QUANTIFIED MULTIMODALITY MR IMAGING BIOMARKERS FOR DIAGNOSIS OF COGNITIVE IMPAIRMENT IN ELDERLY PATIENTS

A Thesis

Submitted By

Muskan Khetan

For the award of the degree of

MASTER OF TECHNOLOGY, CLINICAL ENGINEERING

Jointly offered by

Indian Institute of Technology, Madras

Christian Medical College, Vellore

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum

Is evaluated and approved by

Dr. Ramshekhar N. Menon	Dr. C. Kesavadas	Dr. Santosh Kumar K.
(Guide)	(Co-guide)	(Examiner)

EKHAR MENON
...DM (NIMHANS), EPILEPSY FELLOWSHIP
Consultant Neurologist
Sree Chitra Tirunal Institute
For Medical Sciences & Technology
...m-11; Reg No: TCMC 30958

Dr. C. KESAVADAS
Professor of Radiology
DEPT. OF IMAGING SCIENCES AND INTERVENTIONAL RADIOLOGY
SREE CHITHRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
TRIVANDRUM - 695 011
Email: kesav@sctimst.ac.in, Mob: 9447047002

DEPARTMENT OF IMAGING SCIENCES & INTERVENTIONAL RADIOLOGY
THIRUVANANTHAPURAM
S.C.T.I.M.S.T

CERTIFICATE

This is to certify that the thesis titled **'Quantified Multimodality MR Imaging Biomarkers for Diagnosis of Cognitive Impairment in elderly patients'** being submitted by **Muskan khetan** to SCTIMSTTrivandrum, for the award of degree of **Master of Technology in Clinical Engineering** jointly offered by IIT Madras, CMC Vellore and SCTIMST Trivandrum, is a bonafide record of research work done by her under our supervision. The contents of this thesis in full or in parts have not been submitted to any other Institute or University for the award of any degree or diploma.

The research had been carried out at Sree Chitra Institute of Medical Sciences and Technology (SCTIMST), Trivandrum.

Dr. Ramshekhar N. Menon
Guide
Associate Professor
Department of Neurology
SCTIMST, Trivandrum

Dr. C. Kesavadas
Co-guide
Professor
Department of Imaging Sciences
and Interventional Radiology,
SCTIMST, Trivandrum

Date: 14/6/21

DR. RAMSHEKHAR MENON
MD; DNB; DM (BOMBAY), EPILEPSY FELLOWSHIP
Consultant Neurologist
Sree Chitra Tirunal Institute
For Medical Sciences & Technology
Reg No: TCMC 30958

ACKNOWLEDGEMENT

Foremost, I would like to express my sincere gratitude to my advisor Dr. Ramshekhar N. Menon of Department of Neurology at SCTIMST Trivandrum for the continuous support of my MTech Project study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis.

Besides my advisor, I would like to thank Dr C. Kesavadas as my Co-guide for continuous support and giving me this opportunity to do my research work in this field under his supervision.

My sincere thanks also goes to Mr. P.G. Rajesh (Senior Research Fellow) SCTIMST, for guiding me throughout my project work and helped me clearing my basic doubts instantly. He had consistently allowed me to work and constantly steered me in the right direction whenever he thought I needed it. He had also actively helped in formulating ideas into implementation.

Special thanks to Dr. Ravi Prasad Verma (Associate Professor at AMC, SCTIMST Trivandrum). He has been energetically contributed in statistical analysis during our study. He allowed me to learn and gave me new insights into statistics concepts. His knowledge in the data study helped us in reaching our findings.

I also express my sincere gratitude to Clinical Neuropsychologists Ms Meenu KS and Ms Sushma SR for helping me in extracting data related to neuropsychological tests of each patients. Also, I would like to thank other members in Radiology lab Mr Jithin and Ms Gautami (Research scholars, SCTIMST) who were very supportive during my project, and guided me to deal with small details in the hospital.

Lastly, I also convey my gratitude to Dr Roy Joseph for conducting the whole academic semester smoothly and letting us to be more focus towards the project. His kind help and support was a motivation for me in this duration.

I offer my sincere regards to my parents and batchmates who supported me in all respects during the course of the project.

