Associative learning and face recognition in MCI and early Alzheimer's disease-a Neuropsychological and Brain Volumetric study

Thesis submitted in partial fulfilment of the rules and regulations for the requirement of the degree of DM in Neurology

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2016-2018

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DECLARATION

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ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to Dr Ramshekhar Menon, Additional Professor of Neurology, my guide for the study, for his suggestion of topic, expert guidance, constant review, kind help and keen interest at each and every step of the study. His vibrant ideas and unmatched enthusiasm for the project made this thesis an exciting journey for me. Doing a thesis on cognition was an aspiration for me when I joined this institute and with the vision of my guide this aspiration has turned into a reality.

I express my sincere gratitude to Prof. Muralidharan Nair, Senior Professor and Head, Department of Neurology for his guidance, encouragement and valuable suggestions during the period of the study.

I express my sincere thanks to Dr.Sankara Sarma P, Professor of Biostatistics, Achutha Menon Centre for Health Science Studies for helping me with the statistical analysis of this study and my colleagues in the Department of Neurology for their valuable input and assistance to the study. I would like to thank the neuropsychologist Miss Nandini M for the hard work she has put in testing the patients. The patience with which she has worked and conducted the tests has been exemplary. I would also like to acknowledge the role played by Miss Mridula, Neuropsychologist in the initial implementation of the new tests. My sincere thanks are due to Dr Sheelakumari R for her help in analyzing the volumetric data. Her inputs were of immense help when writing and designing this manuscript. Special acknowledgement has to be made for the VBM maps which have been constructed with so much patience by her.

I would like to thank my wife Dr. Sarita Sanke for being of great help in proof reading the manuscript. I would like to thank her for the unflinching support that she

has provided in doing my thesis work. Her constant motivation has helped me in meeting the deadlines. I would also like to thank my parents Dr. Narinder Nanda and Dr. Meera Nanda; my sister Dr. Neha Nanda for being a source of inspiration and showing me the way. Their constant guidance has been of immense help in helping me compile the thesis.

Last but not the least, I extend my gratitude to all my patients, volunteers and their primary caregivers who willingly participated in this study.

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INTRODUCTION

Dementia is an important cause of morbidity in the elderly age group. Most common cause of dementia in elderly individuals above 60 years is Alzheimer's disease. The worldwide societal and economic burden of patients with dementia is set to increase many fold over the next few decades. A major part of this burden will have to be borne by countries such as India where there is expected to be an exponential growth of the ageing population. The prevalence of MCI(Mild Cognitive Impairment) in subjects aged 70-89 is estimated to be approximately 16%. Subdivided 11.1% had amnestic MCI, and 4.9% had non-amnestic MCI. The prevalence of MCI is higher in men and noted to increase with increasing age.[1]TheAnnual Conversion Rate for progression of MCI to dementia is approximately 5-10% and many people with MCI will not progress to dementia even after 10 years of follow-up.[2] Identifying MCI patients allows for monitoring of progression, provides opportunity for appropriate counselling and offers a possible therapeutic window for intervention in the future. Alterations in brain integrity are evident prior to the manifestation of the cognitive symptoms associated with Alzheimer's dementia (AD), and also predate the symptoms associated with the prodromal stage of MCI.[3] Therefore, considerable research effort is currently focused on finding reliable markers that identify individuals at high risk for AD, even before clinical symptoms are evident. The apolipoprotein E (APOE) ε4 allele is considered a reliable biomarker for increased risk of conversion from MCI to AD, and for the transition from asymptomatic to MCI.[3]

From a neuropsychological perspective, the goal is to identify cognitive domains and specific measures that can identify cognitively intact individuals at

increased risk for subsequently developing MCI and AD. Episodic memory tasks (particularly delayed recall) have generally been considered the most consistent cognitive measure predictive of progression from age-appropriate memory performance to MCI and AD.[4-6] However, it has become increasingly evident that the pre-clinical period is also characterized by prominent difficulties in other cognitive domains including executive functions (attention), language, and working memory.[7] In addition, the deleterious impact for ε4 allele carriers is evident in several other cognitive domains as well.[8]

fMRI(functional magnetic resonance imaging)has been used to demonstrate activity in concerned regions on learning and recognition testing during face name pairs in prior studies.[9]MCI and AD patients have an impaired ability in associating names to faces.[10]We have attempted to analyse this aspect of neuropsychology in our study with regards to changes in volumetry of available imaging data for the same cohort. As there were no tests currently available for use in Indian population for face name pair association we have devised a test for same(South Indian subjects) and after a pilot study have implemented the same in our cohorts.

In our study, we shall also be examining; a person-identity semantic memory test that is recognition accuracy for famous names, as a potential early marker for identification of increased risk of episodic memory decline in asymptomatic individuals. The selection of a famous name recognition accuracy task is based on converging evidence showing a disproportionate impairment for person identity knowledge in MCI and AD compared to general semantic memory.[11,12]. Findings from fMRI studies also indicate that recognition of famous names consistently produced patterns of BOLD(Blood Oxygen Level Dependent) signal activity in a neural network that overlaps with the default network, and includes regions known to

be affected early in the AD process.[13,14]Currently there are no available neuropsychological tests which assess the ability to identify and correctly name famous faces for South Indian subjects. Thus we have devised a tool for same and after a pilot study have used it in our cohort.

We propose this study to fill in the knowledge gap on performance in paired associate learning ability and famous faces recognition in MCI(mild cognitive impairment) patients and Alzheimer`s Disease patients and to correlate these differences with atrophy as ascertained by volumetric studies.

REVIEW OF LITERATURE

For prediction of progression from age appropriate memory performance to MCI and AD the most commonly employed cognitive measure is an episodic memory task specifically a delayed recall.[4,6] From recent studies it is increasingly evident that the pre-clinical period of AD can be characterised by impairment in other cognitive domains which includes executive functions; language and working memory.[7]Likewise the impairment of semantic memory in AD has also been well documented. A historical body of literature suggests a temporal gradient for memory loss in AD dementia,such that newly learned information is thought to be more vulnerable to disease pathology, with relative preservation of early or remote memories. This is known as Ribot's Law.[15] Even in subjects with MCI impairment on tests of semantic memory has been demonstrated.[16] A study in 2007 found deficits in both episodic and semantic memory 3 years prior to progression to AD.[17] The measures usually employed for testing semantic memory include object naming and category fluency. Recently there has been evidence showing a disproportionate impairment in MCI and AD for person identity knowledge compared to general semantic memory.[11,12] In a study,utilising the impact of recognition of famous faces on long term episodic memory decline it was observed that individuals whose episodic memory declined more than 1 SD had performed worse on the test for recent famous names as compared to individuals who had a stable episodic memory. This was also correlated with the finding of smaller baseline hippocampal volume for individuals who had a significant memory decline. The reason put forth for this finding was that some of the recent facescarry an autobiographical significance for a person. Thus this led to the proposal of concept that recognition of famous faces has both a semantic and an episodic component.[18]Even on fMRI studies there has been

greater activation noted in hippocampus for recent famous names as compared to remote famous names.[19,20] From a previous study it is evident that separation of famous names into recent and remote time epochs may be quite informative in predicting the course of episodic memory in the pre-clinical phase.[18]

In a recent study done on normal aged subjects wherein the performance on famous names was correlated with amyloid burden it was shown that individuals who were amyloid positive had worse performance on recent famous names as compared to nosignificant difference for remote famous names. It is possible that recently acquired semantic information is re-encoded poorly and relies on hippocampally mediated consolidation for longer periods of time. Thus it is proposed that as memory traces along age-related lesions, to hippocampally mediated neocortical connections, recent memories still dependent on the hippocampus are lost preferentially.[21]

In a study from UK in 2007 it was observed that MCI patients at risk for subsequent development of dementia were impaired in the naming for faces and buildings more as compared to naming for objects relative to healthy controls. In both MCI and AD groups the pattern of naming accuracy was the same: GNT(graded names test)> GBT(graded buildings test)> GFT(graded faces test). Proper names are thought to be more difficult to retrieve than common nouns due to the weak and arbitrary links between a proper noun and its reference.[22] Similar results were published in 2002 wherein an attempt was made to assess performance of people with AD and MCI in naming tasks pertaining to famous faces; animals and objects. It was observed that individuals with AD as well as MCI had an impairment in naming famous faces; animals and objects but the degree of impairment was more for famous faces.This was in contrast to the control group which did not show any difference in performance for names vis a vis objects or animals. The AD patients were also

impaired on the recognition component of these tests and were noted to have difficulty in identifying which one is the famous [face/name]. Furthermore the decline in semantic memory was proportional to the severity of dementia as assessed by MMSE scores.[23]

Forming an association between random name and a face is a particularly difficult associative memory task, because names and faces are inherently unrelated. It requires the formation of a novel association between inherently unrelated items of information across the verbal and visual domains. In addition to the hippocampus, the encoding of novel face-name associations likely requires the integration of several other brain regions, including the prefrontal and fusiform cortices. A large number ofrecent functional imaging studies have suggested that the dorsolateral prefrontal cortex is activated during memory tasks.[24] As per the findings of an fMRI study involving novel face name pairs it was found that this encoding is subserved by a distributed functional network of brain regions, including the hippocampal formation, dorsolateral prefrontal cortex, pulvinar nucleus of the thalamus, fusiform and adjacent areas of visual association cortex. Further it was noted that the activation in these regions was highly significant and the pattern of activation was very consistent across individual subjects.[25]It has for long been postulated that tasks which require relational or associative processing between stimuli are more likely to activate anterior regions of the hippocampal formation, as opposed to tasks that require processing of single stimuli, which tend to activate posterior hippocampal and parahippocampal regions. Studies in the past have further provided evidence that the successful encoding of novel cross-modal associations is subserved by a specific set of brain regions, in particular, anterior portions of the hippocampal formation bilaterally and the left inferior prefrontal cortex.[26]The utility of face name pairing in explicit memory

training for MCI patients has also been demonstrated by a recent study showing an increase in connectivity and activation of widespread brain cortex following the face-name pairing task.[10]

In a recent study undertaken to assess the performance on associative memory learning the performance of 29 subjects with MCI was compared to 28 controls. The results showed a significant difference between the various parameters on associative recognition between the two groups of MCI and controls.[27]

With regard to imaging basis recent studies have proved a dominant role of fusiform face area in the recognition of famous faces as was demonstrated by a study involving famous faces involving Prime Minister and President of Israel.[28]Previous studies involving comparison among activation patterns in healthy right handed individuals for famous versus unfamiliar faces demonstrated significantly larger intensity changes over widespread areas of the prefrontal and lateral temporal regions. With regard to famous faces increased activity was also observed in hippocampal and parahippocampal regions. Newly learned faces when compared to a novel unfamiliar face produced increased activity in frontal and/or parietal regions. As the famous faces have been proposed to intersect both autobiographical and semantic memory systems thus they lead on to more extensive and bilateral temporofrontal region activation as compared to other types of stimuli. Encoding of unfamiliar faces in the same study showed increased activity in medial and posterior parietal area which was likely related to the structural encoding area. This contrasted with newly learned faces which produced greater activity in the right supramarginal gyrus. This was postulated to represent a temporary storage function of the parietal lobes in mediating recent episodic retrieval operations.[29]

With regards to volumetric studies in AD patients it has been demonstrated that presymptomatic and mildly affected individuals have significantly increased rates of hippocampal atrophy. As the disease advances there is a shift in the pattern of temporal lobe atrophy with the inferolateral regions of thetemporal lobes showing the most significantly increased rates ofatrophy by the time the patients are moderatelyaffected. Significantly increased rates of medial parietal lobeatrophy have been described at all stages, with frontal lobe involvementoccurring only later in the disease. The occurrence of atrophy much before the onset of symptoms in AD patients has also been well described.[30]

Studies in the past which have attempted to predict the conversion of MCI to AD have demonstrated that individuals with MCI who convert to AD have reduced volumes in a number of brain regions, including superior, middle and inferior temporal gyri, anterior hippocampus and amygdala, orbitofrontal cortex, posterior cingulate and the adjacent precuneus, insula, fusiform gyrus, and parahippocampalwhite mattter. Additionally noted was the larger size oftemporal horns of the ventricles. It is therefore evident that MCI who convert to AD have already reached levels of widespread and significant brain atrophy at baseline.[31]

Another study demonstrated that MCI who convert to AD had positive baseline SPARE-AD (Spatial Pattern of Abnormalities for Recognition of Early AD) and atrophy in temporal lobe gray matter (GM) and white matter (WM), posterior cingulate/precuneus, and insula. Even the biochemical pattern ofMCI individuals that converted to AD had mostly AD-like baseline CSF biomarkers. [32]Among the pattern of atrophy in amnestic MCI versus non amnestic MCI it was demonstrated that individuals with amnestic MCI had significant hippocampal atrophy compared with controls and that this pattern did not differ significantly from the patients with AD.[33]

The entorhinal cortex (EC) and the hippocampal formation (HF) are part of the mesial temporal lobe memory system. The EC connects the neocortex with the HF via the perforant path, thereby providing the latter with multimodal sensory information.[34] These brain regions have received special attention in both post mortem and in vivo investigations on the pathophysiology of AD, since memory dysfunction is one of the earliest hallmarks of AD. Post mortem pathological studies have implicated the EC and the transentorhinal region as early sites of involvement in AD and in individuals with mild cognitive impairment.[35]Furthermore, Braak and co-workers have suggested that early AD-related pathology may start in the EC and the transentorhinal region and then spread to the HF.[36] In fact, pathological studies carried out on brains from patients with very mild AD have demonstrated greater neurodegenerative changes (i.e. plaques and tangles) in the EC and HF than in other brain regions.[37]In a study involving 27 MCI participants who were followed up for 36 months of which 10 converted to AD it was found that though both hippocampal and entorhinal volumes were independently predictive of likelihood of conversion to AD; right entorhinal cortex volume had the best concordance rate of 93.5%. The greater atrophy of right entorhinal cortex in prodromal AD is an intriguing finding and needs to be confirmed with larger studies.[38] In another study it was demonstrated that EC volume differentiated those at risk for developing dementiabetter than hippocampal volume. Those subjects with atrophy of the EC werefound to be 1.6 times at greater risk of developing AD compared to those with hippocampal atrophy, who were 1.5 times at greater risk.[39]

Attempts have been made in the past to develop neuropsychological predictors of conversion from MCI to AD. In one such study study, 39 (26.4%) of 148 MCI patients converted

to AD during a mean of 46.6 months. Of the 39 MCIconverters, 38 (97.4%) were classified at baseline asamnestic MCI; 36 (41.4%) of 87 multiple-domain amnestic patients (md-MCI-A) and 2 (9.5%) of 21 pureamnestic patients (MCI-A) converted to AD on follow-up. When the sample was restricted to 3 years of follow-up, the conversion rate was 30.4% (35/115), with50.0% (32/64) of multiple-domain amnestic patientsand 10.0% (2/20) of pure amnestic patients convertingto AD. Among the neuropsychological predictors for AD it was found that that episodic memory and executivefunction deficits are among the most robust and earliestpredictors of AD. This study also put forward the notion that MCI patients with memory plus other cognitive domain deficits, rather thanthose with pure amnestic MCI, constituted the highrisk group for conversion to AD.[40]In another study done to evaluate the neuropsychology profiles of MCI who did not convert to AD even after a period of 10 years, it was shown that those patients had a better performances at baseline in memory (Word Delayed total recall) and non-verbal abstraction tests (Raven's Progressive Matrices)when compared to MCI patients who did convert to AD. Thus better performance in memory tests and non-verbal abstraction tests at screening could help predict clinical and neuropsychological stability in MCI cohort.[41]

In another 3 year follow up study aiming to predict conversion of MCI to AD using neuropsychological parameters it was found that 24 out of 105 subjects with MCI converted to AD. A logistic regression analysis was done which determined that the long delay cued recall and the performance time of the Rey figure test were the best predictive tests of conversion to dementia after an MCI diagnosis. Concerning the sociodemographic factors, sex had the highest predictive power. Thus, it was concluded that the data obtained in the episodic verbal memory tests and tests that assess visuospatial and executive components may help to identify people with MCI

who may develop AD in an interval not longer than 4 years, with the masculine gender being an added risk factor.[42]

In a study aiming to evaluate the prognostic ability of genetic, CSF, neuroimaging, and cognitive measurements obtained in the same participants it was found that patients with MCI converted to AD at an annual rate of 17.2%. Subjects with MCI who had abnormal results on both FDG-PET and episodic memory were 11.7 times more likely to convert to AD than subjects who had normal results on both measures ($p \leq 0.02$). Thus complementary information provided by these biomarkers may aid in future selection of patients for clinical trials or identification of patients likely to benefit from a therapeutic intervention.[43]The data with regards to face-name associative learning and their volumetric correlations in MCI and early AD are lacking.

