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Alzheimer's disease in Sri Lanka

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Introduction

Dementia is one of the most distressing and burdensome health problems associated with aging. The prevalence of dementia increases exponentially with age at least up to age 90 or 95. Studies in North America, United Kingdom and Europe have reported that dementia affects 4-6% of the population over the age of 65 and 15-20% over the age of 80 [Clark and Goate, 1993; Canadian study of health and aging, 1994; Ott et al, 1995] with the annual incidence approximately doubling for every five years of age between the ages of 75 and 89 years [Paykel et al., 1994]. Therefore, dementia has been acknowledged as a major public health problem in Western industrialized countries.

However, the majority of the world's elderly do not live in the West. It has been estimated that approximately 70% of the world's population aged 60 years and older in the year 2020 will be in developing countries, with 14.2% in the Indian subcontinent [World Health Organization, 1998]. In Sri Lanka, the average life expectancy from birth has increased for men from 62 years in 1963 to 70 years in 1991, and for females from 61 years to 74 years [Annual Health Bulletin, 2000]. Thus, the percentage of the population aged over 60 years in this country is expected to increase rapidly from 8.4% currently, to reach 13% in 2010, and 21% in 2025 [De Silva, 1997].

Primarily because of lesser emphasis on the health of older adults and lesser resources for health research compared with the industrialized countries, dementia has not been systematically investigated in Sri Lanka. As a result there are no epidemiological estimates of the disease in this country. Thus, the extent of the public health burden currently posed by Alzheimer's disease (AD) and other dementias in Sri Lanka remains an open question.

Epidemiological studies of dementia require community screening of subjects for cognitive impairment with neuropsychiatric tests suitable for the cultural background of the study population. This requires translation and cultural adaptation of such screening instruments. The lack of such culturally appropriate screening instruments may have precluded studies on dementia in this country.

The main aims of the work reported here were to:

- Translate and validate a culturally appropriate screening instrument to detect cognitive impairment in Sri Lanka.
- Study the prevalence of dementia and AD in a selected study population.
- Study the association between confirmed genetic and non-genetic risk factors for AD.
- Assess the usefulness of medial temporal lobe oriented CT scans for the ante-mortem diagnosis of AD

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Kelaniya.

As a first step, we translated and validated one of the most commonly used screening instruments used to detect cognitive impairment in the community.

Development of a translated and culturally appropriate instrument for cognitive assessment

The Mini Mental State Examination (MMSE; Folstein et al., 1975) is used widely as a screening instrument to detect dementia both in the clinical setting and in community based epidemiological studies, and appears to be the best brief cognitive test currently in use [Brayne, 1998]. The MMSE provides a measure of cognition, which is based on an interviewer asking 17 questions each of which is noted as correct or incorrect. These questions cover a broad set of cognitive domains: orientation, registration, short-term memory, attention, calculation, visuo-spatial skills and praxis. The MMSE, variously modified and translated into several languages, has been used successfully in several independent cross-national studies of dementia epidemiology [Brayne, 1998]. The aim of this study was to develop and validate a translated (to Sinhalese, the language used by more than 80% of the population) and culturally appropriate MMSE that could be used as a screening test for cognitive impairment in Sri Lanka. The resulting

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instrument was used in an epidemiological investigation of dementia to determine the prevalence of, and risk factors for, dementia in a semi-urban population in Sri Lanka.

Material and methods

Subjects

The study was conducted in Ragama where the faculty of medicine and the university hospital are situated. Ragama was chosen as the sample area to take advantage of its dense semi-urban population and its socio-economic heterogeneity. The languages spoken are mainly Sinhalese and English, and the great majority of elderly individuals living in this town is literate and has had at least a few years of formal education.

Ragama is divided into eight primary healthcare divisions (public health midwife areas). 380 subjects over 65 years of age (mean age = 68.2 years; SD = 7.17) were selected randomly from two public health midwife areas (population of 8915) for the survey. All 380 subjects were interviewed to obtain basic demographic data, and scores on the MMSE (maximum score = 30). The subjects were all living in their own homes or in that of a family member. As no local or national age-stratified household lists exist, the initial contact with the subjects was made through a Public Health Midwife. The assessment of all subjects was done at a community centre in the neighbourhood. All subjects had to be fluent in either English or Sinhalese, and those with major illnesses, deafness, blindness, mental retardation, and major physical disability were excluded from the study.