TABLE OF CONTENTS

LIST OF FIGURES:

LIST OF TABLES:

LIST OF ABBREVIATION:

AD:	Alzheimer's disease
ADNI:	Alzheimer's Disease Neuroimaging Initiative
CSF:	cerebrospinal fluid
MCI:	mild cognitive impairment
MMSE:	Mini-Mental State Exam
MRI:	magnetic resonance imaging
sMRI:	structural magnetic resonance imaging
rs-fMRI:	resting state functional magnetic resonance imaging
ROI:	Region of Interest
BOLD:	Blood-oxygen-level-dependent
MNI:	Montreal Neurological Institute
NINCDS–ADRDA:	National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association
NFTs:	Neurofibrillary Tangles
MTL:	Medial Temporal Lobe
RSNs:	Resting state networks
NC:	Normal Control
SPM:	Statistical Parametric Mapping
CONN:	Connectivity
FDR:	False discovery rate
SPSS:	Statistical Package for the Social Sciences
ROC:	Receiver Operating characteristic curve
LASSO:	Least Absolute Shrinkage and Selection Operator

SVM:	Support vector machine
RELM:	Retrieval-Augmented Language Model
MMSE:	Mini-Mental State Examination
ACE-T:	Addenbrooke's cognitive examination
RAVLT-T:	Rey's Auditory Verbal Learning Test
ANOVA:	Analysis of variance
gVol:	grey matter Volume
NV:	Neuropsychiatric variable
GE:	Global Efficiency
LE:	Local Efficiency
AvPL:	Average Path Length
CC:	Cluster Coefficient
BC:	Betweenness Centrality
DMN:	Default Mode Network
PET:	Positron Emisson Tomography
ICA:	Independent Component Analysis
TR/TE:	Time of repeat
FC:	Functional Connectivity
TP:	True Positive
TN:	True Negative
FP:	False Positive
FN:	False Negative

ABSTRACT:

Background: The early diagnosis of mild cognitive impairment (MCI) and prediction of risk of progression to Alzheimer's disease (AD) is still challenging based on structural and functional MRI. the challenge is to diagnose MCI from Normal Control (NC) and AD with higher sensitivity to avoid false-negative results, thus increases the chances of controlling the progression of MCI to AD, prognostication in patients at risk of progression and guiding inclusion of patients with MCI in dementia-specific treatment trials. In our study, we took the help of two widely studied imaging biomarkers with a different approach; we combined two biomarkers indicating structural atrophy and resting-state functional connectivity changes. Structural atrophy was quantified by grey-matter volume calculation [global and regions of interest (ROI)], while functional connectivity was quantified using matrices and features delineated by graph theory (taking ROIs as nodes and correlation values between nodes as edges). Considering the findings of graph theory to functional connectivity and not utilizing it with other biomarkers yet, we hypothesize to study it in combination with cognitive functioning and structural changes.

Methodology: We selected three groups of 23NC, 34MCI, and 29 AD (n=86) in our study who were age (60-79), gender, and educational background (10-20 years) controlled. Both structural and resting-state scans were taken using 3T MRI and 22 ROIs were observed based on Harvard-Oxford Cortical atlas (in CONN18.0) and Neurophometric atlas (in CAT12.0). Also, to relate cognitive changes with each biomarker, we performed comprehensive neuropsychological assessment tests such as MMSE, ACE-total, RAVLT-total, and delayed-RAVLT. However, considering the non-normal distribution of most of the variables, the accuracy of each biomarker in predicting an outcome was assessed using Receiver Operating Characteristic (ROC) analysis. Variables significant at this level were explored further using binary logistic regression. Variables with a p-value less than 0.1 in the binary logistic regression were included in a multivariate backward conditional binary logistic regression model based on likelihood ratios. Additionally, a discriminant function analysis was also carried out to explore prediction group membership in any of the three groups based on biomarker readings.

Results: We observed higher centrality, greater degree, and larger cluster coefficiency of connectivity in AD and MCI patients as compared to the control group, despite showing the decline in grey-matter volume in the patient's brain regions of Amygdala(L), Caudate(R), MidFrontalGyrus(L) and Precuneus. Besides this, the AD subgroup revealed a positive correlation between grey matter volume and cognitive test scores. The AD sub group showed a significantly negative correlation ($r<-0.3$; $p<0.05$) between grey-matter volume and functional connectivity, while MCI patients showed a positive correlation ($r >+0.3$; $p<0.05$) between functional connectivity and cognitive functioning. These results are suggestive of potential compensatory mechanisms in the elderly consequent to early cognitive decline as reflected in this inverse relationship between functional connectivity and brain volumes within ROI. Furthermore, in our multivariate analysis, we looked at two disease classification models using parameters determined as significant on multivariate analysis, viz. grey matter volume of Hippocampus(L) and SupraMarginalGyrus(R). The first model gave us an overall accuracy of 72.5% to classify AD from MCI and NC (to classify AD from NC; accuracy= 88.6%, and to classify AD from MCI; accuracy= 81.2%), while another classification model gave the accuracy of 69.6% to classify MCI from NC.