AIM AND OBJECTIVES

AIM of the study: To assess the visual and verbal associative learning ability and face recognition abilities among Malayalam-speaking subjects with stable amnestic MCI(mild cognitive impairment) relative to early stage dementia due to Alzheimer`s Disease (AD)and cognitively normal healthy controls (NC) and to correlate these differences with regional brain volumes as ascertained by automated regional volumetry (ARV).

Objectives of the study:

1. To develop tests for face-name paired associate learning and recall and famous face recognition for Malayalam-speaking subjects.
2. To compare the visual-verbal associative learning-recall and face recognition abilities in amnestic MCI patients with respect to Alzheimer`s Disease patients and healthy controls.
3. To correlate the above differences with standard neuropsychological tests for verbal and visual memory
4. To determine if associative learning-recall abilities correlate with structural volumetric differences ascertained between the same groups using automated regional volumetry.
5. To determine if deficits in associative learning-recall predate structural volumetric changes in MCI and early AD.

Hypothesis:

1. Verbal-visual paired associate learning-recall and face recognition abilities are affected in amnestic MCI.
2. These tests are useful to demonstrate impairment in patients with early cognitive disorders who are at a risk of progression to dementia even prior to the development of structural volumetric changes on MRI.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING: The study was designed as a hospital based cross sectional observational study. The cases were selected from among patients with cognitive deficits attending the outpatient general neurology and Memory clinic of SCTIMST. The subjects were screened for eligibility for enrolling in the study. If they satisfied the inclusion criteria, the procedure of the study was explained to them and informed written consent was obtained. For the conduct of this study consent was obtained from IEC vide reference no. IEC/978.

Inclusion criteria:

The cases were patients aged above 55 years of age attending the outpatient service in SCTIMST consenting for the study and satisfying the inclusion criteria.

- The lower limit for age was taken as 55 years and the upper limit was selected as 85 years.
- A minimum of 6 years of formal educationwas a prerequisite.
- Subjects were on stable doses of medications for cognition or behaviour for at least 2 months prior to screening.
- Subjects were willing to attend and undergo the recommended tests according to study protocol.
- HADS(Hospital Anxiety and Depression scale) score of <7 in both domains.
- Cases were selected in 2 categories, as characterized below:
    - Amnestic Mild cognitive impairment (MCI) – were classified as per Petersen criteria(2004):[44]

- Memory complaint corroborated by an informant
- Objective memory impairment for age
- Essentially preserved general cognitive function
- Largely intact functional activities
- Not demented

- Early Alzheimer's dementia (AD) – CDR(Clinical Dementia Rating) score of 0.5 to 1[45]; patient's met NIA-AA(National Institute on Aging and Alzheimer's Association) criteria for probable AD[46]; ACE M (Addenbrooke's Cognitive Examination- Malayalam) score ranging from 70-90; MMSE(Mini Mental Status Examination) score of 18 or more.

- **Normal controls (NC)** – CDR(Clinical Dementia Rating) 0-0.5; self-declared no impairment in cognitive or behavioural functions; normal neuropsychology test scores (ACE M score> 80)

Exclusion criteria

- CDR > 1
- Subjects with less than 6 years of formal education
- Any other major psychiatric or neurological disorders known to affect cognitive function or which may make it difficult for the subject to comply with the study requirements long-term
- Uncontrolled hypertension or uncontrolled diabetes that cannot be stabilized with treatment, major organ diseases, head injury, alcohol or other drug abuser, HIV(Human Immunodeficiency Virus) Disease or any other disorder known to affect cognitive or behavioral function or if under any medication known to significantly affect cognitive function; history suggestive of

OSA(Obstructive Sleep Apnoea); history of surgery with prolonged anesthesia;

- Subjects who cannot follow the study protocol for physical (vision; hearing) reasons
- Patients who did not have a primary caregiver
- Subjects with contraindications to MRI (e.g. pacemakers, artificial heart valves etc)
- Subjects (or in the case of patients, their authorized representative) who declined to give written informed consent for the study.
- Subjects who had evidence of extensive white matter changes on MRI(Fazeka's Grade II and above).

Methodology:

Those consenting for the study were interviewed with a structured proforma for details of age and mode of onset of cognitive decline along with relevant history.Consecutive patients presenting to the respective clinics during the study period were selected of which around 40-50 % were expected to be women. No sampling method was used. All the cases were subjected to clinical and neuropsychological evaluation. MRI brain with volumetry analysis was done for the subjects who gave their consent for same. All the subjects selected were interviewed by the primary investigator based on a structured proforma. The details collected included:

History

The demographic profile of the person was collected including: name, age, sex, occupation, educational status and place of residence. Details of disease: age and mode of onset, faculties affected, functional abilities, progression of the disease.

Examination

Detailed neurological examination.

Investigations

1. Standard biochemical tests for workup of MCI and AD with risk stratification for vascular risk factors were done. The biochemical tests involved screening the patients for their HIV status.
2. Neuropsychological tests as outlined below.
3. Imaging studies as outlined below.

Location:

Memory clinic and General Neurology OPD of SCTIMST.

Follow up

The follow up of these patients shall be done at the Memory Clinic(MNC) by both the principal and co-investigator for any deterioration in CDR and ADL.

The neuropsychological tools which were used are:

Standard Neuropsychological tools:

1. M-ACE (Addenbrooke's Cognitive Examination) validated for use in Malayalam speaking population[47]
2. RAVLT(Rey Auditory Verbal Learning test)
3. Weschler memory scale: visual and verbal subsets
5. Wisconsin card sorting test
6. Semantic battery
7. Trail making test A and B
8. Warrington face recognition test

The details of the standard neuropsychological battery are summarised in the appendix on neuropsychological tests.

Existing validated test for verbal paired associate learning:

WMS(Weschler memory scale) paired associate learning test[48]

Neuropsychological Tests which have been developed include:

1. **Face-Name pair association:** to assess the face name associative learning ability of subjects. This test was conducted after a pilot study which wascarried out in SCTIMST. The pilot study was needed as there are no tests to measure this association in Indian subjects.
2. **Famous faces test**: to assess the ability of subjects to assess the ability to recognize famous faces.This test was also conducted after a pilot study in SCTIMST. The pilot study was needed as there are no tests to measure this association in Indian subjects.

The test for face name pair association had to be devised as no such test validated for use in a South Indian population cohort exists. A similar test was devised for English speaking population and was used in an American cohort for assessing the connectivity changes in MCI subjects after giving them explicit memory training using face-name pairing.[10]

Test development:

For the evaluation of visual paired associate learning the following procedure was employed for devising a test:

- 16 random faces were selected from the internet(6 females, 10 males). These faces ranged from age group of 20-70.
- The faces were converted to a greyscale to obviate the effect of colour association for particular names.
- Intimate features such as eyes, facial, cosmetic and dressing/grooming characteristics were retained so as to enable their help(visual clues) in forming

an association for the stimulus.

- A gender and age appropriate name was assigned to each of the face. The names chosen were random and covered names from all three prominent communities endemic to the Malayalam population. The names assigned were one of the common names in a Malayali population.
- The name assigned was displayed in English and Malayalam along side the face in a font size of 44 on a standard power point presentation.
- Each stimulus consisting of 16 faces and their corresponding names in 1 set weredisplayed for 10 seconds each.
- Total time of administration for 1 set: 16*10sec= 160 seconds
- Total 3 such rounds were administered; in a successive fashion with no intervening waiting time.
- At no time was a verbal stimulus given. The patients had to visually associate the face and names.
- After 20 minutes of cessation of 3rd round, a free recall session was there; in which if a name was freely recalled correctly 2 points were given, otherwise 0. The maximum time allotted for free recall was 10 seconds per stimulus. The **FR(free recall)** scores were given as 0 or 2 for each stimulus. The cumulative FR scores were calculated for 16 stimuli and tabulated on Excel sheet.
- After the free recall phase was over, cued recall in which names would be displayed was done.
- The subjects had to choose one among the 3 names which were displayed on the screen. These 3 names were phonetically distinct and consisted of one of the name from the stimulus set(correct response) and 2 distractors. The 2 distractors were again randomly selected from the internet and consisted of

names from all the communities. These were common names in a Malayali population.

- The subjects were given 1 point if correct, 0 if incorrect. This gave the **Cued recall(CR)** scores. The cumulative CR scores for each stimulus were tabulated on Excel sheet.
- The time for this exercise was 5 seconds per stimulus.
- At the end the sum total was to be done in excel sheet.

For the assessment of verbal paired associate learning WMS-verbal paired associate learning for Malayalam speaking subjects was used which was validated before in an earlier published study.[48]The *Verbal Paired Association Learning Test* consisted of ten word pairs, six forming "easy" associates and other four "hard" word pairs that were not really associated. The list was read three times, with a memory recall after each trial. The first of word pair was presented, and the subject had to give the associated pair. When the subject gave an incorrect response, the correct response was told and the test was continued. One credit was given for each correct response. Total score was calculated as one-half of the sum of all the correct answers on easy pairs plus the sum of all correct associations to the hard pairs.

Procedure of developing famous personalities test:

15 random famous personalities were selected from the internet and constituted famous personalities from different walks of life including politicians, sportspersons, musicians, film actors and social activists. These famous personalities were chosen from a wide range of generations and most of them had attained prominence before 2000. Some of them had been active in news recently, whereas others had been out of news from the past decade. One of the personality chosen was Mother Teresa who was no longer alive. The famous personalities chosen were from

Malayali background or people of national importance. No international figures of prominence were used in our test. After choosing the famous personalities a Microsoft powerpoint slide was made in which besides the famous personality 2 distractors were used. The subjects had to correctly identify the famous personality and also had to name them. If they were able to identify the correct famous personality but not able to name them they were scored as 1 and if they were able to name them also they were scored 2.If they were not able to identify the famous personality a score of 0 was given. The time of exposure of the stimulus was fixed at 15 seconds per stimulus. If the subjects were not able to answer by the time allotted time, score of 0 was given and the next stimulus was given without coming back to previous slide. The above mentioned procedure was done for all the stimuli. At the end a total score was calculated in an excel sheet.

Methodology of MRI acquisition:

MRI (Magnetic Resonance Imaging) of Brain including Volumetry studies as described below were done for radiological assessment. Structural MRI was performed to identify the earliest grey matter changes seen in AD and MCI. The image acquisition was performed in 3T GE 750W Discovery with a standard transmit-receive head coil. In all subjects, MR images of the entire brain was obtained using a three dimensional T1 weighted BRAVO sequence(TR=8ms; TE= 3 ms; FA=12°; FOV= 25.6; 172 slices with thickness 1 mm).

Image Analysis

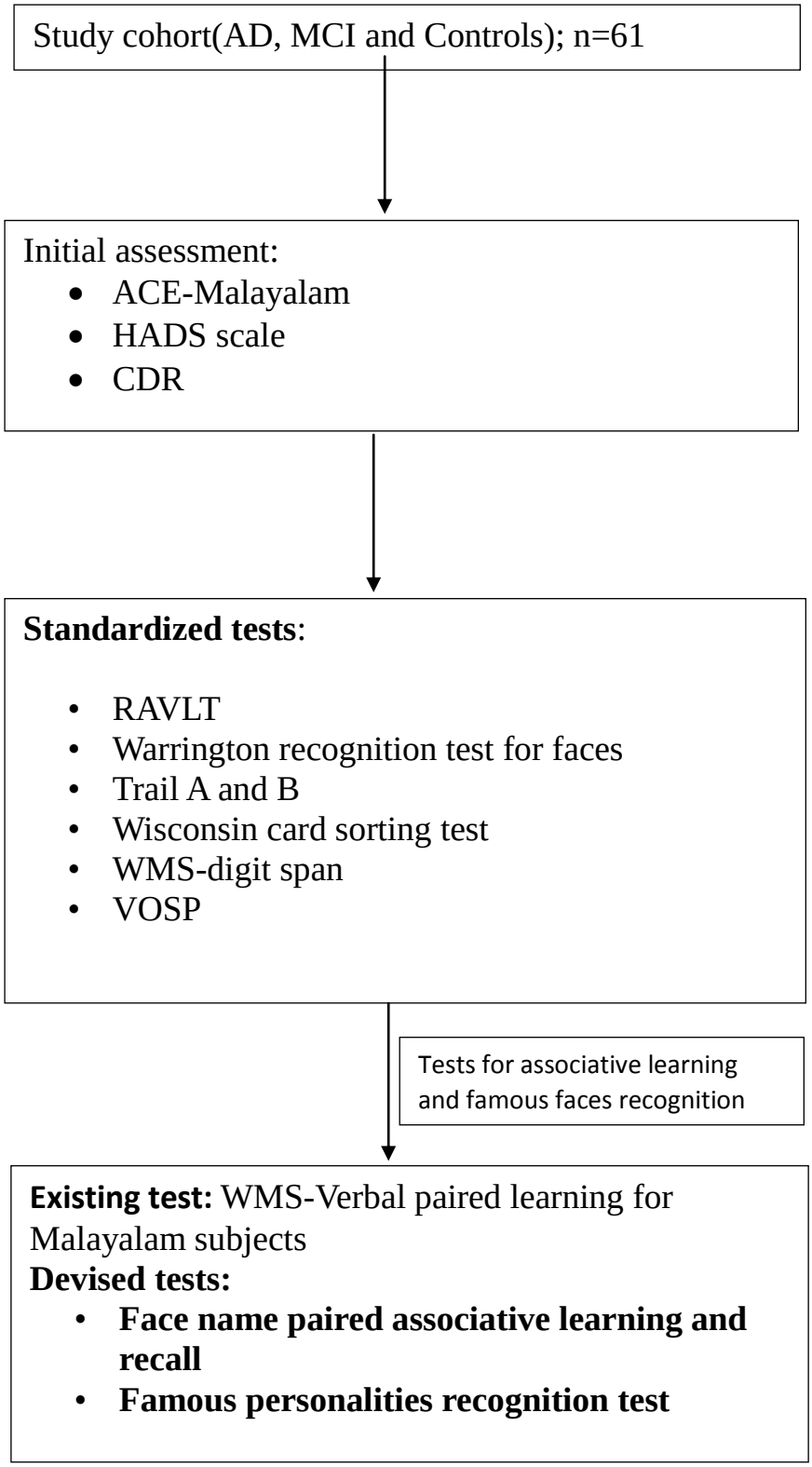
All the images were post-processed using CAT12 software in SPM 12 (Wellcome Department of Imaging Neuroscience, London)for the cortical volume measurement. All the images were oriented in the anterior commissure-posterior commissure (AC-PC) line. The images were segmented into GM, white matter

(WM), and cerebrospinal fluid (CSF), and spatially normalized into the Montreal Neurological Institute (MNI) space using CAT12 DARTEL procedure with custom settings. The segmentation was followed by modulation for preserving the volume of a particular tissue in a fixed voxel, and volumes of GM, WM, CSF, and total intracranial volume (TIV) were calculated. The resulting images were smoothed with an isotropic Gaussian kernel of 8mm full width half maximum (FWHM).

