CHAPTER 3
SUBJECTS AND MATERIALS

3.1 SUBJECTS

The study was approved by the Sree Chitra Tirunal Institute for Medical Science and Technology (SCTIMST) ethics committee and all the participants gave informed consent for their participation in the study. The study participants were recruited from the patients attending the memory & neurobehavioral clinic of SCTIMST. The spouses of the patients and some volunteers were recruited as controls. The data was processed and analyzed in the Collaborative Brain Mapping Unit at SCTIMST funded by the Kerala State Council for Science, Technology and Environment.

All participants, after informed consent, were subjected to clinical examination, consisting of brief structured history of cognition, and other neurological symptoms and risk factors and a detailed neurological examination at baseline and after one year. They were also subjected to a structured neuropsychological test and radiological investigation. Neuropsychological tests have been standardized on the local population and scores on controls or norms on community based population have already been derived. The original set of the studied subjects was 113, including 23 controls, 49 MCI subjects and 41 subjects with AD.

Table 3.1. Descriptive Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>NCI</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>113</td>
<td>23</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>73/40</td>
<td>11/12</td>
<td>32/17</td>
<td>30/11</td>
</tr>
<tr>
<td>Age</td>
<td>65.22±13.36</td>
<td>61.87±10.62</td>
<td>65.77±11.97</td>
<td>69.88±09.08</td>
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<tr>
<td>Education</td>
<td>12.18±04.17</td>
<td>14.52±04.19</td>
<td>12.08±03.96</td>
<td>10.92±03.92</td>
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<tr>
<td>MMSE_V0</td>
<td>23.78±06.50</td>
<td>28.70±00.97</td>
<td>26.57±02.67</td>
<td>17.68±06.93</td>
</tr>
<tr>
<td>MMSE_V1</td>
<td>26.72±02.91</td>
<td>28.02±00.87</td>
<td>25.50±01.98</td>
<td></td>
</tr>
<tr>
<td>ACE_V0</td>
<td>74.15±21.86</td>
<td>93.87±04.74</td>
<td>79.82±11.02</td>
<td>56.32±24.32</td>
</tr>
<tr>
<td>ACE_V1</td>
<td>70.97±14.97</td>
<td>92.95±03.94</td>
<td>69.00±11.14</td>
<td></td>
</tr>
</tbody>
</table>

_V0-baseline visit; _V1-first follow-up visit

Longitudinal volumetric studies that used ROI based measurements and classification methods included 23 subjects with MCI for whom clinical follow up data and brain volumetry data was available. The classification algorithmic and ROI
based volumetric discriminating study included the entire set of 113 subjects, 23 healthy elderly controls, 49 MCI subjects and 41 subjects with AD.

3.1.1 Inclusion and Exclusion Criteria

Inclusion criteria

a) 55 years of age or more
b) ≥ 7 years of education
c) Cardiac disease is stable on appropriate medication for 3 months prior to screening
d) DM (HbAlc concentration of <8% and a BSL < 250 mg/dL), no hospital admission within 3 months due to complications
e) Thyroid disease patients were included if they were euthyroid on treatment
f) No history of epilepsy, stroke, head-injury with loss of consciousness, any other diagnosed disease of central nervous system
g) Patients and study partner are willing to give written Informed Consent

Exclusion criteria

a) Patients without a reliable study partner
b) Patients on cholinesterase inhibitor or memantine
c) Patients with epilepsy, major stroke, head injury, loss of consciousness, any other diagnosed CNS disorder
d) Recent (<3 months) AMI or surgery for PVD, CABG
e) Recent (<2 years) hematologic/oncologic disorders other than basal or squamous cell carcinoma
f) Vitamin B-12 or folate deficiency at screening
g) Clinically significant, active GI, hepatic, or pulmonary disease
h) History (within 2 years) of alcoholism or drug misuse
i) C/o Schizophrenia and unipolar and bipolar depressive illness

Following the screening, for the purpose of this study, all participants were subjected to a baseline visit evaluation and were reclassified into one of the three clinical diagnostic categories: No Cognitive Impairment, Mild Cognitive Impairment and Dementia, as detailed in the section Criteria and Definition below.
3.1.2 Criteria and Definition