Conclusion: The multimodality and graph theory approach allows for the characterization of functional connectivity changes in each diagnostic group, which could be used in further studies to understand relationships between brain connectivity and development of atrophy in specific brain regions. Functional connectivity seems to have a bearing on brain volumes in AD and neuropsychological test performances in MCI. As a diagnostic classifier, region-specific brain volumes appear to have greater accuracy in comparison to connectivity measures. However, larger sample size and a longitudinal study will be required to validate our findings with regard to multimodality integration of imaging biomarkers for screening or classification of elderly patients into diagnostic subgroups.

Keywords: sMRI, rs-fMRI, graph theory, grey-matter volume, functional connectivity, compensatory mechanism, classification model.

CHAPTER 1: INTRODUCTION AND STATEMENT OF PROBLEM

1.1 ALZHEIMER'S DISEASE:

Alzheimer's disease (AD) is a neurologic disorder that causes the brain to shrink (atrophy) and the death of brain cells. Alzheimer's disease is the most critical cause of dementia, which is described as a progressive loss of cognitive, behavioural, and social abilities that impairs a person's ability to function independently.

Prevalence of Dementia and AD:

In the US 65 years of age and over Alzheimer's disease affects around 5.8 million people. 80% of these are about 75 years of age or older. It was anticipated by the United Nations ageing programme and the U.S. centres for disease prevention and control that by 2030 the average number of older people worldwide (65+ years) will increase from the figure of 420 million in 2000 to nearly 1 billion, with the proportion of older people rising from 7 to 12%. The largest increase in the absolute number of older people will be observed in developing countries. The share of the world's ageing population in developing nations is therefore increasing from 59% to 71%.

Surveys suggest that as of 2010, global prevalence of dementia was 35.6 million and India accounts for about 3.7 million people with dementia. Worldwide, the total number of patients with dementia is expected to almost double in every 20 years from 65.7 million in 2030 to 115.4 million in 2050. (India KSD, 2015)

With demographic ageing, comes the problem of dementia and India is expected to have one of the largest number of elders with dementia. In 2015, an estimated of 4.1 million persons aged over 60 years had dementia in India. This is estimated to rise from 6.35 million to 13.33 million in between 2020 to 2050. In the following two-three decades, the burden of dementia in India is already high and is expected to rise rapidly. (Shaji K et al., 2010)

The dementia treatment or service gap for India is estimated at around 90%, with only 1 out of 10 people diagnosed, treated for dementia. This is due to numerous reasons, but the main ones seem very poor awareness of dementia both among society and the health professionals, very low human resources capacity or trained for the treatment of people with dementia and a lack of priorities for dementia for the health sector. (The World Bank, 2017)

According to the World Alzheimer Report 2016, With a large service gap, lack of awareness in the community and lack of adequate specialist trained resources in low and middle-income countries like India, there is a need to involve not only primary care services in the deliver dementia care but also community health workers trained to do specific tasks, in dementia care provision.

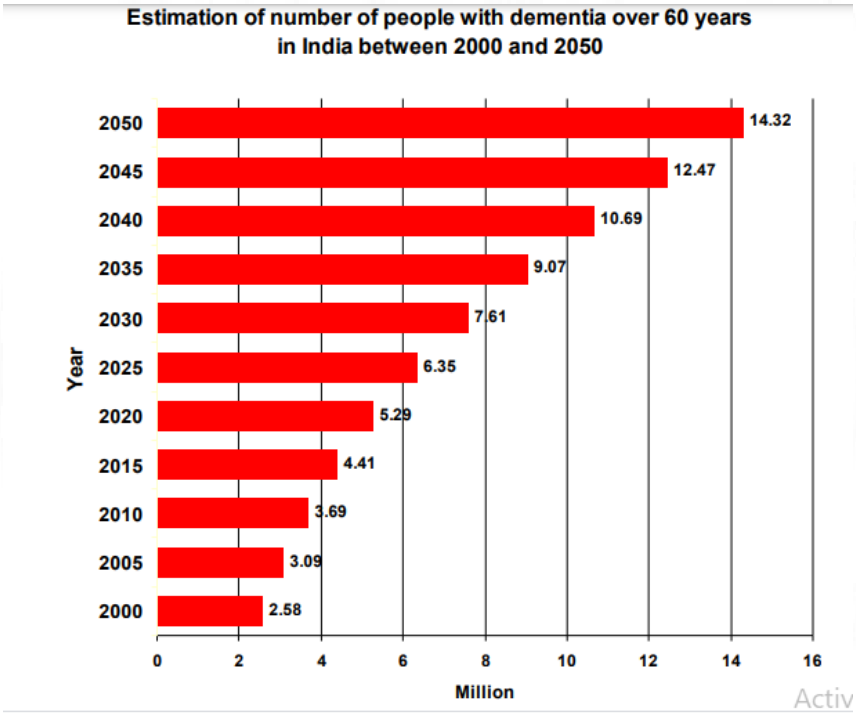


Figure 1: Graphical presentation of prevalence of dementia in India from 2000 to 2050(Dementia India Report, 2010. https://ardsi.org/pdf/annual%20report.pdf)