Regions of interest (ROI) analysis of subcortical volumes

The segmentation algorithm in LPBA 40 atlas in SPM atlas was used to estimate the bilateral volumes of hippocampus, parahippocampus, fusiform gyrus, cuneus and precuneus. The mean values of each regions were obtained and tabulated. Group comparison among MCI, AD, and controls wereperformed by one- way analysis of variance (ANOVA) withinthe Statistical Parametric Mapping (SPM12) general linear model. Group comparisons were tested with a correctedthreshold of $P < 0.05$, which determines the clusters withsignificant differences in GM concentration. Three separate contrasts (NCI vs MCI, NCI vs AD, and MCI vs AD) with age,sex, and total intracranial volume (TIV) as covariates wereused to compare the GM density between participants. The significant atrophic regions were overlaid on T1 weighted standard brain images, allowing the localization of areasof significant GM loss. The atrophic regions are reported inMontreal Neurological Institute (MNI) coordinates with the help of xi view toolbox (http://www.alivelearn.net/xjview/).

STUDY METHODOLOGY:



STATISTICAL ANALYSIS:

Demographic data, neuropsychological measures, and corticalGM volumes between study groups were analyzed using theStatistical Package for the Social Sciences Statistics for Windowsversion 21 (SPSS, IBM. Armonk, NY, USA). As the data contained quantitative continuous variables the measure of assessment was mean with standard deviation. The demographicand cognitive variables were compared across the groups: NC,MCI, and AD, using univariate analysis of variance (ANOVA).Pearson's correlation was applied for determining correlation between volumes and neuropsychology variables. Linear regression analysis was performed to adjust for the effect of age, gender, total intracranial volume and education in the comparisons between volumes and neuropsychology. In all comparisons, the level ofstatistical significance was set at $p< 0.05$.

Ethical considerations

This study had the approval of the Institutional Ethics Committee vide reference no: IEC/978 and informed consent was obtained from all cases and controls.

RESULTS

PILOT STUDY

The pilot study was done by 2 neuropsychologists and a total of 21 healthy subjects across different education and age groups consented for this study. The data parameters are summarised below:

Neuropsychology parameters	Neuropsychologist 1 Mean(SD)	Neuropsychologist 2Mean(SD)
Free Recall (FR)	27.20(6.87)	25.45(6.27)
Cued Recall(CR)	15.70(0.66)	15.91(0.30)
Famous faces score(FP)	27.40(2.78)	27.18(3.46)

Table (i): Comparison of data between 2 neuropsychologists.

The unpaired t-test did not show any significant difference between the FR(p=0.54),CR(p=0.36) and FP(p=0.87) scores among the 2 neuropsychologists.

Neuropsychology parameters	Females(11) Mean(SD)	Males(10) Mean(SD)
Free Recall (FR)	27.82(4.51)	24.60(8.0)
Cued Recall(CR)	15.82(0.60)	15.80(0.42)
Famous faces score(FP)	27.91(1.81)	26.60(4.06)

Table (ii): Study parameters when assessed gender wise in the pilot study group.

There was no significant difference noted between the means of FR(p=0.26); CR(p=0.93) and FP(p=0.34) scores among the 2 genders by unpaired t-test.

Most of the subjects on whom pilot study was done were less than 40 years of age(19/21). The distribution of subjects was even across the 2 genders(11 females versus 10 males).The calculated differences with regards to age distribution were not significant forCR(p=0.632); FR(p=0.120); FP(p=0.139) scores by ANOVA analysis.

Neuropsychology parameters	Below graduation(9) Mean(SD)	Graduation(8) Mean(SD)	Above graduation(4) Mean(SD)
Free Recall (FR)	24.44(8.47)	26.25(4.59)	30.50(1.91)
Cued Recall(CR)	15.78(0.44)	15.75(0.70)	16(0)
Famous faces score(FP)	26.56(4.36)	27.50(1.51)	28.50(1.91)

Table (iii): Study parameters when assessed with respect to education level.

ANOVA(Analysis of variance) showed no significant differencebetween the means of FR(p=0.31); CR(p=0.729); and FP(p=0.585)scores across the different levels of education.

STUDY COHORT

Demographic profile of subjects:

	MCI	AD	Controls(NC)	MCI-NC(p-value)	MCI-AD(p-value)	AD-NC(p-value)
Total	**22**	**19**	**20**	-	-	-
Males	**15**	**10**	**8**	**0.067**	**0.309**	**0.429**
Females	**7**	**9**	**12**	**0.067**	**0.309**	**0.429**
Mean Age(SD)	**69.91(7.65)**	**68.05(6.85)**	**65.05(6.01)**	**0.028**	**0.421**	**0.154**
Mean years of formal schooling(SD)	**13.36(3.47)**	**13.19(3.39)**	**14.36(5.02)**	**0.487**	**0.877**	**0.456**

Table 1: Demographic profile of subjects

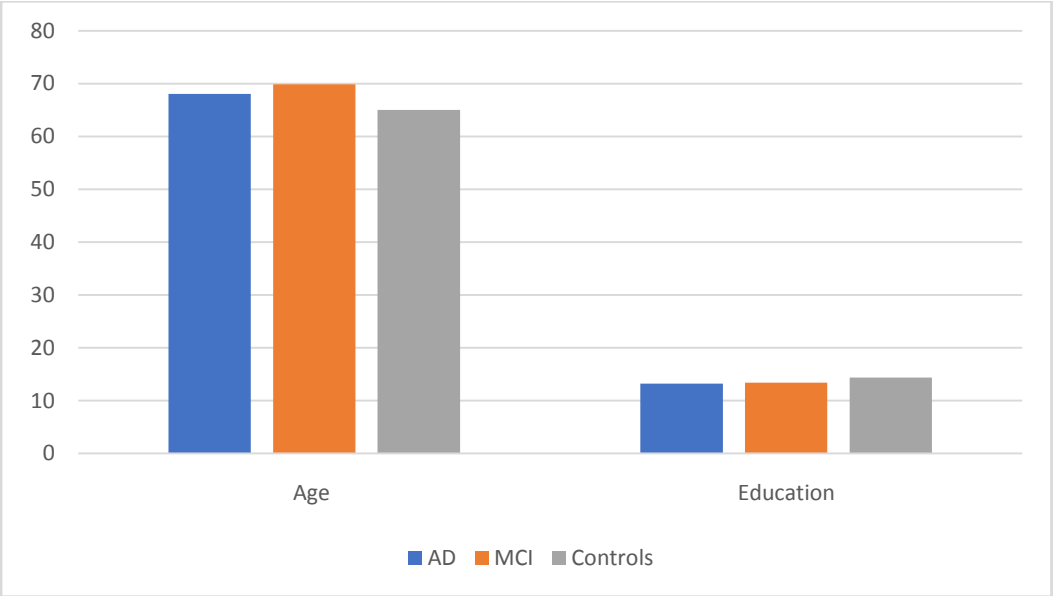


Figure 1: Demographic profile of AD, MCI and controls.

There was no statistically significant difference on ANOVA for age(p=0.421) and education(p=0.877) of MCI subjects versus AD. The ANOVA for age(0.154) and education(0.456) for AD versus controls was also non-significant. ANOVA for age when comparing MCI versus controls was significant with a p-value of 0.028 where as that of education was not significant with a p value of 0.487.

ANOVA: analysis of variance

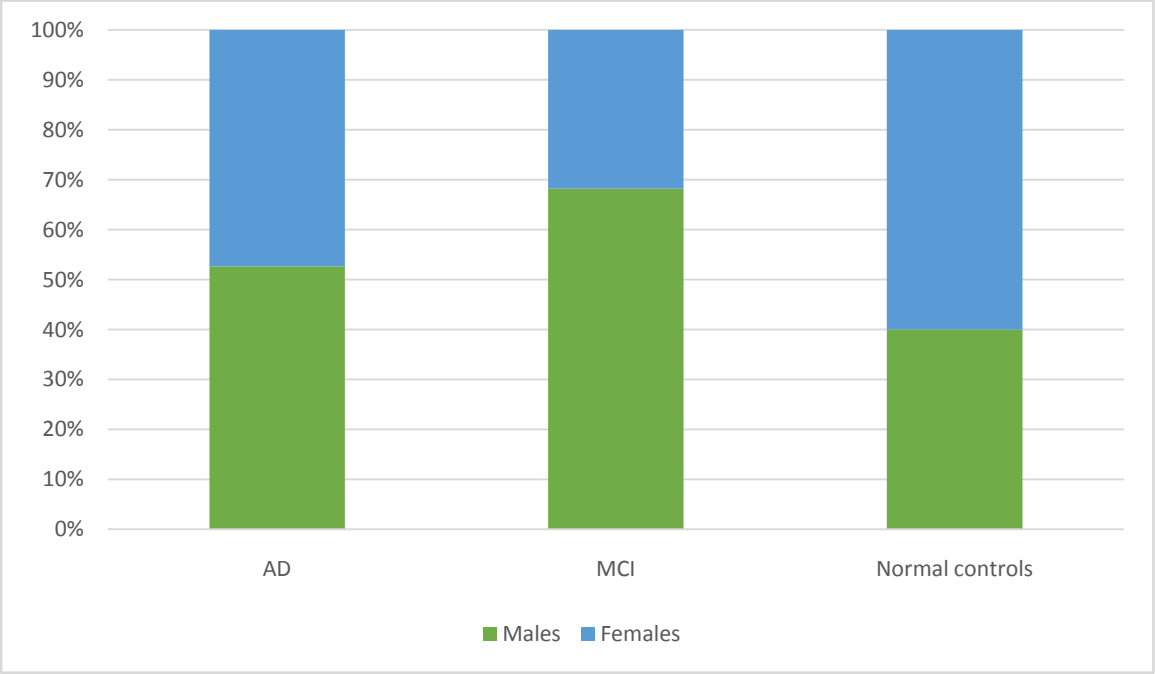


Figure 2: Gender distribution of cases and controls

The chi square test done for comparison of gender between AD and MCI(p=0.309); MCI and controls(p=0.067) and AD and controls(p=0.429) were all non-significant.

Neuropsychology profile:

Screening test results:

Neuropsychology parameter	AD Mean(SD)	MCI Mean(SD)	Controls(NC) Mean(SD)	MCI-NC(p-value)	AD-MCI(p-value)	AD-NC(p-value)
MMSE	24.53(3.02)	27.96(1.59)	29.4(0.91)	**0.001**	**<0.001**	**<0.001**
ACE-Total	74.18(13.74)	83.59(9.88)	93.47(4.72)	**0.001**	**0.023**	**<0.001**
CDR	0.62(0.28)	0.48(0.24)	0.13(0.23)	**<0.001**	0.103	**<0.001**

Table 2: Baseline screening test results for AD, MCI and NC with p-values for group differences.

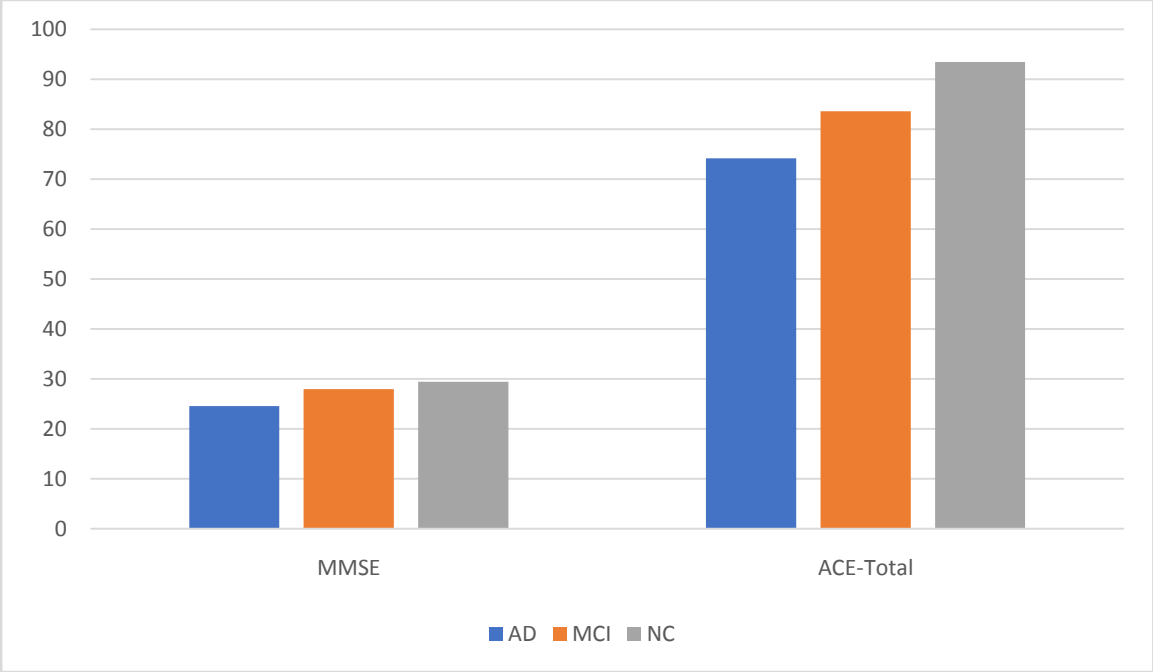


Figure 3: Scoring of AD, MCI and NC cohorts on MMSE and ACE-total scores

Reference Standard test results:

Neuropsychology parameter	AD Mean(SD)	MCI Mean(SD)	Controls(NC) Mean(SD)	MCI-NC(p-value)	AD-MCI(p-value)	AD-NC(p-value)
ACE-Reg/24	17.06(4.84)	18.27(5.50)	21.53(2.30)	**0.037**	0.476	**0.003**
ACE-Language	27.00(.94)	26.86(2.51)	28(0)	0.090	0.833	**<0.001**
ACE-orientation	8.06(2.015)	9.68(0.89)	9.60(1.30)	0.821	**0.002**	**0.017**
ACE-visuospatial	3.59(1.23)	4.09(.97)	4.73(0.46)	**0.023**	0.162	**0.002**
ACE-recall	1.82(2.74)	4.82(2.72)	7.80(1.82)	**0.001**	**0.002**	**<0.001**

Table 3: Neuropsychology profile of AD, MCI and normal controls with p-values, for among group differences.