Instruments

The original MMSE was translated into Sinhalese and back translated by the investigators (who are bilingual), and the accuracy and cultural appropriateness of the translation were assessed by the Linguistics Unit, and the Department of Sinhala, University of Kelaniya, Sri Lanka. In order to make the Sinhalese translation of the MMSE applicable to subjects who are illiterate or who have had little / no formal education as well, some modifications made in the Chinese [Katzman et al., 1988] and Hindi [Ganguli et al., 1995] versions of the test were incorporated where relevant. Table 1 provides a comparative brief description of the original and translated versions of the MMSE.

Table 1. Item-wise comparison of the translated original MMSE and translated version (figures in parentheses in the table show maximum score for each item)

Item	*MMSE*	*Translated version*
1. Orientation to time	Date, day, month, year, season. (5)	Date, day, month, year, time of day. (5)
2. Orientation to place	Country, town, street, place, floor. (5)	Country, town, street, place, floor. (5)
3. Three objects registration	Apple, table, penny. (3)	Orange, table, rupee. (3)
4. Calculation	Subtract serial sevens backwards from 100. (5)	Subtract serial threes backwards from 20**. (5)
5. Recall	Name the three objects learned earlier. (3)	Name the three objects learned earlier. (3)
6. Language (Naming)	Pencil, watch. (2)	Pencil, watch. (2)
7. Repetition	'No ifs, ands or buts'. (1)	'No ifs, ands or buts' in Sinhalese. (1)
8. Language (Comprehension)	Read and follow command 'Close your eyes'. (1)	Asked to follow verbal command, 'Close your eyes'*. (1)
9. Three step task	Follow interviewer's instruction: 'take paper in right hand, fold in half and put on the table. (3)	Follow interviewer's instruction: 'take paper in right hand, fold in half and put on the table. (3)
10. Sentence construction	Write a complete sentence. (1)	*Say* a complete sentence*. (1)
11. Copying figure	Overlapping pentagons. (1)	Overlapping pentagons. (1)

* For illiterate subjects

** For subjects who have had less than 5 years formal education

Most items on the MMSE could be directly translated to Sinhalese at the time of administering the test, and a few required minor modification. Attention was given to make each item in the questionnaire appropriate for use in the Sri Lankan context in terms of familiarity and cultural relevance. The item that required major translation was the repetition phrase, "no ifs, ands, or buts". The modified items in the translated version of the MMSE are briefly discussed below in relation to items in the original English version of the test.

Orientation to time. Since there are no major seasonal variations in the climate in Sri Lanka, the question on 'season' was replaced with a question of 'time of day'.

Registration. 'Apple', 'table' and 'penny' in the original MMSE were substituted with 'orange', 'table' and 'rupee', items more familiar to people in Sri Lanka.

Calculation / attention. Those who had ≤ 5 years formal education were asked to subtract serial threes backwards from 20, while those who had more than 6 years education were asked to subtract serial sevens backwards from 100 as required in the original version of the MMSE.

Language / comprehension. For the reading and writing tests, illiterate subjects were asked to "close your eyes" instead of read and follow a written instruction, and say a sentence instead of write. This was done to guard against failure on these items due to illiteracy.

Survey procedure

Based on the results of two previous studies done in China, the cut-off score on the MMSE suggestive of cognitive impairment was taken as 17 out of a maximum 30 [Li et al., 1989; Zhang et al., 1990]. The lower cut-off, instead of the more standard cut-off point of 24, has been found to be an appropriate score to detect cases of cognitive impairment in community surveys [Brayne and Galloway, 1990]. Non-response or refusals on any specific item was scored as zero in accordance with other studies (Fillenbaum et al., 1984).

Those screening positive for cognitive impairment (MMSE ≤ 17) and a randomly selected sample of those screening negative (MMSE > 17) were asked to undergo a clinical examination and a cognitive assessment with the more comprehensive neuropsychiatric test battery, Cambridge Cognitive Score (CAMCOG), to determine the presence of dementia. The CAMCOG is the cognitive assessment section of the Cambridge Mental Disorders of the Elderly Examination (CAMDEX) [Roth et. al., 1986]. The family and cognitive history of the patient was obtained from the spouse or a family member using section H of the CAMDEX. The clinical examination and the cognitive assessment with the CAMCOG were done in hospital. The CAMCOG was also translated to Sinhalese at the time of administration, and a few items in the test battery were modified to make the test more appropriate to Sri Lankan subjects (e.g. "who is the current Prime Minister of England" replaced with "who is the current President of Sri Lanka"). The cut-off score for CAMCOG to suggest cognitive impairment was taken as 80 out of a maximum possible score of 105 [Roth etal., 1986].

Results

In the sample of 380 subjects, there were 126 (33.1%) males and 254 (66.9%) females, more than half (55.5%) of whom were between 65-70 years of age. Education levels were generally low, especially among the very old subjects and women. More than half (54.2%) has had less than six years of formal education, 11.6% no formal education, and 21 subjects (5.5%) were illiterate.