The prevalence of dementia studies are distributed throughout India, 6 studies in southern India and individual studies in western, eastern and northern regions. In many regions of the country data were lacking and the few studies reported have very different prevalence estimates. While the

coverage of evidence is good in Southern India, large variations in comparison with northern studies are difficult to give the entire country a consistent overview. It is therefore important, by generating quantitative meta-analyse with available evidence, to synthesise evidence on the prevalence of dementia.

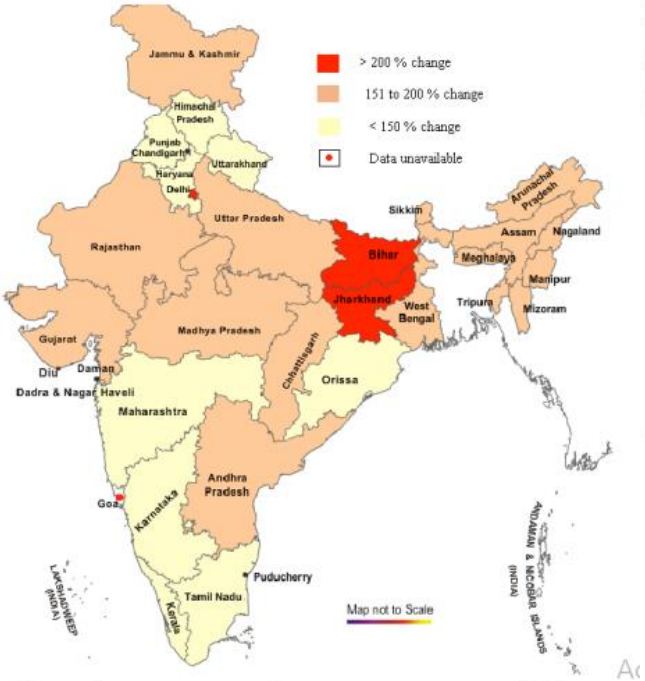


Figure 2: State-wise prevalence of Dementia in India (Dementia India Report, 2010. https://ardsi.org/pdf/annual%20report.pdf)

Alzheimer's Disease Continuum:

Alzheimer's disease take in a continuum of illness, which exhibits changes in brain which are not noticeable to the individual, primarily brain changes which lead to memory problems and ultimately physical disability.

There are three broad phases in this continuum: Alzheimer's preclinical disease, mild cognitive impairment, and Alzheimer's dementia. The dementia phase of Alzheimer's has also been broken down into mild, moderate and severe stages which reflect the degree to which symptoms are interfering in the ability to do daily work.

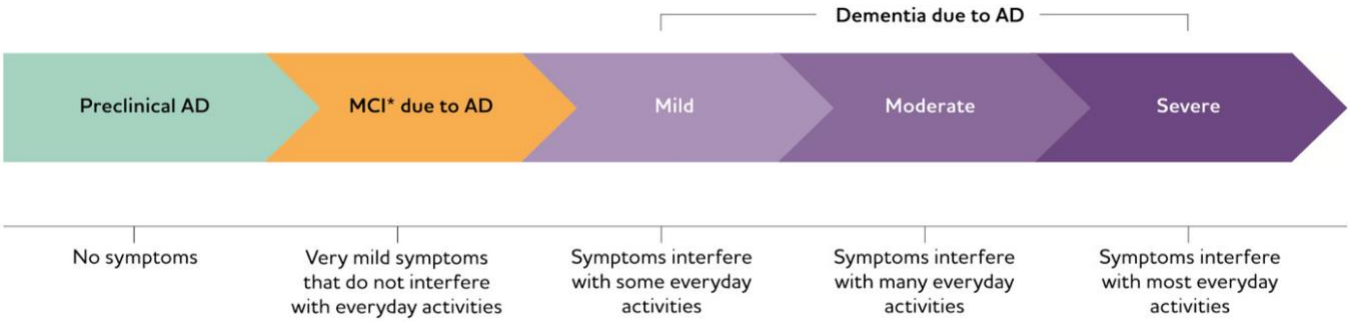


Figure 3: showing continuum of AD (Lazarou L. et al, 2019)