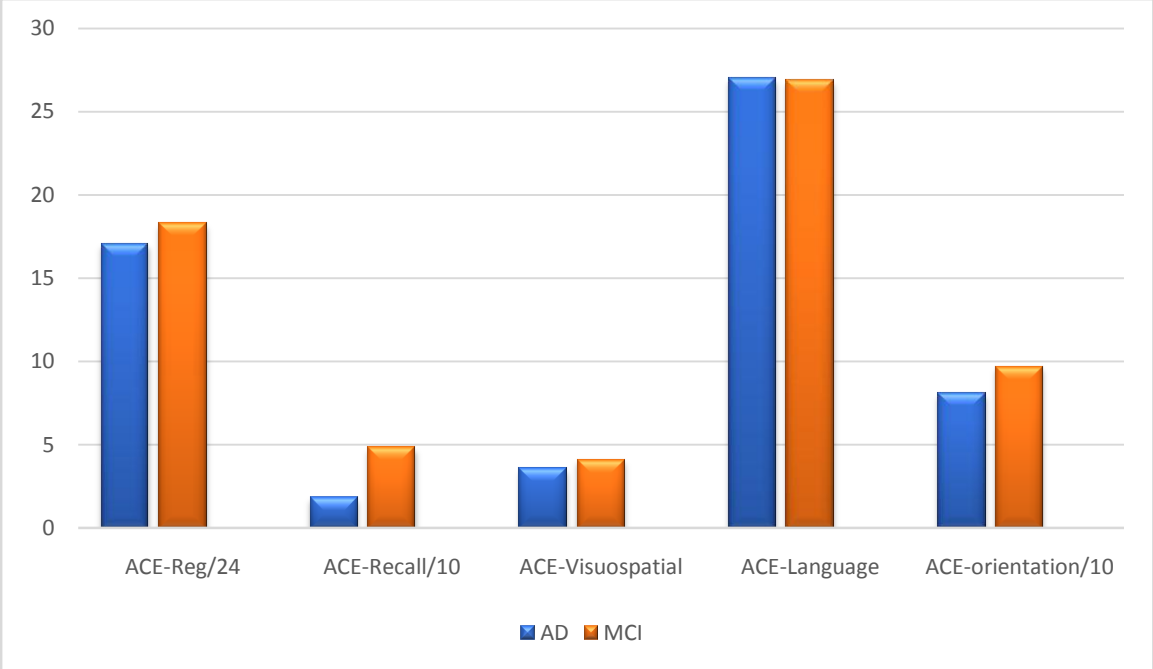


Figure 4: Comparison of ACE scores in AD versus MCI

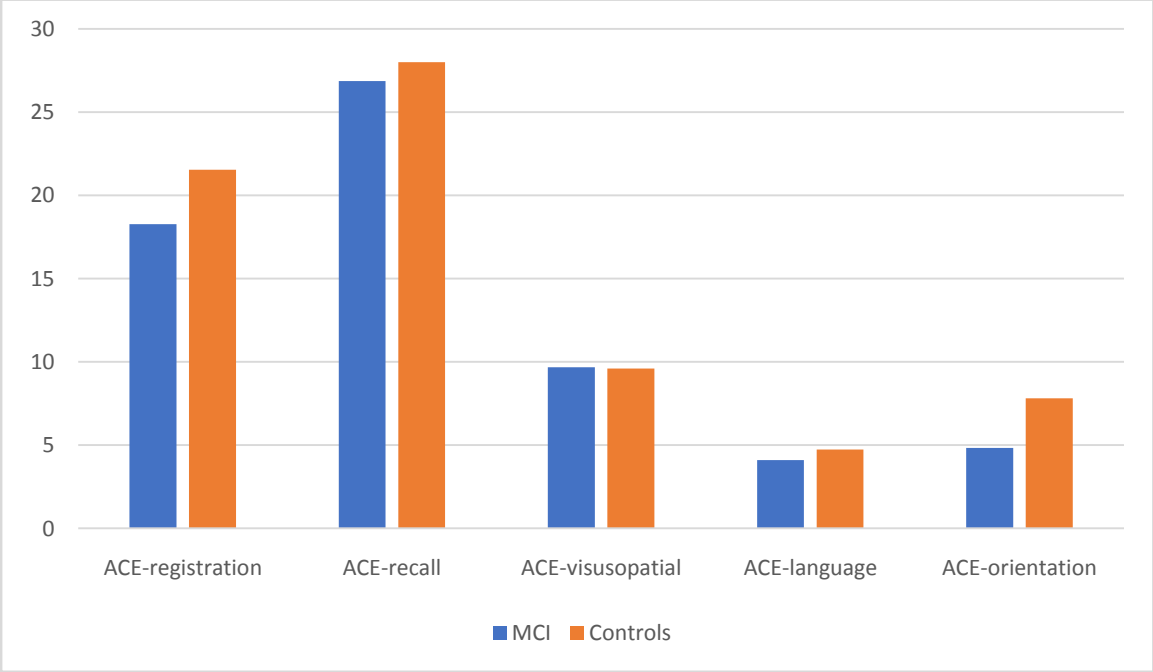


Figure 5 : Comparison of ACE scores in MCI versus controls

Neuropsychology	AD Mean(SD)	MCI Mean(SD)	Controls Mean(SD)	MCI-NC(p-value)	AD-MCI(p-value)	AD-NC(p-value)
RAVLT-Total	27.18(7.32)	31.86(11.72)	49.33(10.50)	**<0.001**	0.157	**<0.001**
RAVLT(recognition)	9(4.36)	10.32(4.48)	13.57(1.56)	**0.013**	0.362	**0.001**
RAVLT-delayed recall	0.94(1.60)	5.36(3.55)	10.6(2.61)	**<0.001**	**<0.001**	**<0.001**
RAVLT-commission	9.82(10.03)	6.64(7.08)	0.71(1.14)	**0.004**	0.252	**0.002**
RAVLT-ommission	5.94(4.34)	4.14(3.64)	1.43(1.60)	**0.013**	0.166	**0.001**
Warrington's faces	14.47(4.19)	18.952(3.80)	23(2.65)	0.091	**0.001**	**0.003**
WMS-logical memory delayed recall	1.94(3.19)	8.05(6.39)	19.57(4.33)	**<0.001**	**0.001**	**<0.001**
WMS-visual delayed recall	3.65(8.51)	11(9.75)	26.79(7.80)	**<0.001**	**0.018**	**<0.001**

Table 4: Neuropsychology profile of AD, MCI and normal controls.

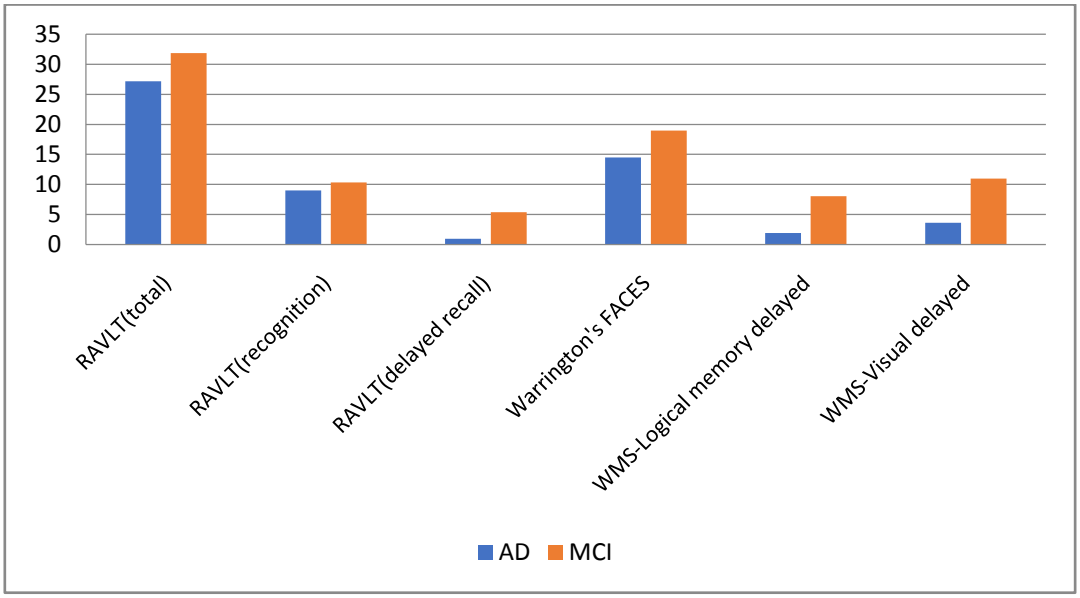


Figure 6: Comparison of RAVLT, Warrington's faces; WMS-verbal delayed memory anddelayed visual memory scores between AD and MCI.

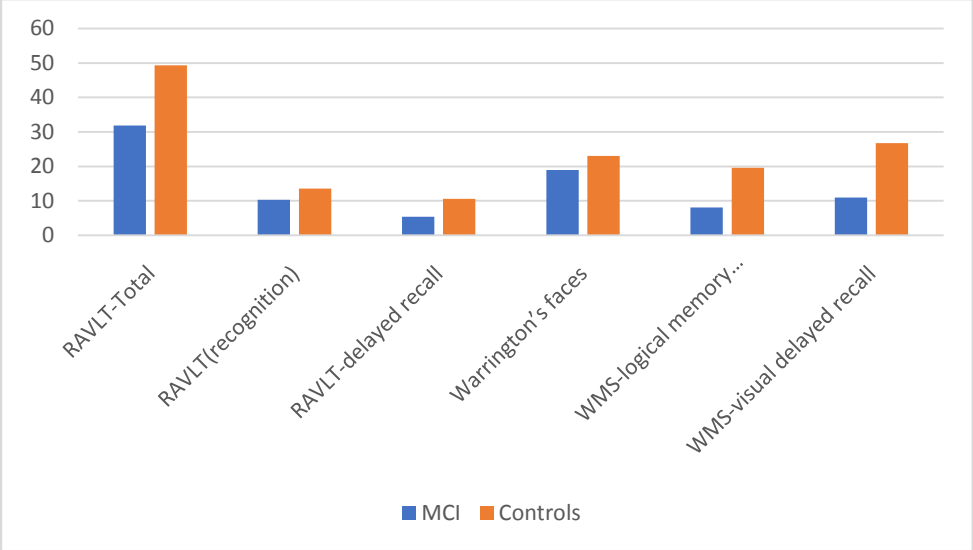


Figure 7: Comparison of RAVLT, Warrington's faces; WMS-verbal delayedmemory and visual delayed memoryscores between MCI and controls.

Tests of Associative learning:

Neuropsychology parameters	AD Mean(SD)	MCI Mean(SD)	Controls Mean(SD)	MCI-NC(p-value)	AD-MCI(p-value)	AD-NC(p-value)
Free Recall (FR)	4.58(4.98)	10.86(5.82)	20.20(5.98)	**<0.001**	**0.001**	**<0.001**
Cued Recall(CR)	8.74(3.91)	12.86(2.89)	14.35(1.73)	0.061	**<0.001**	**<0.001**
WMS-paired associate	6.68(3.19)	10.33(3.41)	12.98(3.11)	**0.040**	**0.002**	**<0.001**

Table 5: Scores in AD, MCI and controls on associative learning with age, gender and education adjusted comparisonsbetween the same cohorts.

Famous personalities test:

Neuropsychology parameter	AD Mean(SD)	MCI Mean(SD)	Controls Mean(SD)	MCI-NC (p- value)	AD-MCI (p- value)	AD-NC (p- value)
Famous personalities(FP)	19.53(4.54)	22.71(4.03)	26.35(2.08)	**0.007**	**0.032**	**<0.001**

Table 6: Scores in AD, MCI and Controls on famous personalities with age, gender and education adjusted comparisons between the same cohorts.

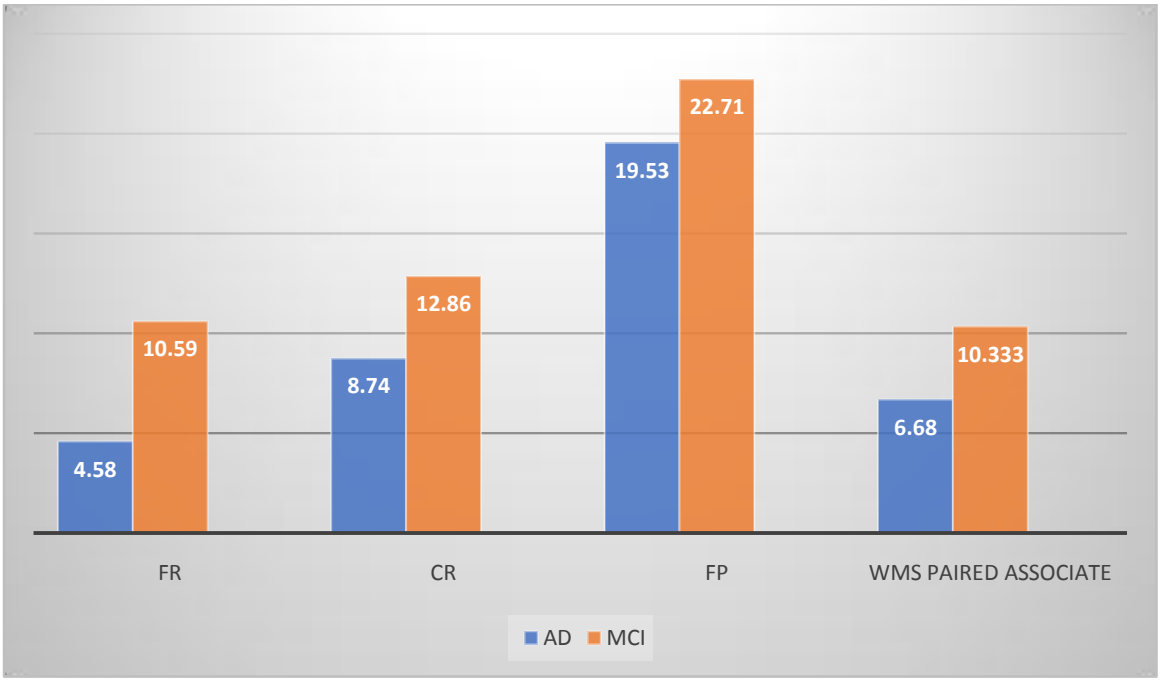


Figure 8: Comparison between means of FR, CR, FP and WMS paired associate learning scores in AD versus MCI cohort.

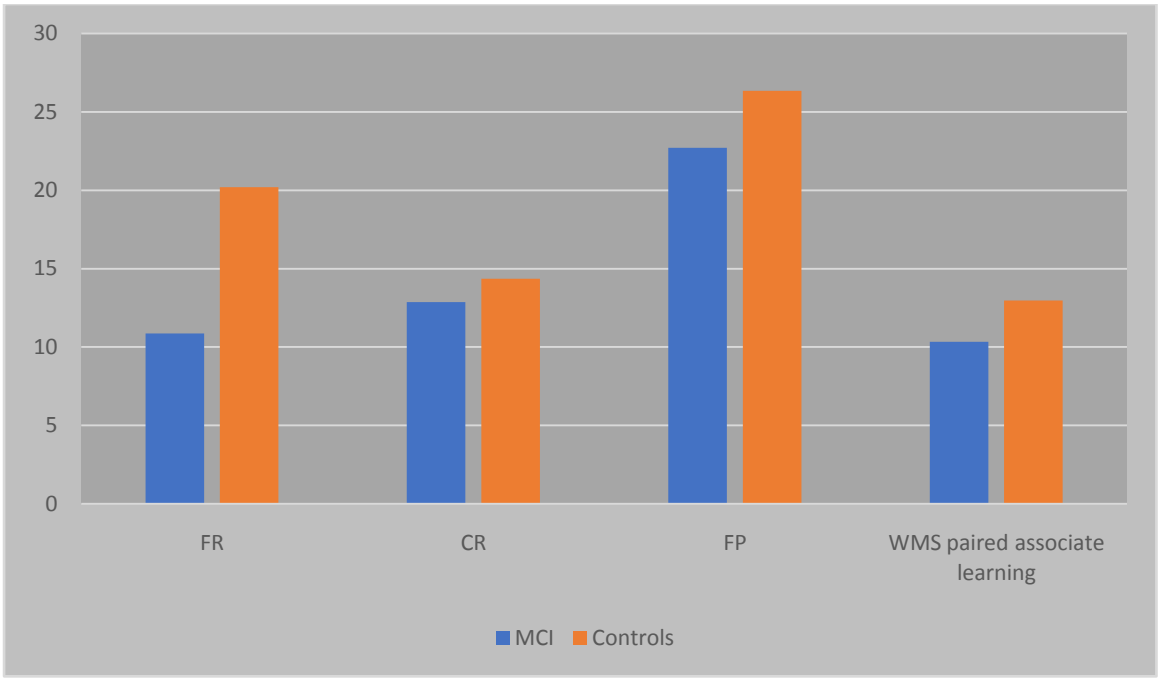


Figure 9: Comparison between means of FR, CR, FP and WMS paired associate learning scores in MCI versus Controls cohort.