Using the clinical, neuropsychological and radiological information, the subjects were classified into one of the three diagnostic outcomes - No Cognitive Impairment, Mild Cognitive Impairment and AD. Subjects classified as MCI were further classified as amnestic-MCI (a-MCI) or other-MCI (o-MCI). The diagnosis of dementia was made using the Diagnostic & Statistical Manual– 4th edition (DSM-IV) criteria (Appendix 2). Those diagnosed as DEM were further classified as AD if they meet the NINDS-ADRDA criteria for AD (Appendix 1) or as non-AD. Diagnosis of MCI was be made using the consensus revised criteria and that of a-MCI using Petersen’s criteria. Subjects who meet the requirement of MCI but not of a-MCI were classified as o-MCI and those who do not meet the criteria of MCI or DEM were considered as NCI.

3.2 NEUROPSYCHOLOGICAL ANALYSIS

The study subjects were administered the Mini Mental State Examination, Clinical Dementia Rating (CDR), Addenbrooks Cognitive Examination (ACE), Ray Auditory Verbal Learning Test (RAVLT), Semantic Battery Verbal Fluency and Trail Making test. The neuropsychological assessment was done at the hospital in a quiet room by a trained neurophysiologist.

3.3 MAGNETIC RESONANCE IMAGING (MRI)

Magnetic Resonance Imaging is non-invasive imaging technique used in radiology to provide an unequaled view of the anatomic configuration of the living human brain. MR images of the soft tissue of the structures of the body such as brain, heart, and liver provide accurate analysis of the anatomy unlike the other imaging modalities. In other imaging modalities the hard bone will obscure the details. MR brain slice images are also used for detection of brain tumors. Neurologists are greatly aided by the MRI as it unfolds the interiors of the most vital of the human organs of the brain. The imaging analysis of the brain helps in the quantitative and qualitative study of the brain which helps the neurologists diagnose and analyze various neural disorders associated with the brain like a tumor, epilepsy, cerebral palsy, hydrocephalus, AD etc.
3.3.1 Working Principle

One of the most recent inventions to have changed the way of medical diagnostics is the MRI machine. Its inventor Dr. Raymond V Damadian received the 2007 national inventor of the year award for the MRI, even though the Nobel Prize on the MRI went to Paul Lauterbur and Sir Peter Mansfield in 2003. The first MRI device for a full body scan was built by 1977. MRI makes use of the property of Nuclear Magnetic Resonance (NMR) to image nuclei of atoms in the body. MRI uses Magnetization and radio waves, rather than X-rays. An MRI scanner is a device in which the patient lies within a great, powerful magnet where the magnetic field is used to align the magnetization of some atomic nuclei in the body, and radiofrequency pulse are given to alter the alignment of this magnetization. This causes the nuclei to generate a rotating magnetic field detectable by the scanner and this information is recorded to construct an image of the scanned area of the body. Magnetic field gradients cause nuclei at different locations to process at different speeds, which permit spatial information to be recovered using Fourier analysis of the measured signal. By using gradients in different directions, 2D images or 3D volumes can be obtained in any arbitrary orientation. MRI machines make use of the fact that body tissue contains lots of water (H$_2$O), and hence protons (1H nuclei), which will be aligned in a large magnetic field. Each water molecule has two hydrogen nuclei or protons. When a person is inside the powerful magnetic field of the scanner, the average magnetic moment of many protons becomes aligned with the direction of the field. A radio frequency current is briefly turned on, producing a varying electromagnetic field. This electromagnetic field has just the right frequency, known as the resonance frequency, to be absorbed and flip the spin of the protons in the magnetic field. After the electromagnetic field is turned off, the spins of the protons return to thermodynamic equilibrium and the bulk magnetization becomes realigned with the static magnetic field. During this relaxation, a radio frequency signal (electromagnetic radiation in the RF range) is generated, which can be measured with receiver coils. Protons in different tissues return to their equilibrium state at different relaxation rates. Different tissue variables, including spin density, T1 and T2 relaxation times, and flow and spectral shifts, can be used to construct images. By changing the settings on the scanner, this effect is used to create contrast between different types of body tissue or between other properties, as in fMRI and diffusion MRI. MRI is used to image every part of the body, and is particularly
useful for tissues with many hydrogen nuclei and little density contrast, such as the brain, muscle, connective tissue and most tumors.