Although we know that the continuum begins with pre-clinical Alzheimer's and ends with severe Alzheimer's dementia, the time spent on each part of the continuum varies. Age, genetics, gender and other factors are used for the duration of each phase of the continuum.

i) Preclinical AD: During this stage, the brain changes are measurable, indicating earliest signs of Alzheimer's disease (biomarkers) but symptoms, for example, memory loss, have not yet been developed. The abnormal levels of beta-amyloid as shown in positron emission tomography (PET scans, CSF) analysis and decreased glucose metabolism, as shown in PET scans, include measurable brain changes. When Alzheimer's early changes occur, the brain compensates them so that people can continue to work normally.

While research environments have the tools and expertise to identify some of Alzheimer's early brain changes, further research is necessary to adjust the exactness of the instruments prior to their being available to be widely used in hospitals, doctor's offices and other clinical environments. It is important to note that not every person with proof of changes in the brain associated with Alzheimer continues to develop MCI symptoms or Alzheimer's dementia, for example some people have beta-amyloid plaques at death but have no memory or cognitive problems.

ii) MCI due to AD: People with MCI from Alzheimer's disease have biomarker evidence of Alzheimer's brain changes and subtle memory and thought problems (for example, abnormal levels of beta-amyloid). Such cognitive issues can not interfere with the individual's ability to do everyday activities and can be noticeable to family members and friends. Moderate changes in

thinking skills happen when the brain can no longer compensate for nervous cell damage and death due to Alzheimer's disease.

One analysis of those with MCI showed that 15 percent of people over 65 years have developed dementia after two years of follow-up. Another study found that within 5 years Alzheimer's dementia was developed by 32 percent of people with MCI. A third study has found that 38 percent developed dementia among people with MCI who were tracked 5 years or longer. In some individuals, however, MCI returns or remains stable to normal cognition. In other cases, like when a drug causes cognitive changes inadvertently, MCI has been erroneously diagnosed and cognitive changes occurs with changes in the drug formulation can be reversed.

iii) Dementia due to AD: Alzheimer's dementia is characterised by signs of significant deficits, in memory, thinking and behavior affecting the ability of a person to function in their everyday lives, along with evidence of brain changes. People with dementia of Alzheimer have several symptoms that change over several years. The degree of nerve cell damage in different sections of the brain is shown by these symptoms. The rate of progression from gentle to moderate to severe dementia symptoms varies from individual to individual.

- Mild AD: At the mild stage of Alzheimer's dementia, most people are able to work independently in many areas but may need assistance to maximise independence. You can still drive, work and take part in preferred events.
- Moderate AD: In the moderate stage of Alzheimer's dementia, which is often the longest stage, individuals may have difficulties communicating and performing routine tasks, including activities of daily living (such as bathing and dressing); become incontinent at times; and start having personality and behavioral changes, including suspiciousness and agitation.
- Severe AD: At the severe stage of Alzheimer's dementia, people need assistance in daily activities and may need care 24 hours a day. In this phase, especially the consequences of Alzheimer's disease on the physical health of people become obvious. Due to damage to moving areas of the brain, people become tied to bed. Bed-boundness makes them vulnerable to body-wide inflammation and organ failure, including blood clots, skin

infections, and sepsis. Damage is difficult to eat and drink to the areas in the brain that control swallowing.

- Pseudo-dementia: (PSD) As the name suggests, it has all the symptoms of dementia but not having any neurological significance or neurodegeneration. Mostly occur in the patients having depression, thus also contribute in giving false negative and false positive result of dementia. (Kang H et al, 2014)

Etiologyof MCI:

Mild cognitive impairment (MCI) is both heterogeneous in its clinical and etiological manifestations. Since amnestic MCI is often caused by AD, it is not surprising that the majority of amnestic MCI patients will progress into clinical AD within 6 years. Non amnestic forms of MCI may be due to cardiovascular disease, Lewy's body dementia, Parkinson's disease, frontotemporal dementia, Alzheimer's atypical disease and any specific pathological pathology that is underlying.