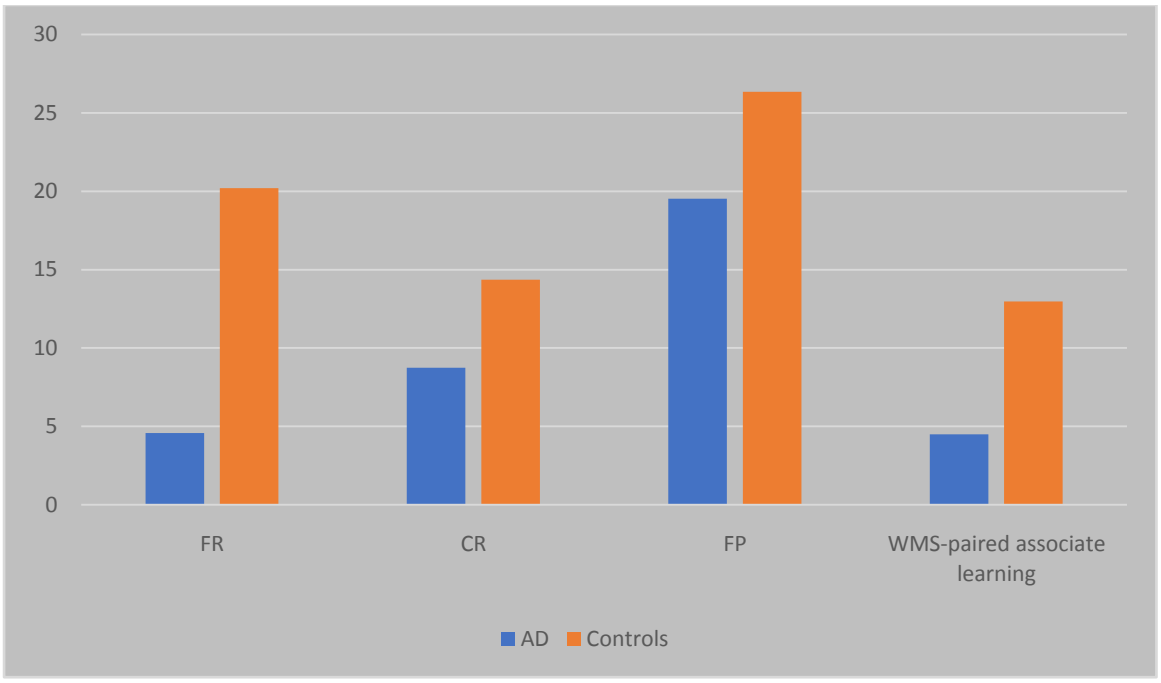


Figure 10: Comparison between means of FR, CR, FP and WMS paired associate learning scores in AD versus Controls cohort.

Correlation of associative learning recall with standard tests:

FR correlations	ACE-Recall (p-value; r-correlation coefficient)	RAVLT-delayed recall (p-value; r correlation coefficient)	WMS-delayed logical memory recall(p-value; r correlation coefficient)	WMS-delayed visual memory recall(p-value; r correlation coefficient)
Controls	**0.035; 0.546**	**0.002; 0.732**	**0.027; 0.589**	0.200; 0.364
MCI	0.199; 0.292	**0.012; 0.536**	0.050; 0.433	0.473; 0.166
AD	**0.015; 0.577**	**0.033; 0.518**	**0.003; 0.668**	0.079; 0.438

Table 7: Correlation of FR scores with ACE-recall; RAVLT-delayed recall; WMS-delayed logical memory and WMS-delayed visual memory scores in controls, MCI and AD cohort.

Free recall scores:

With regards to correlation analysis between the Free recall(FR) scores against the pre-existing standardised tests significant and moderate strength correlation was observed with ACE-Recall (p=0.015; r=0.577); RAVLT delayed recall (p=0.033; r=0.518) and WMS-delayed logical memory recall (p=0.003; r=0.668) among the cohort of AD patients. Compared to MCI subjects for whom a significant and moderate correlation could be observed for RAVLT-delayed recall (p=0.012; r=0.536).The controls showed significant correlation between FR scores and ACE-recall scores(p=0.035; r=0.546); RAVLT delayed recall (p=0.002; r=0.732) and WMS-delayed logical memory recall (p=0.027; r=0.589) scores. **There was no significant correlation observed for FR scores and WMS-visual delayed recall for any of the group of subjects.**

Cued Recall scores:

CR correlations	Warrington's FACES(p-value; r-correlation coefficient)	RAVLT-delayed recall (p-value; r correlation coefficient)	WMS-delayed logical memory recall(p-value; r correlation coefficient)	WMS-delayed visual memory recall(p-value; r correlation coefficient)
Controls	0.667; -0.50	0.906; -0.046	0.908; -.034	0.519; -.188
MCI	**0.011; 0.554**	**0.022; 0.707**	**0.001; 0.654**	**0.048; 0.436**
AD	0.228; 0.308	**0.296; 0.392**	**0.048; 0.485**	0.092; 0.421

Table 8: Correlation of CR scores with Warrington's Faces; RAVLT-delayed recall; WMS-delayed logical memory and WMS-delayed visual memory scores in controls, MCI and AD cohort.

The CR scores had a moderate and mildly statistically significant correlation with WMS-logical memory delayed recall(p=0.048; r=0.485) for AD patients but there was a significant and moderate strength correlation observed with RAVLT-delayed recall(p=0.022; r=0.707); WMS-visual memory delayed(p=0.048; r=0.436) and WMS-logical memory delayed recall(p=0.001; r=0.654) scores in MCI patients. The CR scores were noted to have a significantly positive correlation for MCI subjects with FACES score on Warrington's test(p=0.011; r=0.554). The CR scores had no significant correlation with FACES score for AD and controls. There was no significant correlation observed in controls with CR scores against standardised tests.

There was significant correlation observed between FR and CR scores for controls(p=0.010); MCI(p=0.001) and AD(p=0.006) subjects.

Famous Personality scores:

FP correlations:	Warrington's FACES(p-value; r-correlation coefficient)	RAVLT-delayed recall (p-value; r correlation coefficient)	WMS-delayed visual memory recall(p-value; r correlation coefficient)
Controls	0.967; 0.052	0.182; 0.364	0.719; -0.106
MCI	0.076; 0.406	**0.028; 0.479**	**0.035; 0.462**
AD	0.039; 0.505	0.443; 0.199	0.293; 0.271

Table 9: Correlation of FP scores with Warrington's Faces; RAVLT-delayed recall; WMS-delayed visual memory scores in controls, MCI and AD cohort.

A significant association was noted for FP scores only in MCI subjects with regards to RAVLT-delayed recall(0.028) and WMS-delayed visual recall scores(0.035). In addition the FP scores correlated well with FACES scores in AD subjects(r=.505; p=.039). There was no significant correlation observed between the same 2 variables for MCI(p=0.076;r=0.406) and controls(p=0.967; r=0.052) cohort.

WMS paired associate learning scores

WMS paired associate learning scores correlations	RAVLT-delayed recall (p-value; r correlation coefficient)	WMS-delayed visual memory recall(p-value; r correlation coefficient)
Controls	0.832; -0.060	0.192; -0.371
MCI	**0.047; 0.438**	0.145; 0.329
AD	**0.005; 0.650**	**0.001; 0.796**

Table 10: Correlation of WMS-paired associate learning scores with RAVLT-delayed recall and WMS-delayed visual memory scores in AD, MCI and controls.

The WMS paired associate learning scores had a moderate and statistically significant correlation with RAVLT-delayed recall($p=0.005$;$r=0.650$) and WMS-visual delayed($p<0.001$;$r=0.796$) scores for AD subjects. As for MCI subjects this correlation was significant and moderate with respect to RAVLT delayed recall scores($p= 0.047$; $r=0.438$).

Correlation between Visual and Verbal paired associate learning:

After adjusting for age, gender and education there was moderate but non-significant correlation observed between FR scores and WMS paired associate learning scores in MCI ($p=0.058$; $r=0.616$) and AD cohort($p=0.137$; $r=0.536$). The correlation was negative for controls($p=0.365$; $r=-0.344$). There was significant correlation observed between WMS paired associate learning scores and CR scores for AD($p=0.001$; $r=0.711$) and MCI cohort($p<0.001$; $r=0.810$) whereas this was not significant for controls cohort($p=0.202$; $r=0.298$).

Volumetric analysis(Subset analysis)

This analysis was done for 13 controls, 14 MCI subjects and 15 AD subjects as rest of the subjects did not give a consent for MRI. **The comparisons between the different groups are made after adjustment for age, gender and TIV(total intracranial volume) of the cohorts.**

ROI	AD(15)	MCI(14)	NC(13)	AD-MCI (p-value)	MCI-NC (p-value)	AD-NC(p-value)
TIV	1379.8(119.51)	1347.57(126.13)	1301.39(109.69)	0.499	0.321	0.084
GM	411.93(52.80)	369.87(69.85)	328.46(53.64)	**0.011**	0.852	**<0.001**
WM	538.27(49.51)	551.57(51.21)	547.23(40.34)	**0.013**	0.836	**0.001**
CSF	426.47(41.57)	421.71(44.44)	421.85(45.94)	0.189	0.763	**0.027**
lHip	2.74(0.67)	3.44(0.70)	3.68(0.27)	**0.006**	0.346	**<0.001**
rHip	2.68(0.74)	3.56(0.70)	3.82(0.25)	**0.003**	0.315	**<0.001**
lPhg	3.16(0.47)	3.40(0.50)	3.57(0.33)	0.062	0.144	**<0.001**
rPhg	3.44(0.48)	3.77(0.44)	3.94(0.39)	**0.022**	0.142	**<0.001**
lCun	3.19(0.65)	3.05(0.53)	2.98(0.27)	0.445	0.801	0.770
rCun	3.44(0.48)	3.32(0.42)	3.46(0.48)	0.960	0.106	0.244
lPrecun	5.85(0.79)	6.07(0.78)	5.97(0.81)	0.428	0.850	**0.038**
rPrecun	5.87(1.03)	6.01(0.88)	5.96(0.79)	0.284	0.629	**0.017**
lFusG	6.39(0.99)	6.67(0.92)	6.63(0.58)	0.231	0.881	**0.035**
rFusG	6.41(0.94)	6.90(0.79)	6.85(0.70)	**0.019**	0.966	**0.002**

Table 11: Values of volumetric analysis for MCI; AD and normal controls as per region of interest and age, gender, TIV adjusted differences between the cohorts as depicted by p-values.GM: Gray matter; WM: White matter; CSF: Cerebrospinal fluid; lHip: left hippocampal; rHip: right hippocampal; lPhg: left parahippocampal gyrus; rPhg: right parahippocampal gyrus; lCun: left cuneus; rCun: right cuneus; lPrecun: left precuneus; rPrecun: right precuneus; lFusG: left fusiform gyrus; rFusG: right fusiform gyrus

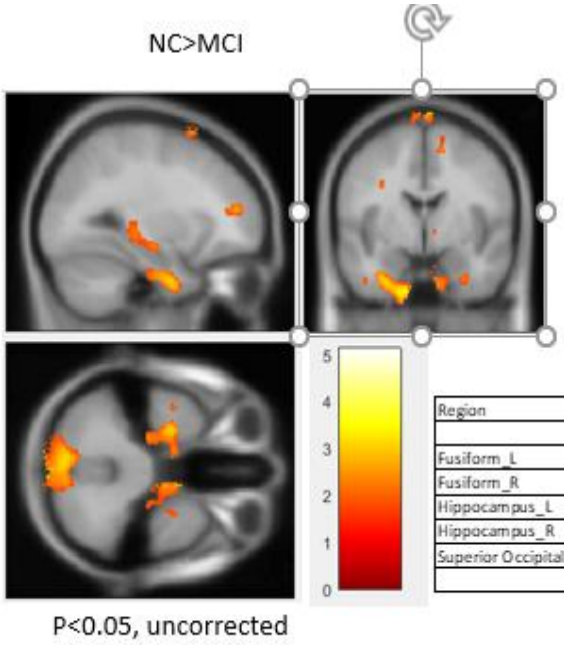
There was no statistically significant difference found for the tested ROI volumes among the two cohorts of MCI and controls after adjustment for the covariates.

Between AD and MCI significant difference on volumetry was found for left hippocampal ($p=0.006$); right hippocampal ($p=0.003$); right parahippocampal gyrus ($p=0.022$); White matter ($p=0.013$); gray matter ($p=0.011$) and right fusiform gyrus(0.019) volumes.

With reference to volumetry between AD and controls significant differences were observed between left parahippocampal gyrus($p<0.001$); right parahippocampal gyrus($p<0.001$); left hippocampal gyrus($p<0.001$); right hippocampal gyrus($p<0.001$); left precuneus ($p=0.038$); right precuneus ($p=0.017$); left fusiform gyrus($p=0.035$); right fusiform gyrus(0.002); White matter($p=0.001$) and Grey matter($p<0.001$) volumes. There was no statistically significant difference observed between the volumes of right and left cuneus volumes between AD and controls.

VBM group comparison results:

MCI-controls

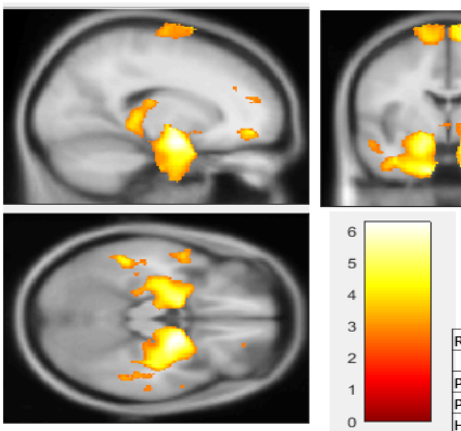


Region	MNI Co-rdinates			Z-score
	x	y	Z	
Fusiform_L	-27.92	-3	-37.83	3
Fusiform_R	28.5	-12	-33	2
Hippocampus_L	-31.5	-16.5	-13.5	2.15
Hippocampus_R	30.62	-13.5	-13.52	1.77
Superior Occipital_R	18	-96	16.5	1.97

Figure 11 : VBM analysis between MCI and controls with age, sex and TIV as covariates

AD-controls

NC>AD

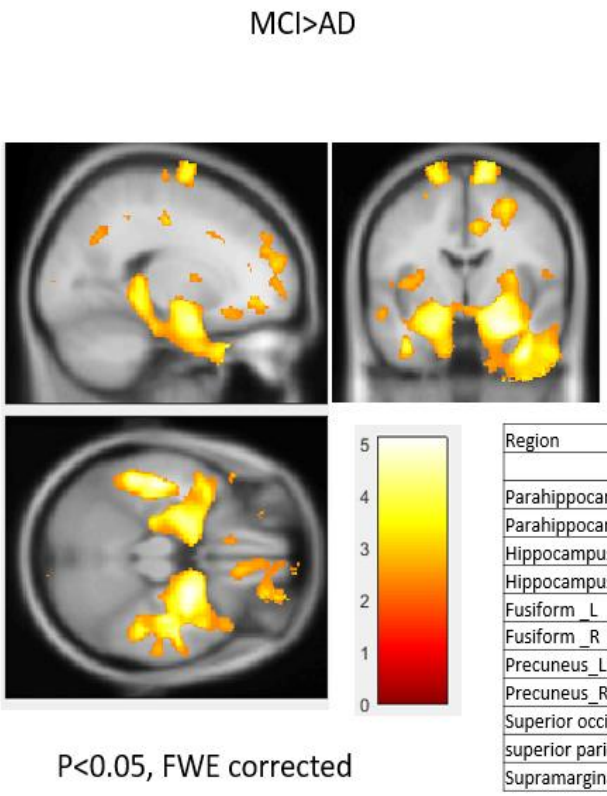


P<0.05, FWE corrected

Region	MNI Co-rdinates			Z-score
	x	y	Z	
Parahippocampus_L	-15	-3	-30	3.73
Parahippocampus_R	-18	-3	-18	6.25
Hippocampus_L	-15	-3	-21	4.92
Hippocampus_R	30.62	-13.5	-13.52	1.77
Fusiform _L	-25.5	-18	-30	3.63
Fusiform _R	30	-10.5	-34.5	3.34
Inferior orbitofrontal_L	-29.7	36	-12.62	3.52
Inferior orbitofrontal_R	31.5	36	-12	3.7
Cuneus_L	-15	-72.02	36.91	
Inferior parietal_L	-39	-33	37.5	2.13
Supramarginal gyrus_R	60	-21	37.5	2.41

Figure 12 : VBM analysis between AD and controls with age, sex and TIV as covariates

AD-MCI



Region	MNI Co-rdinates			Z-score
	x	y	Z	
Parahippocampus_L	-13.5	-3	-19.5	3.95
Parahippocampus_R	15	-1.5	-19.5	4.62
Hippocampus_L	-17.11	-3.59	-19.5	2.24
Hippocampus_R	27	-10.5	-19.5	4.32
Fusiform _L	-39	-18	-30	3.63
Fusiform _R	42	-46.5	-21	1.83
Precuneus_L	-10.5	-58.5	49.5	2.01
Precuneus_R	4.5	-76.53	42.31	1.81
Superior occipital_L	-21.61	-67.5	33.31	2.35
superior parietal_L	-39	-45	57	2.3
Supramarginal gyrus_L	-64.5	-33	33	3.38

Figure 13 : VBM analysis between AD and MCI with age, sex and TIV as covariates

Correlation between Neuropsychology and Volumetry:

All correlations were made after adjusting for age, gender and education of individual cohort(s).

