of AD pathology is positively correlated with CAA severity [63, 64]. In our study, samples with AD neuropathologic change for intermediate and high level presented CAA grades 2 and 3 respectively ($p = 0.003$, Kendall's tau b correlation coefficient (τ) = 0.654). The presence of CAA also showed a significant association with leukoaraiosis ($\tau = 0.324$). Leukoaraiosis is increasingly recognized as a feature of sporadic CAA [65]. Amyloid deposits are found in more proximal portions of the small penetrating arteries and the contribution of amyloid deposits alone without downstream arteriosclerosis are not clear in the pathogenesis of leukoaraiosis. However, leukoaraiosis found in some of the genetic amyloid angiopathies suggest that amyloid angiopathy alone is sufficient to cause this feature [26, 65]. Besides the associations with AD and CAA, the presence of leukoaraiosis

also showed a significant association with Lewy body pathology and this is opposing to Zijlmans *et al* [66] findings where, in the absence of Lewy bodies, involvement of microscopic small vessel diseases were significantly demonstrated for the aetiology of vascular Parkinsonism. Another co-morbid pathology noted in these elderly brains is hippocampus cell loss in DG and CA1 regions and are considerably higher in Colombo samples (51% and 53% vs. 14% and 17%). The CA1 region of the hippocampus is vulnerable to both AD type neurofibrillary degeneration and anoxia-ischemia and is more consistent in AD than in ischemic vascular dementia [67]. In our study, a higher degree of association between CA1 cell loss and Braak NFT stages III-VI ($p = 0.036$) and between CA1 cell loss and spongiform changes of neuropil ($p = 0.005$) were observed with positive correlations (Table 5). Later one is opposed to the study of Masullo and Macchi [68] where they demonstrated the resistance of hippocampus, particularly, pyramidal cell layer of CA1 in Creutzfeldt-Jakob disease, group of subacute spongiform encephalopathies of animals and man. On the other hand, cell loss in DG region (almost similar percentage to CA1 cell loss) was neither related AD related pathologies nor cerebrovascular pathologies. But, it was significantly related to increasing age (OR = 1.07, 95% CI = 1.0-1.1). These observations are further strengthened by Small *et al.* [69] review on pathophysiological framework of hippocampal dysfunction in ageing and disease. The hippocampal formation has been implicated in a growing number of disorders, from AD and cognitive aging to Schizophrenia and depression. They pinpointed that these disorders differentially target distinct sub regions of the hippocampal circuit. AD, vascular disease and ageing all contribute to hippocampal alterations in late life and their direct comparison suggests that entorhinal cortex is differentially associated with AD and the CA1 with vascular disease, whereas ageing process *per se* seems to differentially target the DG. On the whole, our observations further support the possible pathological pathways for hippocampal dysfunction during aging.

There are some limitations in our study. Sample size is small and represents an elderly community. Therefore, our study is not reported the prevalence of AD neuropathologic changes in Sri Lankan and Indian general populations. Incomplete clinical history of the recruited samples lacking objective psychometry and dementia scores, ubiquitin immunohistochemistry for labelling Lewy bodies instead of α -synuclein and non-availability of some brain regions as specified under minimum tissue requirements by NIA-AA guidelines are also considered as important limitation factors of this study. As this was a retrospective study, case history was obtained through informants who were familiar with intellectual and functional status of the subjects before death. However, uncertainty in cognitive status of the subjects at the time of tissue removal affects the final diagnosis of AD based on NIA-AA guidelines. For the LBDs, although H and E staining may be used to detect Lewy bodies, greater sensitivity can be achieved with α -synuclein immunohistochemistry and is recommended as preferred method in the NIA-AA guidelines. In our study, however, in addition to H&E staining, ubiquitin instead of α -synuclein immunohistochemistry was used in labelling Lewy bodies which was recommended in CDLB 1996 guidelines [70]. Further, the assessment of

some cerebrovascular changes (leukoaraiosis, spongiform changes & dilated perivascular spaces) and hippocampus cell loss in DG and CA1 regions were based on their presence in fronto temporal cortex regions and in the hippocampus, and didn't apply any staging or grading system. In fact, due to the high variability of cerebrovascular changes associated with cognitive impairment, no validated neuropathological criteria are currently available for VaD or mixed dementia [71]. Finally, in our study, specific brain sections from deceased victims were collected between May 2009 and March 2010. As a result, some of the brain sections, as specified in the NIA-AA guidelines (2012), were not available in this study but will be included in future concerns.

CONCLUSION

Between these two elderly Colombo and Bangalore samples, AD neuropathologic changes didn't show any significant variations. However, pathologies associated with Parkinsonism (PD and PSP), cerebrovascular changes (spongiform changes and dilated perivascular spaces) and granular cell loss in DG region are frequently found in elderly Colombo samples. Taken together, in comparison with elderly Indians, elderly Sri Lankans are more vulnerable to aging cytoskeletal pathologies and this might be due to their genetic, dietary and/ or environmental variations.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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KRD organized the institutions for research activities and had final responsibility for manuscript content as the principal investigator. PW carried out research activities, analysis and wrote the manuscript under the supervision of KRD, SKS and YTC. SKS provided necessary assistances for neuropathological screening and performed neuropathological diagnosis. YTC trained PW on neuropathological diagnosis and evaluation. All authors (PW, SKS, YTC, CG, DA, SH, KSK, KS, YHS, HWS and KRD) contributed to the design, interpretation of the results and critically reviewed the paper.

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