Mood disorders, medical conditions and drugs can affect cognition so that a patient meets the MCI criteria (usually nonamnestic MCI). When these patients reevaluated after a year, they will get normal neuropsychiatric test results. (Mehta S.,2019)

Some of the possible conditions include:

- Depression, anxiety, and stress
- Thyroid, liver or kidney misfunctioning
- Apnea in sleep, and other sleep deficits
- Diseases or conditions that impact brain's blood flow (blood clots, tumors, stroke, normal pressure, traumatic brain injury, and hydrocephalus)
- Optic (Eye) or hearing problems
- Low vitamin B12 levels or other nutrient levels
- An infection
- History of alcoholism

- Side effects of certain prescription (for example, Parkinson’s disease and depression) or illegal drugs (Rodakowski J., 2014)

Causes of AD:

Alzheimer's disease is a condition of unknown exact causes. However, brain proteins fail at a fundamental stage and disturb the workings of brain cells (neurons) and cause a cascade of toxic events. Neurons weaken, lose ties, and eventually die.

The function of two proteins is being studied by researchers trying to figure out what causes Alzheimer's disease:

- **Plaques.** A part of a larger protein, beta-amyloid, is found in the brain. These fragments tend to have a toxic effect on neurons and impede cell-to-cell contact as they cluster together. These clusters combine to form amyloid plaques, which contain additional cellular debris.
- **Tangles.** In the neurons internal support and transportation system for the carrying of nutrients and other essential materials, tau proteins play an important role. Tau proteins change their shape and organise in structures known as neurofibrillary tangles in Alzheimer's disease. The tangles can interrupt the transport system.

The early signs of the disease include the forgetfulness of recent events. A person with Alzheimer's condition is increasingly suffering from severe memory impairment and loses their ability to perform daily tasks.

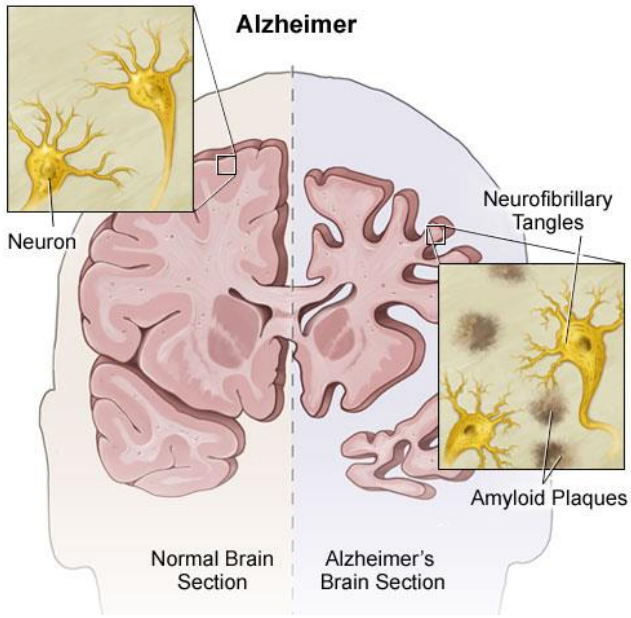


Figure 4: Anatomy in Alzheimer's patient. (Source: https://thealevelbiologist.co.uk/genetics-control-homestasis/the-effect-of-ageing-on-the-nervous-system/)

How is Alzheimer disease diagnosed?

There is no single diagnosis of Alzheimer's disease. First, a healthcare provider excludes other conditions. But only examining the brain after death can confirm the diagnosis of Alzheimer's. An autopsy may show changes in the disease's brain.

It is important to determine whether dementia is due to a disease that can be treated. A medical provider will carry out thorough nervous system examinations. The supplier can also:

- **Complete health history**. This may include questions about the person's overall health and their past health problems. Evaluation of the person's ability to perform daily tasks. Assessment of behavior and personality and verify the same with family members on account of the changes they noted.
- **Mental status test.** This include tests of problem-solving, memory, attention, language, and counting. Neuropsychological testing can also be done. This might include a series of

tests that can analyze one's brain functioning. It usually involves questionaire and letting them do certain tasks.

- **Other lab tests.** This include blood and urine tests to determine the possible causes of the problem.
- **Brain imaging tests.** CT, MRI, or position emission tomography (PET) may be used to rule out other causes of the problem.

Why need of research related to AD?

Alzheimer's disease causes memory loss and language deficits, poor planning and problem solving skills, diminished judgement, and other cognitive changes that can make treating other illnesses more difficult. A person with Alzheimer's disease can be unable to:

- Explain symptoms of another illness
- Communicate that he or she is feeling some pain
- Adhere to a care plan that has been prescribed to you.
- Describe how medications can cause side effects.

When Alzheimer's disease progresses to its final stages, physical functions such as chewing, balance, and bowel and bladder control begin to be affected. These side effects will make you more vulnerable to other health issues.