AD cohort:	FR (p-value; r value)	CR(p-value; r value)	WMS-paired associate learning (p-value; r-value)
Left hippocampal	0.521; 0.247	0.560; -0.226	0.595; 0.206
Right hippocampal	0.856; -0.071	0.577; -0.216	0.457; 0.285
Left parahippocampal	0.140; 0.532	0.959; 0.020	0.189; 0.481
Right parahippocampal	0.713; 0.143	0.985; 0.007	0.258; 0.422
Left cuneus	**0.005; 0.833**	0.099; 0.583	0.067; 0.633
Right cuneus	**0.003; 0.861**	**0.023; 0.739**	**0.002; 0.871**
Left precuneus	0.137; 0.535	0.327; 0.370	0.131; 0.543
Right precuneus	0.115; 0.562	0.267; 0.415	0.080; 0.612
Left fusiform gyrus	0.233; 0.442	0.893; 0.053	0.116; 0.561
Right fusiform gyrus	0.462; 0.282	0.670; 0.166	0.125; 0.549
GM	0.877; 0.061	0.485; 0.269	0.462; 0.282
WM	**0.041; 0.686**	0.704; 0.148	0.176; 0.494

Table 12: Correlation of associative learning scores with volumetry in AD cohort.

r: Pearson's correlation coefficient

With respect to AD cohort significant and strong correlation was observed between all 3 parameters of paired associate learning i.e FR, CR and WMS-paired associate learning scores and right cuneus volume. In addition the FR scores also had a significant correlation with left cuneus and white matter volume. The other correlations were non significant.

AD cohort:	FP(p-value; r-value)	RAVLT- delayed recall(p-value; r-value)
Left hippocampal	0.541; 0.236	0.113; 0.565
Right hippocampal	0.471; 0.277	0.090; 0.596
Left parahippocampal	0.919; 0.040	**0.029; 0.720**
Right parahippocampal	0.878; -0.060	0.102; 0.579
Left cuneus	0.851; 0.074	0.108; 0.572
Right cuneus	0.449; 0.290	**0.012; 0.785**
Left precuneus	0.699; -0.151	0.432; 0.300
Right precuneus	0.850; -0.074	0.288; 0.398
Left fusiform gyrus	0.832; -0.083	0.113; 0.565
Right fusiform gyrus	0.971; -0.014	0.249; 0.429
GM	0.927; -0.036	0.434; -0.300
WM	0.905; -0.047	0.120; 0.556

r: Pearson's correlation coefficient

Table 13: Correlation of FP and RAVLT-delayed recall scores with volumetry in AD cohort.

With regard to FP scores no significant correlation was observed between FP scores and volumetry. RAVLT-delayed recall scores were significantly associated with right cuneus and left parahippocampal gyrus volumes.

MCI cohort:	FR(p-value; r-value)	CR(p-value; r-value)	WMS-paired associate learning(p-value; r-value)
Left hippocampal	0.645; -0.167	0.770; 0.106	0.961; -0.018
Right hippocampal	0.743; -0.119	0.752; 0.115	0.882; 0.054
Left parahippocampal	0.902; -0.045	0.337; 0.340	0.552; 0.214
Right parahippocampal	0.449; -0.271	0.966; -0.015	0.844; -0.072
Left cuneus	0.303; -0.363	0.901; 0.046	0.944; 0.026
Right cuneus	0.451; -0.270	0.825; -0.081	0.716; -0.132
Left precuneus	0.407; -0.296	0.566; 0.207	0.332; 0.343
Right precuneus	0.138; -0.503	0.908; 0.042	0.622; 0.178
Left fusiform gyrus	0.593; -0.193	0.866; 0.062	0.879; -0.056
Right fusiform gyrus	0.478; -0.254	0.908; 0.042	0.987; -0.006
GM	0.904; -0.044	0.921; -0.036	0.790; -0.097
WM	0.726; -0.127	0.573; -0.203	0.375; -0.315

r: Pearson's correlation coefficient

Table 14: Correlation of associative learning scores with volumetry in MCI cohort

There was no significant correlation observed between any of the paired associate(FR,CR,WMS-paired associate learning) scores and volumetry results in MCI cohort.

MCI cohort:	FP (p-value; r value)	RAVLT- delayed recall (p-value; r value)
Left hippocampal	0.551; 0.215	0.736; -0.123
Right hippocampal	0.892; 0.049	0.651; -0.164
Left parahippocampal	0.952; -0.022	0.927; -0.034
Right parahippocampal	0.984; 0.007	0.528; -0.227
Left cuneus	0.405; -0.297	0.171; -0.469
Right cuneus	0.485; -0.250	0.136; -0.506
Left precuneus	0.543; 0.219	0.817; -0.084
Right precuneus	0.611; 0.184	0.618; -0.180
Left fusiform gyrus	0.443; 0.274	0.566; -0.207
Right fusiform gyrus	0.717; 0.132	0.363; -0.323
GM	0.953; -0.022	0.128; -0.514
WM	0.073; -0.589	0.441; -0.276

r: Pearson's correlation coefficient

Table 15: Correlation of FP and RAVLT-delayed recall scores with volumetry in MCI cohort.

Even the FP scores and RAVLT delayed recall scores had no significant association with any of the volumetry studies in MCI cohort.

Controls cohort:	FR (p-value; r value)	CR(p-value; r value)	WMS-paired associate learning (p-value; r value)
Left hippocampal	0.789; 0.105	0.162; 0.508	0.203; -0.469
Right hippocampal	0.207; 0.465	0.843; 0.077	0.332; -0.367
Left parahippocampal	0.656; 0.173	0.182; 0.488	0.010; -0.799
Right parahippocampal	0.360; 0.347	0.475; 0.274	0.047; -0.673
Left cuneus	0.533; -0.241	0.387; 0.329	0.041; -0.686
Right cuneus	0.531; 0.242	0.108; 0.571	0.074; -0.622
Left precuneus	0.506; -0.256	0.304; 0.387	0.051; -0.663
Right precuneus	0.307; -0.385	0.050; 0.666	0.077; -0.617
Left fusiform gyrus	0.669; 0.166	0.416; 0.311	0.064; -0.639
Right fusiform gyrus	0.148; 0.524	0.947; -0.026	0.278; -0.406
GM	0.086; 0.602	0.086; -0.603	0.690; 0.155
WM	0.506; 0.256	0.473; -0.276	0.012; -0.784

r: Pearson's correlation coefficient

Table 16: Correlation of associative learning scores with volumetry in controls cohort

The measures of associative learning(FR and CR) scores were not significantly associated with any of the volumetry scores in controls. However for WMS-paired associate learning significant negative correlation was observed with left cuneus, left fusiform gyrus, white matter, left and right parahippocampal gyrus volumes.

Controls cohort:	FP (p-value; r value)	RAVLT- delayed recall (p-value; r value)
Left hippocampal	0.505; -0.256	0.425; 0.305
Right hippocampal	0.127; -0.548	0.290; 0.397
Left parahippocampal	0.372; -0.339	0.449; 0.290
Right parahippocampal	0.420; -0.308	0.285; 0.400
Left cuneus	0.507; -0.255	0.852; -0.073
Right cuneus	0.885; 0.057	0.320; 0.375
Left precuneus	0.514; -0.252	0.937; 0.031
Right precuneus	0.673; -0.164	0.656; -0.173
Left fusiform gyrus	0.116; -0.561	0.543; 0.235
Right fusiform gyrus	0.178; -0.493	0.242; 0.435
GM	0.514; -0.251	0.428; 0.303
WM	0.473; -0.276	0.506; 0.256

r: Pearson's correlation coefficient

Table 17: Correlation of FP and RAVLT-delayed recall scores with volumetry in controls.

There was no significant association between FP scores and volumetry in controls. In addition no association was noted between RAVLT-delayed recall scores and volumetry in control subjects.

DISCUSSION

The pilot study for the new tests was undertaken to note the reproducibility of results with regards to age, gender and education differences among the study population. We found good reproducibility of the tests with regards to test results by 2 different neuropsychologists. There was good reproducibility among the different genders and education level of pilot study participants. The reproducibility among different age groups could not be ascertained as most of the subjects in pilot study were less than 40 years of age. The interrater reliability could not be tested for these tests as repeated testing by the neuropsychologists would have resulted in a recall bias. The sensitivity and specificity of these tests cannot be commented upon as there is no gold standard to compare these tests with. The reliability of this test could not be determined because of the potential for recall bias on performance in these tests. As there are no similar tests in Indian Population the development of new test as done by us for visual associative paired learning(face-name pairing) needs to be emphasised and studies on larger control groups are needed to ascertain the final validity of our tests.

The demographic data between MCI, AD and controls were education matched for all the subcategories being studied. The age distribution was well matched between MCI and AD but there was statistically significant difference between MCI and controls. This was accounted by the older age of recruited MCI subjects. There were 5 MCI subjects with age more than 75 years(one of the subject was aged 84 years) as compared to none of the controls who were aged more than 75 years. This would explain the significant age difference between the 2 groups. The AD cohort also had 4 individuals who were >75 years of age, this would explain the

lack of significant difference between AD and MCI. The 3 cohorts of MCI, AD and controls were gender matched as proved by non-significant values on chi square test when comparing between the groups.

Neuropsychology parameters

Even after adjustment for age, gender and education the performance on neuropsychological tests showed statistically significant differences between MCI and AD cohorts for the tested parameters of Free recall and cued recall on face name pairing test. When comparing between MCI and controls the free recall scores were found to be statistically significant with no significant difference between Cued recall scores. This would lead us to drawing a conclusion that face name pairing is affected in mild cognitive impairment but this is amenable to improvement provided clues are provided thereby indicative of a retrieval deficit rather than an associative-encoding problem. This is in agreement with a recent study which demonstrated a significant impairment of tasks involving associative learning in MCI subjects as compared to controls.[27]The design parameter in above mentioned study also utilised the ability to form an association between faces and names as a measure of associative learning. As compared to our study which utilised non presented names as distractors the above mentioned study utilised the previously presented names for other faces as distractors. The above mentioned study did not have a provision for free recall and instead subjects had to choose between the correct name and distractor which were presented. This was similar to cued recall scoring on our test paradigm. As there was no provision for free recall thus the cued recall scores on their test can be taken to represent the 1st objective measure of face name pair associate learning in that study. Our study in comparison can be considered to be a more robust measurement of associative learning in that there is no associative clue which is being given in the 1st

attempt thus offsetting the advantage being posed by name(item) familiarity. The methodology also differed in our study as we utilised three trials for better chances of recollection of names considering a free recall component. As compared to our study in which there was a gap of 20 minutes between the last exposure and free recall the study mentioned above does not have a mention of same. This was done as we needed to assess delayed recall memory of face name pair association.

For the assessment of verbal paired associate learning we used a Malayalam adaptation of WMS-paired associate learning which is a validated scale for use in our population cohort.[48]

As per our results there was a statistically significant impairment of WMS paired associate learning which was age, gender and education matched between the groups of MCI versus controls and AD versus MCI. These results represent the verbal aspect of associative learning which is also significantly impaired in MCI compared to controls. The effectiveness of WMS IV-verbal paired associates in distinguishing amnestic MCI from healthy controls was assessed at case level and group level in a study comprising of 77 subjects each of MCI and healthy adults. This study also demonstrated the effectiveness of verbal paired associate learning in distinguishing the 2 cohorts at group level with large effect size and significant values. But this test failed to identify 70% of MCI as impaired at case level when the existing normative data were used. In the same study it was demonstrated that the delayed recall component of verbal paired associate learning was slightly better in identifying MCI as impaired as compared to immediate recall. In this study it was demonstrated that there are ceiling effects evident for healthy controls on easy pairs whereas floor effects are observed in MCI for hard pairs. This limitation was largely offset in our cohorts as we sought out to find out the differences between MCI and controls cohorts

based on verbal paired associate learning rather than aim to classify them as MCI or normal controls based on the normative values. In this regard our results go in good agreement with the mentioned study aiming to use verbal paired associate learning for differentiating MCI from controls on a group basis.[49]

The scores on famous personality test were significantly different between MCI and controls and AD and MCI cohort. As discussed in the review the test for famous personalities aims to assess famous personalities with autobiographical significance for adults. The famous personalities included in our test had been active in the news till recently. Thus this test aimed to test retrograde and visual semantic memory as is applicable in a real-world scenario. Given the fact that most patients with early cognitive impairment report inability to recall faces and names of people who were well-known to them in the distant past we wanted to evaluate this capability specifically in MCI. Literature is lacking with regard to this aspect of famous faces recall abilities. Our study thus brought about several interesting findings. Our test utilised a bipartite scoring model in which the subject had to 1st identify the famous personality from among the 2 distractors; than had to name him. Thus this test aimed to identify remote memory component attributed to famous faces. These findings are in good agreement with the study by Ahmed et al wherein it was demonstrated that both MCI and AD subjects performed significantly worse on Graded faces name test as compared to normal controls. The test mentioned above utilised famous personalities from the past 5 decades some of whom might have been out of limelight recently.[22]Our study however attempted to assess performance on naming famous personalities who were popular in the study population and had been in limelight till recent past. The utility of recent famous faces in predicting declining episodic memory from stable episodic memory has been demonstrated in another study. The

mentioned study aimed to assess the performance of cognitively healthy elderly subjects on time limited temporal gradient of famous faces for decline in episodic memory which was to be retested after a period of 18 months. In this study it was found that the individuals who were progressing(declining in episodic memory) had a poorer performance at baseline for recognising recent famous names. The performance on remote names was comparable between declining and stable group. This study attempted to use performance on the test of recent famous faces as an index of rapidity decline in episodic memory.[18] Extrapolating these findings for the rapidity of conversion to AD in MCI it can be postulated that performance for recent famous names can help identify MCI individuals at risk for progression to AD. Further follow up for episodic memory decline was not done in our study. The performance on RAVLT delayed recall was significantly affected in MCI when compared to controls and AD when compared to MCI. The utility of RAVLT delayed recall in predicting subjects who would convert to AD in future has been demonstrated in a previous study. This study involved administering RAVLT to 116 subjects referred for subjective memory complaints who were then followed up for next 2 years for evidence of evolution. Of the 70 patients included in final analysis 27 developed probable AD and 26 were diagnosed to have MCI. The authors further found that the subjects who were diagnosed to have probable AD later showed lower scores and more frequently scored 0 at delayed test recall. They also more often had a >75% of forgetting. There were significant group differences which were noted in the performance at baseline for all immediate recall and delayed recall trials of RAVLT in the subjects who subsequently evolved to MCI or AD as compared to controls with no cognitive impairment. Nearly 50% of subjects who were diagnosed to have AD on subsequent follow up had a delayed recall score of 0 and ~ 60% of the same cohort

had a (%) of forgetting >75% from trial 5 to trial 6.[50]In agreement to the study quoted above in our cohort 10 of 17 early AD patients for whom RAVLT delayed recall scores were available scored 0 as compared to 1 in 22 MCI subjects and none of 15 controls who scored 0.