Of the 380 subjects who were interviewed 33 scored ≤ 17 on the MMSE (screened positive), and were thus considered cases of suspected dementia. All 33 subjects who screened positive, and 24 randomly selected subjects who scored > 17 on the MMSE (screened negative), thus considered cognitively normal, underwent a brief clinical examination and a neuropsychological assessment with the CAMCOG (total of 57 subjects). There were two illiterate subjects among the screen positives and one illiterate subject among the selected screen negatives. There were seven subjects with < 6 years of formal education among the screen positives and five with < 6 years of formal education among the selected screen negatives.

After assessment with the CAMCOG and clinical examination, 29 out of the 33 subjects who screened positive on the MMSE showed evidence of dementia while four subjects scored above cut-off on the CAMCOG and showed no clinical signs of dementia. Of the 24 randomly selected subjects who screened negative on the MMSE, 22 showed no evidence of dementia while two scored below cut-off on the CAMCOG and showed clinical evidence of dementia. Therefore, the sensitivity and specificity of the translated version of the MMSE used by us (when 17 was used as the cut-off point) were respectively 93.5% and 84.6%. The sensitivity and specificity and other validity measures such as, likelihood ratios for some cut-off points of the Sinhalese version of the MMSE used by us during this study are shown in table 2. Since this is the first time this version of the MMSE has been used in any population, an item-wise performance of the instrument in relation to CAMCOG scores obtained by all 57 subjects is shown in table 3.

Table 2. Validity measures – sensitivity and specificity, likelihood ratios and Youden's indices – for some cut-off points for the Sinhalese version of the MMSE validated against CAMCOG scores (n=57)

MMSE cut-off score	*Sensitivity %*	*Specificity %*	*Likelihood ratio*	*Youden's index*
23	100	69.2	3.3	0.69
22	100	73.1	3.7	0.73
21	100	76.9	4.3	0.77
20	100	80.8	5.2	0.81
19	100	84.6	6.5	0.85
18	96.8	84.6	6.3	0.81
17*	93.5	84.6	6.1	0.78
16	74.2	96.2	19.3	0.70

*Cut-off point used by us

Table 3. Item wise performance by the two groups at the translated MMSE (figures in parentheses for each subtest / item show maximum score for each item)

Subtest / item	*Non-demented sample CAMCOG (-) (n=26)*		*Demented sample CAMCOG (+) (n=31)*	
	Score	*%*	*Score*	*%*
Orientation to time (5)	4.1 (0.8)	82	3.4 (1.2)	69
Orientation to place (5)	4.5 (0.7)	90	3.3 (1.0)	66
3-object registration (3)	2.6 (0.5)	86	1.9 (0.5)	63
Serial sevens / three (5)	3.8 (1.4)	76	0.5 (0.8)	10
3-object recall (3)	1.9 (1.1)	63	0.8 (0.6)	27
Name pencil & watch (2)	2.0 (0)	100	1.8 (0.6)	90
Repeat phrase (1)	0.8 (0.4)	80	0.3 (0.5)	30
3-step task (3)	2.7 (0.6)	90	1.7 (0.8)	57
Read / verbal command (1)	0.9 (0.3)	90	0.2 (0.4)	20
Write / say sentence (1)	0.6 (0.5)	60	0.2 (0.4)	20
Copy drawing (1)	0.6 (0.5)	60	0.2 (0.4)	20

Comment

The MMSE, variously translated and adapted to suit many cultures, has been used as a screening instrument among elderly people in many countries, which is generally considered as the first step in prevalence surveys in the identification of individuals with a high probability of being demented [Launer 1992].

Based on the results of two previous studies [Li et al., 1989; Zhang et al., 1990], one of which used level of education-dependent cut-off points to screen for dementia [Zhang et al., 1990], we used 17 as the cut-off for our version of the MMSE instead of the more standard cut-off point of 23. We did this to minimize the likelihood of not detecting subjects with cognitive impairment during screening.

The main purpose of the present study was to develop a version of the MMSE, translated to Sinhalese and made more appropriate and relevant

to the cultural context of Sri Lanka. In order to evaluate the sensitivity and specificity of the modified instrument, the MMSE scores of all subjects in our study were evaluated against performances at the CAMCOG, the cognitive section of the CAMDEX. The CAMCOG allows observation of a broad range of impairments associated with dementia in a relatively short time (20-30 min). Thus, in comparison to the MMSE, CAMCOG provides a wider coverage of cognitive functions and detects milder degrees of cognitive impairment. This makes the CAMCOG a very comprehensive test battery to diagnose dementia, and also to evaluate the sensitivity and specificity of screening instruments such as the MMSE [Roth et al, 1986].