The GBD (Global Burden of Disease) report shows that dementia is one of the major causes of later life disability. In particular, older people are likely to have multiple conditions of health. Chronic physical conditions affecting various systems of the organ may coexist with mental and cognitive disorders. These several pathologies interact in difficult manners so that important activities and tasks (disability) are difficult to accomplish and care needs are determined (dependence). Dementia often affects the ability for independent living disproportionately.

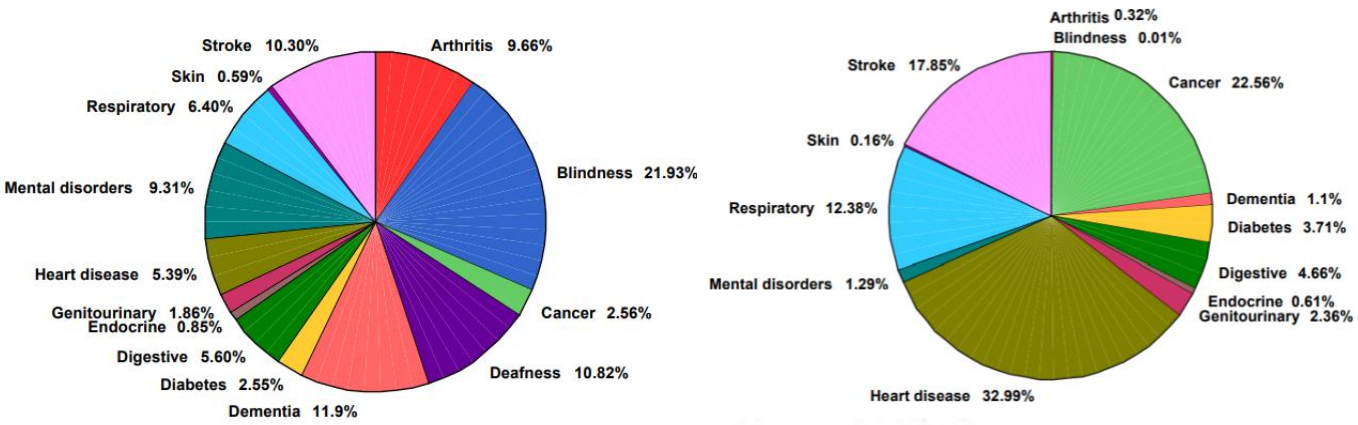


Figure 5: A) Contribution of chronic diseases to years lived with disability (Dementia showing 11.9%) B) Contribution of chronic diseases to years life lost (Dementia with 1.1%) (Source: Dementia India Report, 2010. https://ardsi.org/pdf/annual%20report.pdf)

In a 10 year analysis of India's research effort on Pub Med, 1,426 cancer, 604 cardiovascular, 917 mental-related, 94 stroke-related, 119 arthritis-related, and 76 dementia-related publications have been revealed. It shows the correlation between research effort (number of publications), mortality (years of loss of life) and incapacity (years of disability). Clearly, the effort in the research of dementia is the least and the contribution of these chronic disabilities and research efforts have an inverse correlation. The more disabled the condition, the less research has been done. In contrast, the connection between life-lost years and research efforts is significantly positive. The need for cost-effective and effective interventions to develop in the community can only provide proper guidance on good quality research. Dementia is a priority health condition for future research by research funding agencies such as ICMR, DST and DBT.

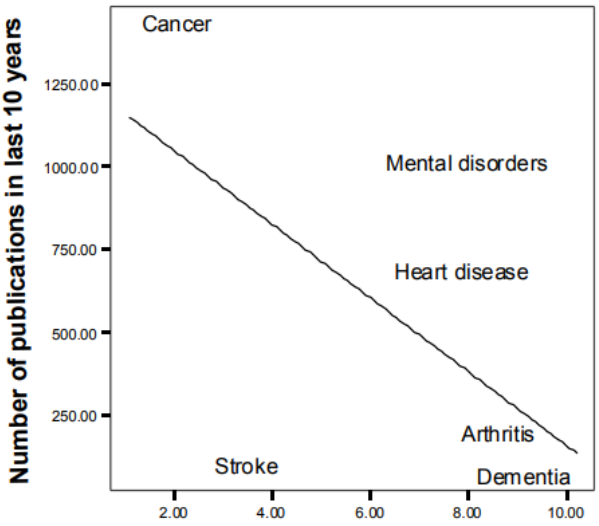


Figure 6: GBD- contribution of years lived with disability (Source: Dementia India Report, 2010. https://ardsi.org/pdf/annual%20report.pdf)

1.2 RESTING STATE FUNCTIONAL MRI:

(Vemuri P et al., 2012) The spontaneous low frequency variations (~0.01–0.15 Hz) in the BOLD signal are the subject of rs-fMRI. Biswal et al. presented the practical importance of these fluctuations for the first time in 1995. No cognitive, language, or motor tasks were provided to the participants in this study. These low-frequency variations have since been shown to be unique to grey matter and can be used to determine the spatial extent of temporally associated structural and functional communication networks within the brain. The lack of a predetermined experimental task in rs-fMRI led to the coining of the word "resting state" to characterise the technique and the networks discovered (that is, resting-state networks).