In order to test the validity of the new scale which was developed the results on FR scores were correlated with another score of delayed recall namely RAVLT-delayed recall and as per our results the same was concordant for cohorts of MCI, controls and AD. With regards to WMS-delayed logical memory recall significant correlation was observed in AD and controls cohort. The good correlation of our test with other validated measures of delayed memory gives credibility to our testing method. In addition there was a good correlation observed between WMS-paired associate learning and FR scores for both AD and MCI cohorts. This observation becomes important in the context of affirming a similar degree of affection of visual and verbal paired associate learning among MCI and AD cohorts. Although traditionally thought of, to subserve two different systems the affection of both visual and verbal memory to a similar degree in MCI subjects would point towards an affection of both the systems early on in cognitive decline ascribed to MCI.

Volumetric Analysis

Both the volumetric and group analysis revealed significant differences between AD and MCI with respect to bilateral hippocampus and right parahippocampal gyrus volumes. There was also a significant reduction noted in the volume of right fusiform gyrus. Furthermore the reduction in volume of hippocampi was more significant as compared to reduction in volume of parahippocampal gyrus. This is in accordance with previous studies which have demonstrated significant reductions in the volume of hippocampus and parahippocampal cortex in AD patients

compared to a-MCI subjects. The greater relative reduction of volume of hippocampi as compared to reduction in the volume of parahippocampal gyrus is also in accordance with the findings by this study.[51]The reduction in the volume of fusiform gyrus would be indicative of volume loss spreading to adjacent areas as the dementia is advancing.

In our cohort there were no significant differences found in the volumetryand group analysis of studied regions between a-MCI and normal controls. This is in contrast to the previously discussed study[51] which has demonstrated significant differences in the volumetry of hippocampi and entorhinal cortex in MCI as compared to normal controls. The heterogeneity in the degree of cognitive impairment in 2 MCI cohorts being compared here might explain these non-concordant findings. In our MCI cohort the mean MMSE score was 27.96 as compared to 24 for the above mentioned study. This might imply a relatively advanced stage of MCI or even prodromal AD in their cohort . The degree of atrophy in MCI subjects is also expected to progressively increase as the severity increases. Further the limited sample size of our cohorts available for volumetry is a factor which needs to be considered when interpreting the results.

With regards to volumetric and group differences between AD and controls significant differences were observed with regards to both the hippocampi, parahippocampal gyrus, bilateral precuneus, bilateral fusiform gyrus and gray and white matter volumes. These findings of atrophy in hippocampus and parahippocampal gyrus are in accordance with the previously mentioned study.[51]The atrophy in fusiform gyrus would be explained by the progression of atrophy to nearby regions in AD as the disease progresses. The presence of gray matter atrophy is also concordant with a previous study which demonstrated significant reductions in the

volume of caudate nucleus in AD subjects as compared to controls.[52]The presence of significant white matter atrophy in AD as compared to controls would represent atrophy of the adjoining white matter tracts as the disease advances a fact confirmed by DTI demonstrating significant reduction in volume of bilateral superior longitudinal fasciculus in AD as compared to controls.[53]Significant atrophy of precuneus in AD as compared to normal controls has also been described previously.[54]

Correlation between associative learning and volumetry:

With regards to associative learning significant positive correlation was demonstrated with regards to performance on FR, CR and WMS verbal paired associate learning tests when compared to right cuneus volume. The FR scores also had a positive significant correlation to left cuneus and white matter volumes. In a recent study done to assess the structural brain substratesof face name associative learning it was concluded that the same is a higher order cognitive process and requires the activation of multiple brain structures and processes.[55]This finding is corroborated by another study in which the face name pairing was shown to activate a widespread cortical network including, medial frontal, left frontal operculum, angular gyrus, left lateral temporal, parietal and occipital cortices.[10] The activation of striate and extrastriate cortex while encoding for novel face-name pairs has been demonstrated in a previous study.[25]In this context our findings of correlation of only right cuneus volume to associative learning tests(visual and verbal) represent a novel finding which needs to be confirmed by other similar studies. With respect to MCI cohort no significant correlation could be derived between the associative learning scores and volumetry analysis. As there were significant differences in associative learning with no significant volumetric differences in MCI subjects compared to

controls, **it can be concluded that dysfunction on neuropsychology testing predates the appearance of regional brain atrophy in MCI subjects.** This would lead us to opine that MCI cohort is a structurally and functionally different cohort from that of AD with its own characteristics. In respect to controls cohort there was no correlation noted between visual associative learning and volumetry. But there was significant negative correlation noted between performance in WMS paired associate learning and volumetry of bilateral parahippocampal gyrus, left cuneus, left fusiform gyrus and white matter volumes. As these ROIs were more concerned with visual encoding of information it is possible that verbal paired associative learning might have had a negative correlation with the volumes of ROIs in controls cohort. These results need to be interpreted with caution with regards to the small sample size of the controls cohort. These findings need to validated on a larger cohort of controls.

Famous personalities and volumetry:

There was no significant correlation observed between the scores on recognition of famous faces and volumetry for any of the cohorts including in the areas considered important for face recognition; i.e PHG and fusiform gyri. Our results in this aspect are discordant from the demonstration of involvement of right fusiform face area in the recognition of famous faces.[28]A previously done study also had shown the correlation of performance on task for recent famous names with regards to hippocampal volume. This study did not find a significant correlation with regards to hippocampal volume for remote famous names.[18] Our study used remote famous names and this might explain the lack of a correlation with hippocamapal volumes. In this regard our results need to be validated on a larger cohort of subjects.

Correlations between Volumetry and RAVLT delayed recall scores:

In AD cohort there was significant correlation observed between performance on RAVLT delayed recall test and left parahippocampal gyrus, right cuneus volume. For MCI and controls cohort no significant association between the same variables could be demonstrated. There was no significant correlation between RAVLT-delayed recall scores and hippocampal volumes. The correlation of RAVLT delayed recall scores(an index of measure of impairment in AD) with volume loss in left parahippocampal gyrus would be in accordance with Braak's[56] pathological findings whereintransentorhinal and entorhinal cortex were the first regions to show neurofibrillary tangles in AD.

Our test results indicate the significant differences on neuropsychological test scores between the 3 cohorts and are indicative of classification accuracy between the 3 groups as mandated by the inclusion criteria. Memory domain specific impairment was demonstrated on all tests including ACE-recall; WMS and RAVLT-total. The profile of neuropsychology tests in MCI indicate that this cohort might have been multidomain rather than pure amnestic one as indicated by impairment in ACE-visuospatial scores. **Additionally the MRI volumetric differences between the AD-MCI cohort and AD-controls cohort may indicate phenotypic differences between the groups although functional differences in terms of neuropsychology were most significant.**

STRENGTHS:

1. Ours is the first study to comprehensively look at the face-name pairing associative ability, famous faces recognition using Indian faces and verbal paired associate learning and compare the performance with regional volumetric differences.
2. The presence of 3 cohorts i.e. MCI, AD and Controls to compare is an added advantage being put forward by our study.
3. The attempt to derive a correlation between visual and verbal paired associate learning is a novel enterprise from our side.
4. The tests used for associative learning though non validated for use were applied after a pilot study and have been compared to standard tests for delayed recall.
5. The diagnosis of MCI and AD has been confirmed in our study by the existing criteria.
6. The presence of confounders has been eliminated to a large extent by selective case selection: exclusion of patients with manifest psychiatric symptoms.
7. The volumetric analysis among groups was done after adjusting for age, gender and total intracranial volumes of the individuals.
8. The correlation between volumetry and neuropsychology was arrived at after adjusting for age, gender and education in the individuals.

LIMITATIONS:

1. The small size of the cohorts cautions against the generalizability of our results.
2. The cross sectional design of our study with a lack of follow up period has diluted our findings with respect to evolution of MCI to early AD. The characteristics of converters from non-converters could thus not be assessed.
3. There was a lack of consent for obtaining MRI in the entire cohort which might have affected our correlation analysis as only a sub-group could be systematically studied for neuropsychology-MRI correlations.

CONCLUSIONS:

1. There were significant differences observed between MCI-controls and MCI-AD cohorts with respect to visual and verbal associative learning.
2. With reference to Famous personalities there were significant differences observed between MCI-control and AD-MCI cohorts.
3. There was good concordance which was observed between the newly devised test of visual associative learning and results on other standardized measures of delayed recall.
4. The results of famous personalities were concordant with measures of delayed recall in MCI cohort.
5. There were no significant differences among tested ROIs between the volumes of MCI and control cohort.
6. The AD cohort had significant reduction in volume of bilateral hippocampus, right parahippocampal and right fusiform gyrus when compared to MCI cohort.
7. In addition there was significant atrophy in white matter and gray matter of AD cohort versus MCI cohort.
8. The newly developed tests of paired associate learning had a good concordance with reduction in volume of right cuneus in AD cohort.
9. There was no significant correlation of newly developed tests of associative learning with atrophy in MCI and controls cohort.
10. There was no correlation of Famous personalities with volumes of tested ROIs in any of the cohorts.

11. **Overall these results point towards a rather broad affection of cognition in MCI patients as compared to the initial belief of isolated episodic memory affection.**
12. **The tests for visual and verbal paired associate learning, face recognition have demonstrated considerable potential in diagnosing latent (early) cognitive impairment in the elderly even prior to development of MRI volumetric changes and its potential as a novel neuropsychological biomarker in early cognitive impairment is apparent.**

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Abbreviations:

MCI	:	mild cognitive impairment
a-MCI	:	amnestic Mild cognitive impairment
md-MCI	:	Multidomain mild cognitive impairment
AD	:	Alzheimer's disease
NC	:	normal controls
FR	:	free recall
CR	:	cued recall
FP	:	famous personalities
MMSE	:	Mini mental status examination
ACE	:	Addenbrooke's cognitive examination
ACE-M	:	Addenbrooke's cognitive examination-Malayalam
RAVLT	:	Rey auditory verbal learning test
WMS	:	Wechsler memory scale
TIV	:	total intracranial volume
GM	:	gray matter
WM	:	white matter
CSF	:	cerebrospinal fluid
MRI	:	Magnetic resonance imaging

fMRI	:	functional Magnetic resonance imaging
lHip	:	left hippocampal
rHip	:	right hippocampal
lPhg	:	left parahippocampal gyrus
rPhg	:	right parahippocampal gyrus
lCun	:	left cuneus
rCun	:	right cuneus
lPrecun	:	left precuneus
rPrecun	:	right precuneus
lFusG	:	left fusiform gyrus
rFusG	:	right fusiform gyrus
NIA-AA	:	National Institute on Aging and Alzheimer's Association

PROFORMA

1. Number:
2. Age:
3. Sex:
4. Education(formal years of education):
5. Occupation:
6. Residence:
7. Clinical History:

   Symptoms:

   Age of Onset:

   Mode Of Onset:

   Faculties Affected:

   Progression of the disease:

   Functional abilities:

8. Neurological Examination:

   MMSE:

   Lobar Function test:

   Frontal:

   Temporal:

   Parietal:

   Occipital:

   Relevant other neurological systems involvement if any:

9. Investigations:

   Standard(routine) biochemical tests including Thyroid Function tests and

   Serum Vitamin B12 levels:

10. Neuropsychological Tests:

a) ACE-R(Adenbrooke's Cognitive examination-Revised)

b) RAVLT(Rey auditory verbal learning test)

c) WMS(Weschler Memory scale): visual and verbal subsets

d) WMS paired associate learning test

e) Wisconsin Card sorting

f) Semantic battery

g) Trail making test A and B

h) Warrington Face Recognition test

i) Face Name pair association test:

j) Famous faces test

11. MRI of Brain including Volumetry Studies:

CONSENT FORM CONTROLS:

STUDY TITLE: Associative learning and face recognition in MCI and early Dementias: a Neuropsychological and Brain Volumetric Study.

INVITATION

You are being invited to take part in a cross sectional study. This study attempts to understand about the changes in neuropsychological parameters that are associated with Mild Cognitive decline(MCI) and dementing illness such as Alzheimer's Disease(AD). This is being done to detect early changes in certain neuropsychological functions that may impact the diagnosis of these conditions. This will be correlated to changes in MRI scans. You will have to undergo brain magnetic resonance imaging (MRI) and a comprehensive neuropsychological analysis which are essential for the diagnosis of MCI/dementia. Before you agree to participate in this research study, it is important that you read and understand this information sheet which will give you all information about this study so that you can make a well informed and considered decision about your participation. In addition, the doctors and his team members will be happy to answer any questions that you have as well as explain the details about this research study, the procedure involved and the issues related to it. You may ask them any questions you may have regarding the study, or ask them to explain any word or information that you can't clearly understand.

WHAT IS THE PURPOSE OF THIS STUDY?

The study is proposed to analyze whether there are neuropsychological correlates which shall help in the early diagnosis of Alzheimer's Disease and also to help predict whether a patient who is normal or with early memory and behavioral problems is at a risk for Alzheimer's disease.

WHY I HAVE BEEN CHOSEN?

You are being asked to consider participation in this study because you are a healthy subject who has no major medical, psychiatric or cognitive problems and aged between 55-75 years with at least 6 years of formal school education.

DO I HAVE TO TAKE PART?

It is entirely up to you whether or not to take part. If you decide to take part you will be given this information sheet to keep and be required to sign the consent form. Even after you decide to take part, you are still free to withdraw your participation at any time after notifying the study team, without any form of penalization, and your treatment at this hospital, if any, for any medical illness will continue as before.

WHAT DO I HAVE TO DO IN THE STUDY IF I TAKE PART?

You will have to permit the investigator to run neuropsychological tests to assess your various cognitive domains. This shall require patiently sitting for the length of the questionnaire which can be 1 hour. You will also have to undergo an MRI scan along with a special MRI techniques such as MRS, DTI and rs-fMRI. This will involve lying down in the MRI scanner in a relaxed manner. You will be visually shown a crosshair images. You may have to spend 1-2 hours within the scanner. An MRI scan acquisition will be going on simultaneously while you are supine in the scanner. You may have to undergo repeat neuropsychological and cognitive assessment to assess the progression of your disease if any.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS OF TAKING PART?

There are no disadvantages/risks involved to you by participating. No expenditure will be incurred by you for participation in this study. If you are selected for the MRI scan then you need to know that the scanner has a magnet within. If you have a cardiac pacemaker or any other biomedical device (metal objects like surgical clips, devices, or implants that are in or on your body, then you cannot undergo an MRI. The doctors will check for these things before subjecting you to MRI. If you have any history of head or eye injury involving metal fragments, if you have ever worked in metal shop, you should notify the operator /investigator. During the scan, you may have to put up with a humming noise that the scanner makes. If you feel discomfort at any time, you will be able to inform the operator and you can discontinue the exam. The scans performed in this study arespecific to research purposes only. However, if

the investigator notices a finding on an MRI scan that seems abnormal then he/she will inform you about it and may advise you to take appropriate consultation for the same. In such an event the decision as to whether to proceed with further examination or treatment lies solely with you and your physician. The images which are collected in this study are generally not be made available for diagnostic purposes, but will be made available to you if there are any abnormalities found in it.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

Taking part in this research study may benefit in understanding as to which neuropsychological correlates are significantly impaired and which brain regions and networks are affected in an Indian patient with early memory and cognitive impairment. By identifying these neuropsychology and imaging biomarkers we can identify which elderly subjects with early cognitive disturbances need to be kept under close follow-up/ subjected to early treatment interventions which are likely to be developed with research in this area that is ongoing across the world. You are unlikely to be directly benefitted from the study.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Yes. During this study only your unique study code will identify you. Your name will not be shown in any reports/presentations resulting from the study. Also all information provided by you will be kept confidential and used only for study analysis.

WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

At the end of the study, the results of this study are published in a medical journal so as to make it available to the scientific community for facilitating the development of better treatment options. As stated above, no study participant will be identified in any of the scientific output emerging from this study.

WHO IS ORGANIZING THE RESEARCH?

The study is jointly organized by the Cognitive &Behavioural Neurology Section of the Dept. of Neurology, SCTIMST and the Dept. of Imaging Sciences and Interventional Radiology

in collaboration with the Cognitive Science Research Initiative (CSRI) of Dept. of Science and Technology, Govt. of India

WHO HAS REVIEWED THIS STUDY?