This survey is the first time that a translated MMSE has been used as a screening instrument for cognitive impairment in Sri Lanka, and this study has shown that the modified version of the MMSE used by us to be simple to use and appropriate to the population studied. Therefore, we conclude that the slightly modified Sinhalese translation of the MMSE described here is a useful and sensitive instrument to screen for cognitive impairment in Sri Lanka.

With the modified Sinhalese translations of neuropsychiatric test batteries [De Silva & Gunatilake, 2002], we proceeded to study the prevalence of dementia in a well-defined community dwelling population in Ragama.

The prevalence of dementia and Alzheimer's disease

Methods

Subjects

703 subjects aged 65 years and older were selected randomly from four public health midwife areas (total population = 15828) for the survey. As no local or national age-stratified household lists exist, the initial contact with the subjects was made through a Public Health Midwife (a community healthcare worker), who arranged for an assessment at a community centre in the neighbourhood. All subjects had to be fluent in either English or Sinhalese, and none of the subjects who agreed to join the study was excluded.

General study design

To ascertain cases of dementia in the identified community we used a two-stage study design. During phase I, all subjects who agreed to join the study were administered a brief cognitive test (screening test) to discriminate between subjects with and without possible cognitive impairment. In phase II, all subjects who screened positive were clinically evaluated to confirm or exclude a diagnosis of dementia. Subjects affected by dementia were further investigated to distinguish AD from other dementing disorders.

Phase I and screening instrument

The screening instrument was the Sinhala version of the Mini Mental State Examination (MMSE), which we had previously developed by translating and modifying slightly the original MMSE to make the test appropriate for use in Sri Lanka [De Silva and Gunatilake, 2002]. Subjects were also screened for visual and hearing impairment in order to determine whether they could be cognitively tested.

After informed consent all 703 subjects who agreed to join the study were interviewed to obtain basic demographic information, and screened for evidence of impaired cognition. We used 17 (out of a maximum score of 30) as the cut-off score to screen for dementia with the Sinhala MMSE, which we have previously shown to have good sensitivity and specificity [De Silva and Gunatilake, 2002]. All subjects who scored 17 or less on the MMSE (screen positives) were regarded as suspected dementia cases.

Phase II and clinical diagnosis

All subjects who screened positive in phase I were recruited to this phase for a comprehensive clinical and diagnostic evaluation using a standardized diagnostic protocol which included the following:

- Structured neuropsychiatric assessment with the Cambridge Examination for Mental Disorders of the Elderly CAMDEX [Roth et al., 1986], which comprises a cognitive test battery assessing a broad range of cognitive functions (Cambridge Cognitive Examination; CAMCOG). This test was translated into the vernacular at the time of administration, and a few items in the test battery were modified to make the test more appropriate to Sri Lankan subjects [De Silva and Gunatilake, 2002].
- Informant interview using the Everyday Abilities Scale for India (EASI) [Fillenbaum et al., 1999] to assess evidence for functional limitation in activities of daily living (ADL).
- Full physical and neurological examination.
- Biochemical analysis of blood and axial CT scans of the brain to exclude less common secondary causes of dementia.

The clinical diagnosis of dementia was based on the Diagnostic and Statistical Manual of Mental Disorders-fourth edition (DSM-IV) criteria [American Psychiatric Association, 1994], which require the demonstration of both cognitive and functional (social and occupational) impairment.

We used the 11-item EASI to assess the regular activities and functions performed by and expected of our elderly population, and to determine the presence of functional deterioration. This scale was administered to a responsible household member (usually a child) of each subject, who was asked whether the subject performed each listed activity regularly and adequately. ADL screening by informant report was obtained for two subjects who were severely impaired and could not complete formal cognitive assessment. For this study, inability to perform any three or more of the eleven items designated the subject as functionally impaired.

Cognitive deficit was determined by direct testing of the subject, and was based on MMSE and CAMCOG scores. The diagnosis of possible or probable AD was based on the National Institutes of Neurology and Communicative Disorders - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [McKhann et al., 1984]. For AD prevalence data reported here, we grouped probable and possible AD together. The diagnosis of vascular dementia utilized the Hachinski score [Hachinski et al., 1975] and DSM-IV criteria. The diagnosis of possible or probable dementia with Lewy bodies (DLB) was based on other operative criteria [McKeith et al., 1996]. Diagnoses of depression or other psychiatric disorders were made after evaluation by a consultant psychiatrist working in the same hospital who was blind to cognitive assessment scores.