MRI scans require a magnetic field with two properties, uniform field density and strength. The magnetic field cannot vary more than 1/10,000 to 1% and field strength ranges (depending on the scanner) from 0.2 to 3 Tesla in strength in scanners currently used clinically, with research scanners investigating higher field strengths such as 7 Tesla. MRI contrast agents may be injected intravenously to enhance the appearance of blood vessels, tumors or inflammation. Contrast agents may also be directly injected into a joint in the case of arthrograms.

In clinical practice, MRI is used to distinguish pathologic tissue (such as a brain tumor) from normal tissue. One advantage of an MRI scan is that it is harmless to the patient. It uses strong magnetic fields and non-ionizing electromagnetic fields in the radio frequency range, unlike CT scans and traditional X-rays, which both use ionizing radiation.

3.4 IMAGING PROTOCOL

Whole brain MRI scans were obtained on Siemens Magnetom-Avanto SQ engine, 1.5 Tesla MR Scanner. Whole brain volume was acquired by the 3D flash spoiled gradient echo sequence using standard parameters, TR=11msec, TE=4.95, flip angle=150, slice thickness=1mm, matrix=256x256, 112 axial plane images were made to cover the whole brain. The images were post processed in the fully equipped brain mapping unit of Cognitive and Behavior Neurology Section (CBNS).

3.5 MR IMAGE FORMAT

MRI can be stored in numerous different formats, and there are two general classifications of formats for storing MRI. The first is a scanner format, a format in which the MRI is output from the machine that captures the images. The other is an image processing format which is obtained through a conversion of the MRI from the original scanner format. For the purposes of this study, we are interested primarily in the image processing format of MRI, specifically the Digital Imaging and Communications in Medicine (DICOM) formats. For now, we leave the conversion from the scanner format to our supported formats up to the user. The DICOM format is adapted from the BRAINVOAGER software. This software
converts the scanner format to '.dcm' format using 'rename dicom' files in the option menu.

3.5.1 Dicom Files to Analyze Standards

'MRIconvert' is a medical image file conversion utility that converts DICOM files to analyze format. The Analyze format consists of two files, an .hdr file and an .img file. The header contains information about the image file, such as the data type, image dimensions, and voxel scale. To choose files to convert: choose "Input/Add" from the menu, or click on the "Add" button. This will bring up a directory selector dialog. Choose a directory containing the files you wish to convert. All DICOM files in this directory and its subdirectories will be added to the "DICOM input" panel. Files must end in ".dcm" and must conform to the DICOM standard to be included. The input files are organized by subject, study, and series into a tree structure. Subjects, studies, or series that you don't wish to convert may be deleted from the input list by choosing "Input/Remove" from the menu or by clicking on the "Remove" button. Individual files may not be deleted from a series. Before converting files, the user may view images, DICOM header information, or a summary of information for a series. Individual images may be converted to JPEG, BMP, or TIFF formats. MRI convert creates a directory structure based on series and subject. Directories and output files are given default names based on subject, study, and series information. The user may change these names by selecting the file or directory to be renamed and choosing "Output/Rename" from the menu or by clicking on the "Rename" button. To change to root directory for output, choose "Output/Directory" from the menu or click on the "Directory" button. DICOM information may be saved to a text file from the DICOM viewer. Six output formats are supported: FSL NifTi, SPM Analyze, Meta Image, NifTi, Analyze 7.5 and Brain Voyager.

3.6 SUMMARY

This chapter discusses a subject selected for the proposed study and provides the details of the image acquisition parameters we have used to develop our application. This chapter also discusses the various image format and various methods used for the early diagnosis of AD.