The study has been reviewed and approved by the Institute Ethical Committee of SCTIMST

CONTACTS AND QUESTIONS

If you have any questions regarding this research study, you may contact the study doctors -

Dr. Satyan Nanda: Resident; Department of Neurology, SCTIMST

Dr. Ramshekhar Menon, Associate Professor, Cognitive & Behaviour Neurology Section of the department of Neurology and Dr.C. Kesavadas, Dept. of Dept. of Imaging Sciences and Interventional Radiology, SCTIMST, as detailed below.

Dr. Mala Ramanathan, Member Secretary; IEC, SCTIMST.

Email: mala@sctimst.ac.in

Name and address of the Project Implementing Agency	Sree Chitra Tirunal Institute for Medical Sciences & Technology (SCTIMST), Thiruvananthapuram
Contact Person 1	Dr.Satyan Nanda, Resident Department of Neurology, SCTIMST, Thiruvananthapuram - 695011 Phone: 7860536645
Contact person 2	Dr.Ramshekhar Menon. Associate Professor, Department of Neurology, SCTIMST, Thiruvananthapuram - 695011 Phone: 0471-2524481
Contact person 3	Dr.C. Kesavadas. Professor of Neuroradiology Department of IS&IR, SCTIMST, Thiruvananthapuram - 695011 Phone: 0471-2524215

Thank you for reading and considering participation in this study.

I have been explained the purposes of the research being undertaken, and have understood them. ☐

I have had the opportunity to ask questions and am satisfied with the answers provided to me. ☐

I have been informed of the risk of participation.

I have been informed of and I am satisfied with the steps implemented for protecting my privacy and confidentiality. ☐

I have been informed and I am aware that I may not derive any direct immediate benefits from this research results. ☐

I hereby give an informed voluntary consent to participate in this study and its procedures. ☐

I have received a copy of this consent form.

For any queries/further explanation please contact the Secretary of IEC:

Phone number: 0471-2524234

E-mail: iec.mem.sec@sctimst.ac.in

Study Participant:

_____	_____	_____
Name	Signature	Date

Witnessed by:

_____	_____	_____
Name	Signature	Date

Investigator:

_____	_____	_____
Name	Signature	Date

Consent form For Patients: Mild cognitive Impairment and Alzheimer's disease

STUDY TITLE: Associative learning and face recognition in MCI and early Dementias: A Neuropsychological and Volumetric Study.

INVITATION

You are being invited to take part in a cross sectional study. This study attempts to understand about the changes in neuropsychological parameters that are associated with Mild Cognitive decline(MCI) and dementing illness such as Alzheimer's Disease(AD). This is being done to detect early changes in certain neuropsychological functions that may impact the diagnosis of these conditions. This will be correlated to changes in MRI scans. You will have to undergo brain magnetic resonance imaging (MRI) and a comprehensive neuropsychological analysis which are essential for the diagnosis of MCI/dementia. Before you agree to participate in this research study, it is important that you read and understand this information sheet which will give you all information about this study so that you can make a well informed and considered decision about your participation. In addition, the doctors and his team members will be happy to answer any questions that you have as well as explain the details about this research study, the procedure involved and the issues related to it. You may ask them any questions you may have regarding the study, or ask them to explain any word or information that you can't clearly understand.

WHAT IS THE PURPOSE OF THIS STUDY?

The study is proposed to analyze whether there are neuropsychological correlates which shall help in the early diagnosis of Alzheimer's Disease and also to help predict whether a patient who is normal or with early memory and behavioural problems is at a risk for Alzheimer's disease.

WHY I HAVE BEEN CHOSEN?

You are being asked to consider participation in this study because you are a patient diagnosed with Mild Cognitive Impairment (MCI)/Subjective Cognitive Impairment (SCI)/ Mild Behavioural Impairment (MBI) with memory or cognitive problems or early dementia.

DO I HAVE TO TAKE PART?

It is entirely up to you whether or not to take part. If you decide to take part you will be given this information sheet to keep and be required to sign the consent form. Even after you decide to take part, you are still free to withdraw your participation at any time after notifying the study team, without any form of penalization, and your treatment at this hospital for any medicalillness will continue as before.

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WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

At the end of the study, the results of this study are published in a medical journal so as to make it available to the scientific community for facilitating the development of better treatment options. As stated above, no study participant will be identified in any of the scientific output emerging from this study.

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Dr.Ramshekhar Menon, Associate Professor, Cognitive & Behaviour Neurology Section of the department of Neurology and Dr.C.Kesavadas, Dept. of Dept. of Imaging Sciences and Interventional Radiology,SCTIMST, as detailed below.

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Name	Signature	Date

Witnessed by:

_____	_____	_____
Name	Signature	Date

Investigator:

_____	_____	_____
Name	Signature	Date

STANDARD NEUROPSYCHOLOGY TEST BATTERY AND INTERPRETATIONS

Neuropsychological assessment

The following neuropsychological tests for verbal and visual memory were administered to both the patient and control groups separately**.**

Verbal memory

The tests for verbal memory included Wechsler Memory Scale-Revised (WMS-R) logical memory test[1], Rey Auditory Verbal Learning Test (RAVLT)[2], and WMS-R Paired Associate Learning Test.[1]

The WMS or WMS-R contains sub-tests like Logical Memory Passage, Visual Reproduction and Paired Associate Learning. Logical Memory Passage is a test of paragraph or prose recall and has an immediate recall and delayed recall. The examiner reads two stories, stops after each reading,andaskfor an immediate free recall. After a delay of 30 minutes, delayed recall is taken as an attempted verbatim recitation. Story 1 contains 24 memory units and Story 2, 23 memory units. The total score is the total number of ideas recalled for both stories together.

The *Verbal Paired Association Learning Test* consists of ten word pairs, six forming "easy" associates and other four "hard" word pairs that are not really associated. The list is read three times, with a memory recall after each trial. The first of word pair is presented, and the subject has to give the associated pair. When the subject gives an incorrect response, the correct response is told and the test is continued. One credit is given for each correct response. Total score is one-half of the sum of all the correct answers on easy pairs plus the sum of all correct associations to the hard pairs.

The *RAVLT* consists of word lists A and B. There are 15 words in each list. List A is read out first at the rate of one word per second. The subject has to recall as many words as possible, in any order. List A is repeated 5 times, the total leading to a maximum possible score of 75 (15 x 5). Then the examiner presents a second list of 15 words (List B). The subject has to recall the words from this list also. Immediately following this, the subject is asked to recall as many words as possible from List A. Delayed recall of List A is given after 20-30 minutes. The score for each trial is the number of words correctly recalled. After the delayed recall, recognition is tested by asking the respondent to indicate which of the 50 words in a list read aloud (a mix of words from both lists A and B, as well as semantically or phonemically similar words to Lists A and B) from List A, and which were not. The learning score over the five trials and the delayed recall score was used in the study to assess verbal learning and memory.

Visual memory

The visual memory tests were *WMS-R visual reproduction subsets*[1]and Warrington Recognition Memory Test for Faces (RMF)[3]

The *WMS-R Visual Reproduction Subsets* requires the subject to draw from memory simple geometric figures. Each of the visual reproduction cards is shown for ten seconds. Following each presentation,immediate recall is tested. The subjects then draw from memory what they remember of the design. A delayed recall is taken after 30 minutes.

In the *RMF*, subjects are presented with black and white photos of 25 unfamiliar men. Immediately following presentation of the target photos, memory for the photos is tested with a forced choice between a target photo identical to that presented earlier, and 25 other distracter photos presented in pairs. A delayed recall test is also carried

out in this study to evaluate the retrieval of faces with the scoring similar to that of the immediate recall.

The standard neurological tests which were done included ACE-Malayalam and MMSE:

1. ACE-Malayalam[4]: the divisions of ACE as in registration; orientation, language, visuospatial and recall ability have been retained. The score distribution (mean +/- 1SD), percentage at ceiling, and relative difficulties across items is comparable between the UK and the educationally equivalent Indian groups. Language, Naming, Attention and Orientation are relatively easy (≥ 80% at ceiling) and Recall and Verbal fluency are relatively difficult (≤22% at ceiling). Although the percentage at ceiling are lower for the Indian group, the order of relative difficulty is similar and the percentage scoring at floor is ≤10% on all except visuospatial item.
2. MMSE[5]: as detailed into its components of orientation to time, place, attention, registration, recall, language and comprehension was applied.

.

References

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5. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. Journal of psychiatric research. 1975 Nov 1;12(3):189-98.

FACE NAME PAIRING TEST

Subramanian സുബ്രമണ്യൻ

Varghese വർഗ്ഗീസ്

Thankamma തങ്കമ്മ

Raju രാജു

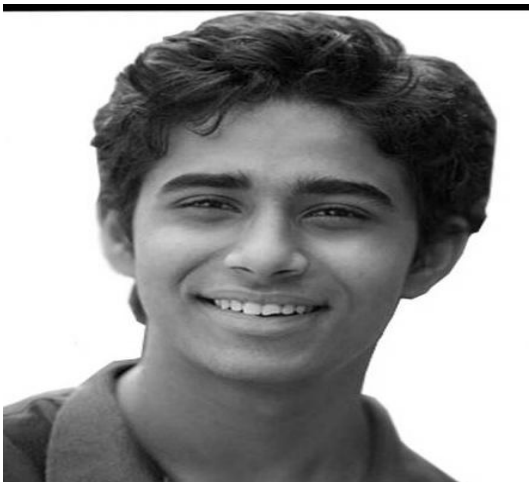
Nandini നന്ദിനി

Jamshed ജംഷദ്

Varun വരുൺ

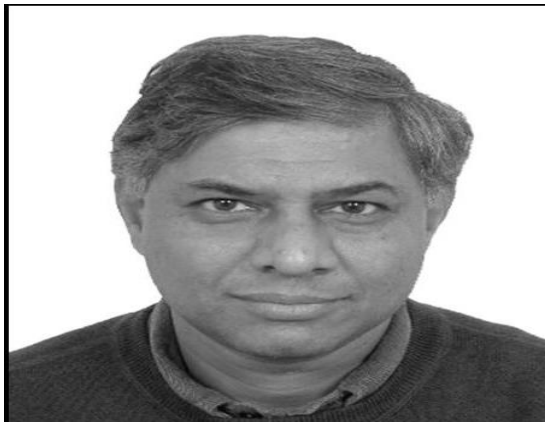
Prabha പ്രഭ

Yousuf യൂസഫ്



Roni റോണി

Ratnamma രത്നമ്മ



George ജോർജ്ജ്

Lakshmi ലക്ഷ്മി

Syam ശ്യാം

Mridula മൃദുല

Usman ഉസ്മാൻ

Distractors name:

1. Muralidharan മുരളീധരൻ
2. Ramachandran രാമചന്ദ്രൻ
3. Augustine അഗസ്റ്റിൻ
4. Thomas തോമസ്സ്
5. Saraswathy സരസ്വതി
6. Santha ശാന്ത
7. Mohan മോഹൻ
8. Binu ബിനു
9. Sruthi ശ്രുതി
10. Revathy രേവതി
11. Musthafa മുസ്തഫ
12. Hameed ഹമീദ്
13. Anil അനിൽ
14. Rajesh രാജേഷ്

15. Sulekha	സുലേഖ
16. Soumya	സൗമ്യ
17. Jalaludeen	ജലാലുദീൻ
18. Shareef	ഷരീഫ്
19. Jijo	ജിജോ
20. Sijo	സിജോ
21. Rajamma	രാജമ്മ
22. Kamalamma	കമലമ്മ
23. Mathew	മാത്യു
24. Jose	ജോസ്
25. Sreeja	ശ്രീജ
26. Vineetha	വിനിത
27. Sandeep	സന്ദീപ്
28. Sajith	സജിത്ത്
29. Manju	മഞ്ചു
30. Poornima	പൂർണ്ണിമ
31. Ansar	അൻസാർ
32. Salim	സലിം

FAMOUS PERSONALITIES TEST

V S Achuthanandan

Indira Gandhi

Narendra Modi

K R Gowri Amma

Mammooty

Kamala Surayya

Mohan Lal

P T Usha

Oomen Chandy

Sugatha Kumari

Sathyan

Aishwarya Rai

Sachin Tendulkar

P T Usha

Mother Teresa

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, त्रिवेन्द्रम
तिरुवनन्तपुरम - ६९५०११, केरल, इंडिया
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY, TRIVANDRUM
Thiruvananthapuram - 695 011, Kerala, India
(An Institute of National Importance under Govt. of India)
Grams : Chitramet, Phone : +91-471-2443152, Fax : +91-471-2550728 / 2446433, E-mail : sct@sctimst.ac.in, Website : www.sctimst.ac.in

Institutional Ethics Committee
(IEC Regn No. ECR/189/Inst/KL/2013)

SCT/IEC/978/OCTOBER-2016 19.04.2017

Dr. Satyan Nanda
Senior Resident
Department of Neurology
SCTIMST, Thiruvananthapuram

Dear Dr. Satyan Nanda,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "ASSOCIATIVE LEARNING AND FACE RECOGNITION IN MCI AND EARLY ALZHEIMER'S DISEASE-A NEUROPSYCHOLOGICAL AND BRAIN VOLUMETRIC STUDY" (IEC/978) on 14th October, 2016.

The following documents were reviewed:

Original submission

1. Covering letter addressed to the Chairperson, IEC, SCTIMST, dated 22.09.2016 with check list
2. TAC Approval Letter
3. Forwarding Letter from Dr. Ramshekhar N Menin, Associate Professor, Department of Neurology, SCTIMST addressed to the Chairperson, IEC, SCTIMST, dated 22.09.2016
4. IEC Application Form 5.Project Proposal 6. Proforma
7. Informed Consent Forms for patients in English and Malayalam
8. Informed Consent Forms for healthy volunteers in English and Malayalam
9. CV of Principal Investigator and Co- Investigators

Revised submission

1. Covering letter addressed to the Chairman, IEC, SCTIMST, dated 21.03.2017 with check list
2. Copy of the IEC Recommendation Letter dated 07.11.2016
3. TAC Approval Letter
4. Forwarding Letter from Dr. Ramshekhar N Menin, Associate Professor, Department of Neurology, SCTIMST addressed to the Chairperson, IEC, SCTIMST, dated 21.03.2017
5. IEC Application Form 6.Project Proposal 7. Proforma
8. Informed Consent Forms for patients in English and Malayalam
10. Informed Consent Forms for healthy volunteers in English and Malayalam
11. CV of Principal Investigator and Co- Investigators
12. Results of the tools: Famous faces recognition and Face Name pair association test

The following members of the Ethics Committee were present at the meeting held on 14th October, 2016 at G. Parthasarathi Board Room, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Justice Gopinathan. P.S	BSc. LLB	Male	Legal Expert (Chairperson)	No
2.	Dr. Asha Kishore	MD, DM	Female	Clinician (Neurologist)	Yes
3.	Dr. Harikrishna Varma PR	PhD	Male	Biomedical Scientist	Yes
4.	Dr. Meenu Hariharan	DM	Female	Clinician (Gastro-Enterologist)	No
5.	Dr. Rema M. N	MD	Female	Pharmacologist	No
6.	Dr. R V G Menon	PhD	Male	Lay Person	No
7.	Smt. Sathi Nair	MA	Female	Lay Person	No
8.	Dr. V. Raman Kutty	MPH(Harvard) MPhil, MD	Male	Public Health	Yes
9.	Dr. K R S Krishnan	ME, PhD	Male	Biomedical Scientist/Engineer	No
10.	Dr. Kala Kesavan. P	MD	Female	Pharmacologist	No
11.	Dr. Christina George	MD	Female	Psychiatrist	No
12.	Dr. P. Manickam	PhD	Male	Scientist - Epidemiologist	No
13.	Dr. Mala Ramanathan	MSc, PhD, MA	Female	Ethicist/Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

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