Results

From the total resident population of 15828 selected for the study, 703 subjects (4.4%) who were 65 years or older on 30 April 2000 were screened. The age, sex and education composition of the study sample is shown in table 4. In the sample of 703 subjects, there were 429 (61%) females and 274 (39%) males. The mean age of the study population was 69.4 ± 6.37 years (SD), and 86% were between 65-75 years. Education levels were generally low, mainly among the very old and women. Less than six years of formal education was reported by 57% of women and 29% of men, whereas 69.7% of men and 36% of women had more than six years of education, reflecting the perceived value in the community of education for women of this generation. Illiteracy, defined as the inability to read and write in at least one language, was reported by 33 (4.7%) of the total cohort. Of these, 31 (94%) were women.

Of the 703 individuals who were screened, 42 (5.9%) scored ≤ 17 on the MMSE (screened positive), and were thus considered cases of suspected dementia. The average time between screening and clinical assessment was 7.7 months. Clinical evaluation was not done in 14 out of the 42 subjects selected for this evaluation in phase II of the study. Four had died, one had a stroke, and one had moved out of the area since the screening. Two subjects and their families refused clinical evaluation. Two had psychiatric illnesses, and four subjects did not have any evidence of dementia.

Table 4. Age, sex and education distribution of the study population

	Male		*Female*		*Total*	
	n	*%*	*n*	*%*	*n*	*%*
Age (years)						
65-75	233	33.1	373	53.1	606	86.2
76-85	40	5.7	48	6.8	88	12.5
>85	1	0.1	8	1.1	9	1.3
Total	274	39	429	61	703	100
Education (years)						
Illiterate	2	0.7	31	7.2	33	4.7
< 6 years	81	29.6	243	56.7	324	46.1
≥ 6 years	191	69.7	155	36.1	346	49.2

We found 28 subjects suffering from dementia yielding a crude prevalence rate of at least 3.98% (95% CI = 2.6%-5.7%) among those over 65 years of age in our study area. The distribution of patients with dementia of all types by age and sex and the corresponding prevalence rates are summarized in table 5. Increased age was accompanied by increased prevalence of all dementias in both men and women (Table 5), and the overall dementia prevalence was higher in females than males (M: 3.3%; F: 4.4%). However, this gender difference did not reach statistical significance. Among the 28 subjects with dementia, 20 (71.4%) were classified AD, four (14.3%) as vascular dementia, and two (7.1%) as mixed dementia (AD and vascular). In addition, one subject with probable DLB, and another with dementia due to syphilis were found in phase II of the study. Of the 20 subjects who had AD, 14 had probable AD and six had possible AD. The diagnosis of dementia due to syphilis was made on positive serology (VDRL and TPFIA) and clinical evidence of dementia.

Table 6 shows the distribution of demented subjects according to the level of education. Although there was a higher prevalence of dementia among illiterate people (9.1%) than literate people (3.7%), the prevalence of dementia was not significantly different between those who had less than six years of education and those who had six or more years of education.

Comment

We have reported on the results of a prevalence survey of a population aged 65 years and older in a well-defined community dwelling population in a semi-urban area of Sri Lanka. Despite the limitations of a small sample, this is the first report on the prevalence of dementia and AD in a community survey of older persons in Sri Lanka. Furthermore, these findings assume importance in the context of Sri Lanka's rapidly aging population who will constitute about one fifth of the entire population in the next 20 years.

We selected Ragama as the target area for the study because the Faculty of Medicine, University of Kelaniya, and the University Hospital are located in the area providing easy access to the study population. Furthermore, the selected study population in Ragama does not vary significantly in education, literacy and cultural sophistication from other urban/semi-urban areas of the country, and is representative of the general Sri Lankan population. All subjects in our study population were living with their families at the time of the survey. This allowed us always to question a relative, which was particularly useful when subjects were cognitively untestable due to severe impairment.

Table 5. Age- and sex-specific prevalence rates of dementia of all types

Age (years)	*Male*		*Female*		*Both sex*	
	n	*%*	*n*	*%*	*n*	*%*
65-75	7	3	13	3.4	20	3.3
76-85	2	5	5	10.4	7	7.95
> 85	-	-	1	12.5	1	11.1
Total	9	3.3	19	4.4	28	3.98

Table 6. Distribution of demented subjects according to level of education

Level of education	*Male*		*Female*		*Both sex*	
	n	*%*	*n*	*%*	*n*	*%*
Illiterate	_	_	3	9.7	3	9.1
< 6 years	1	1.2	11	4.5	12	3.7
≥ 6 years	8	4.1	5	3.2	13	3.8
Total	9		19		28	3.98%

Increasing age and illiteracy are associated with higher prevalence of dementia [Launer et al., 1999]. This pattern is also seen in the present study (Tables 5 and 6). Of the 28 subjects identified as demented in total, 71.4% satisfied the criteria for AD and 14.3% satisfied the criteria for vascular dementia. This finding is at variance with observations made in other Asian countries such as China, Japan, Taiwan [White, 1992] and India [Shaji et al., 1996] where the prevalence of vascular dementia was higher than AD. However, for most European and American populations prevalence rates for AD have been comparable to our rate, while rates for vascular dementia have varied between 0 and 30% [White, 1992].

The major finding from this population was that the overall prevalence of dementia of all types was 3.98%. This is similar to those reported from Western Europe and the United States [Hendrie, 1999]. The prevalence rate of dementia in this study could actually be higher because seven subjects who screened positive in phase I could not undergo clinical evaluation in phase II for reasons including death, disability, migration and refusal to consent. Therefore, it is possible that the prevalence rate of dementia reported here may be an wider-estimate of the true rate.

A few methodologically robust and comparable studies from India have reported varying total and age-specific prevalence rates of dementia suggesting regional differences in prevalence within the sub-continent. Overall dementia prevalence was 1.36% in the Ballabgarh sample [Chandra et al., 1998], which was 73.3% illiterate; 2.7% in the Madras sample [Rajkumar & Kumar, 1996], which was 53.5% illiterate; 3.5% in the Thiroporur sample [Rajkumar et al., 1997], which was 91.2% illiterate; and 3.4% in the Thiruvaniyoor sample [Shaji et al., 1996]. This difference in disease frequency may be due to geographical variation in the distribution of critical risk and protective factors for and against dementia.

However, it is also possible that lower life expectancy and high mortality among those suffering from dementia in developing nations [Katzman et al., 1994] compared with those in the developed nations [Heyman et al., 1996], may contribute to the varying total and age-specific prevalence of dementia reported from India. Therefore, the differences between our prevalence rate and those of some studies from India may partly reflect true regional differences in incidence, and may partly be a function of lower life expectancy and differential survival.

After establishing the prevalence rate of dementia and AD in the survey area, we proceeded to study the association between AD and medial temporal lobe atrophy, and two confirmed risk factors in the cohort of patients identified from the prevalence survey.

Medial temporal lobe atrophy, apolipoprotein genotype and serum homocysteine in Sri Lankan patients with Alzheimer's disease

AD is likely to be caused by a combination of aging, genetic and environmental factors. Susceptibility to AD has also been linked to a polymorphism of the apolipoprotein E (apoE) gene on chromosome 19 [Roses, 1996] and elevated serum total homocysteine (tHcy) [Clarke et al., 1998].

The apoE gene constitutes a major susceptibility factor for the development of the familial and sporadic forms of late-onset AD [Saunders et al, 1993; Corder et al., 1993]. Three common alleles ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) encode the three major apoE isoforms. The risk for AD is higher, and the age of onset lower, for $\epsilon 4$ heterozygotes and even more so for $\epsilon 4$ homozygotes compared with the risk and onset age in patients with other genotypes [Saunders et al., 1993].

Homocysteine levels rise with age [Koehler et al, 1996]. In community-dwelling cohorts of older people, cognitive impairments relate to higher homocysteine [Riggs et al., 1996; Budge et al., 2000], and elevated serum tHcy is associated with dementia and AD [Clarke et al., 1998; Seshadri et al., 2002]. A prospective, observational study has shown that an increment in the serum tHcy level of 5|nmol / 1 increases the risk of AD by 40% [Seshadri et al., 2002], and this association appears to be independent of age, sex, apoE genotype and other putative risk factors for AD. Furthermore, higher serum tHcy levels predicts greater decline of medial temporal lobe widths in patients with AD [Clarke et al., 1998], and medial temporal lobe atrophy is associated with AD [Jobst et al., 1992].

The medial temporal lobe (MTL) of the brain is important for normal cognitive function, notably for memory, and is the region with the most extensive pathological change and atrophy in AD [Ball et al., 1977]. Longitudinal studies have shown that early thinning of the MTL structures predicts cognitive dysfunction [de Leon et al., 1996; Jack et al., 1999], and detecting medial temporal lobe atrophy on CT scans improves the accuracy of ante-mortem diagnosis of AD [Jobst et al., 1992].

In the present study, we examined the association of AD with serum tHcy levels and apoE genotype, and the usefulness of measuring MTL thickness for the diagnosis of AD in Sri Lankan patients using a case-control design.

Materials and methods

Patients and controls

23 patients with a clinical diagnosis of AD were drawn from the cohort of patients with dementia detected during our community prevalence survey [De Silva et al., 2002; De Silva et al., 2003]. The 21 controls without detectable cognitive deficit were drawn from volunteers from the same geographical area. Written informed consent was obtained from both patients and control subjects or their primary caregivers, after discussion of risks and benefits of participation. Diagnosis of AD was made on established criteria [American Psychiatry Association, 1994; McKhann et al., 1984] described in detail above [De Silva et al., 2003]. All subjects underwent measurement of body mass index (BMI) and cognitive assessment by CAMDEX [Roth et al., 1986], from which Mini Mental State Examination (MMSE) and Cambridge Cognitive Examination (CAMCOG) scores were derived.

Radiology

Temporal lobe oriented brain CT imaging was done in all subjects as previously described [Jobst et al., 1992]. The minimum width of each MTL was measured from the hard copies of scans by a Consultant Radiologist who was blind to the diagnoses and cognitive assessment scores. The width reported here is measured in millimeters at the narrowest point, irrespective of side.

Serum homocysteine and apoE renotyping

Non-fasting blood samples were collected from all subjects into EDTA rubes, centrifuged immediately, and stored in aliquots of plasma and packed cells at -70C. Total time from blood sampling to storage was always less than 30 minutes. Plasma and packed cell samples were transported in dry ice to the Oxford Project to Investigate memory and Aging, University of Oxford, by courier for determination of serum tHcy and apoE genotype. Serum tHcy levels were determined by high performance liquid chromatography with fluorescence detection [Clarke et al., 1998], and apoE genotyping was done using polymerase chain reaction techniques [Wenham et al., 1991]. All measurements were obtained in a blind manner to each other and to the diagnoses.

Statistical analysis

The differences between means for numeric variables between AD patients and controls were tested with Student's unpaired t-test. Distribution of apoE alleles was analysed using Fisher's exact test.

Results

Characteristics of the study population are shown in Table 7. The patients and controls were well matched for age, sex, full-time education, and body mass index. The cohort included 28 women and 16 men. The mean age of the study population was 71.2 ± 5.6 (SD), and the majority of subjects were between 65-75 years (69.5% of patients and 85% of controls). The disease severity among the patients is reflected by the low cognitive scores (MMSE and CAMCOG).

The mean serum tHcy was significantly higher in patients with AD than in controls (Table 7). This association was not significantly altered by adjustment for age, sex, BMI, serum folate and vitamin B_{12}, and serum creatinine. The mean minimum medial temporal lobe thickness was significantly less in AD patients compared to that of controls (Table 7). AD patients with one or two alleles of apoE4 had a higher mean serum tHcy and a lower mean minimum medial temporal lobe thickness than controls (data not shown). However, these differences were not significant.

Table 7. Characteristics of the study population

	AD patients (n=23)	*Controls (n=21)*
Age	72 (6.8)	70.5 (3.9)
Sex, % male	34.8	38.1
Full-time education	7.4 (4.2)	7.9 (4.1)
BMI	25 (1.4)	24.5 (1.5)
Systolic BP	132 (21)	127 (21)
Blood urea	27 (9.5)	26.2 (6.1)
Serum creatinine	1 (0.3)	1.1 (0.2)
Total cholesterol	205 (47)	228 (39)
Vitamin B_{12}	483 (134.5)	494 (139)
Serum folate	7 (3.7)	8.7 (4.3)
MMSE score (maximum 30)	8.5 (6.2)	27.1 (1.6)
CAMCOG score (maximum 105)	26.1 (22.8)	88.8 (4.8)
Serum homocysteine (μmol/L)*	13.3(5.3)	8.3 (2.2)
Minimum MTL thickness (mm)*	5.6(2.1)	14.9 (2.2)

*P<0.001 (t-test)

The distribution of apoE alleles in the study population is shown in Table 8. The apoE4 allele frequency was 30.4% in AD patients compared with 4.5% in controls. After adjusting for differences in age, sex and years of full-time education, the OR of AD for the presence of one or more apoE4 alleles compared with none was 10.39 (p=0.010).

Table 8. Distribution of apoE alleles in the study population

	AD patients (n=23)	*Controls (n-21)*
ApoE 2	1	3
ApoE 3	31	37
ApoE 4†	14	2

†P=0.003 (Fisher's test)

Comment

In the absence of a biological marker, the diagnosis of dementia is still largely based upon clinical and neuropsychological criteria and AD, in particular, is usually diagnosed by exclusion of other known causes of dementia. Because no peripheral biochemical marker for AD has been found, a definitive diagnosis of the disorder can be made only if histologic confirmation is obtained by performance of a cerebral biopsy or an autopsy. In the absence of such histopathological analysis, a provisional diagnosis of probable or possible AD can be reached only after extensive neurological and neuropsychological testing.

Structural imaging with CT scans to detect medial temporal lobe atrophy, a feature of AD, is a valuable aid for the diagnosis of AD, particularly in subjects with early symptoms of the disease [de Leon et al., 1989; Jobst et al., 1992]. It may also be useful as a radiological marker for the progression of the disease, and to monitor the effectiveness of therapies designed to slow or arrest the neurodegenerative process. Although AD is not the only cause of atrophy of the medial temporal lobe, it is by far the commonest cause in the elderly [Jobst et al., 1992]. In this study we have shown that medial temporal lobe atrophy is associated with AD in Sri Lankan patients. Therefore, MTL-oriented CT scanning should improve the accuracy of ante-mortem diagnosis of this disease in our patients. This will benefit selection of patients and make treatment cost-effective, which is particularly relevant for developing countries such as Sri Lanka.

Risk factors for developing AD include age, low levels of education, Down's syndrome, high plasma homocysteine, and the presence of apoE4 allele. Of the many putative genetic risk factors for AD, only the gene for apoE has thus far been shown to be associated with both early- and late-onset AD of sporadic and familial varieties [Corder et al., 1993; Saunders et al., 1993]. The E4 allele of the apoE gene has been shown to be associated with AD in studies of white [Cummings et al., 1998], Chinese [Katzman et al., 1997] and Indian [Ganguli et al., 2000] populations, and probably represents a major risk factor for AD in all ethnic groups across all ages between 40 and 90 years [Farrer et al., 1997]. We have previously shown that AD was associated with increasing age and low levels of education in Sri Lankan patients [De Silva et al., 2003]. In the present study, we have reported from Sri Lanka for the first time that the presence of apoE4 and high tHcy are strongly associated with AD compared to non-demented people. The association between AD high serum tHcy was independent of age, sex, BMI, serum folate and vitamin B_{12} and serum creatinine, and that between AD and apoE 4 was independent of age, sex and years of full-time education. These results are in agreement with previous studies [Ganguli et al., 2000; Seshadri et al., 2002], and confirm that high serum tHcy and the presence of one or more apoE 4 alleles are independent risk factors for AD in Sri Lankan patients.

The prevalence of AD and other dementias among the elderly in Ballabgarh, India, was reported to be one of the lowest in the world [Chandra et al., 1998]. However, the strength of association between apoE4 with AD, even within the Ballabgarh study cohort, was similar to those reported elsewhere [Evans et al., 1997; Hofman et al., 1997; Myers et al., 1996]. The odds ratio for this association in the Ballabgarh cohort was within the range reported from other community-based studies [Evans et al., 1997; Hofman et al., 1997; Myers et al., 1996], and is also similar to the findings reported here. As such, the differences in disease prevalence among elderly people within the Indian subcontinent may not be explained by differential risk with respect to the apoE polymorphism. Differences in other genetic and non-genetic risk and protective factors such as serum homocysteine, or survival effects may be alternative explanations for differences in disease prevalence within the subcontinent.

Conclusion

Sri Lanka has one of the fastest aging populations in Asia, and the reported prevalence of dementia is one of the highest in the Indian subcontinent. To our

knowledge this is the first prevalence study of dementia, using culturally appropriate tools and established diagnostic criteria, reported from this country. These prevalence rates will increase further as the proportion of elderly in our population increases. Therefore, dementia will pose a major challenge in the future, in general to the health sector in and in particular to families who care for these patients.

We have shown that AD is strongly associated with established risk factors, such as age, low levels of education, the presence of apoE4 genotype and high serum tHcy in this patient group. Raised serum tHcy is potentially reversible by increasing the dietary intake of vitamin B_{12} and folate. Furthermore, we have demonstrated the association between MTL atrophy and AD in our patients, and thereby the value of MTL-oriented CT scans to improve the accuracy of ante-mortem diagnosis of AD. Accurate diagnosis will enable more targeted treatment with expensive cholinesterase inhibitors, which are effective in slowing the rate of cognitive decline.

Interestingly, comparable studies from various parts of India have reported varying, but low prevalence rates of dementia and AD. The rates reported in Sri Lanka are much higher, and are comparable to those reported from the US and Europe. Furthermore, the prevalence of AD was much higher than vascular dementia in our study population, which is at variance with reports from India where vascular dementia accounted for a greater percentage of dementia patients than AD. This suggests that there are regional differences in dementia prevalence within the subcontinent. The difference in disease frequency, if genuine, might suggest geographical variation in the distribution of critical risk and protective factors for and against AD. Therefore, multicentre surveys with comparable methodology within the Indian subcontinent may help determine new risk and protective factors, which would even be useful for the formulation of new aetiological hypotheses for AD.

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