Functional (fMRI) is a form of magnetic resonance imaging (MRI) technique, a method that detects blood flow changes linked with brain activity. The low frequency BOLD signal in the brain is used to measure brain activity in more detail. The technique is similar to MRI except that it measures the difference in magnetization between oxygen-rich and oxygen-poor blood. Since noise from different sources often corrupts this measurement, statistical methods are used to isolate the underlying signal. The resulting brain stimulation can be visualised graphically by color-coding the intensity of activation around the brain or in a specific area of the brain. The technique can locate activity within millimetres, but with standard techniques, only a few seconds away.

Rs-fmri is an intrinsic brain connectivity imaging technique that is seen as an excellent biomarker for Alzheimer's disease (AD), since changes in functional connectivity brains are believed to be seen prior structural atrophy in the brain's areas. Earlier studies show that functional relaxation connectivity is sensitive to functional brain changes in the clinical spectrum related to AD pathology. Resting-state functional connectivity changes were seen in healthy age, mild cognitive impairment, prodromal stage of AD and AD within the standard mode network, originally identified by Raichle and colleagues.

However, some additional issues still remain to be addressed before functional connectivity can be implemented as a biomarker for AD. Using fMRI data on a single-subject level is currently very limited (that is, the resting-state data as well as task related fMRI data). Changes in fMRI data acquisition could potentially increase the data signal-to-noise ratio, thereby increasing the power of our statistical analyses. Changes in the analytical methods also can increase measurement sensitivity.

The creation and subsequent use of a functional brain atlas instead of a structural brain atlas for evaluating functional connectivity strength is one example of this improvement in analytical methods. Additionally, there is a high level of variability among individual patients and this may also explain why classification procedures have proved difficult to attain adequate sensitivity and specificity. By observing functional linkages and further investigating the very early (i.e. latent) stage of AD, we can understand the changes that take place throughout the trajectory of the disease. This knowledge may help us improve our ability to appropriately classify new subjects and ultimately allow us to use resting-state functional connectivity as a biomarker for AD. (Damoiseaux JS et al, 2018)

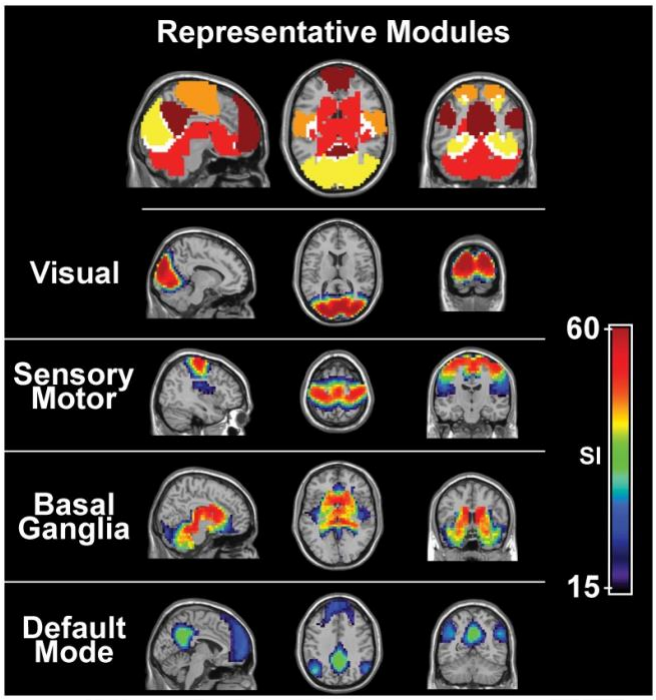


Figure 7: showing four functional networks that were found to be highly consistent across subjects. These modules include the visual (yellow), sensory/motor (orange) and basal ganglia (red) cortices as well as the default mode network (precuneus/posterior cingulate, inferior parietal lobes, and medial frontal gyrus; maroon). Overlap among these modules was present but minimal (white) (Malaak N. Moussa, 2012)

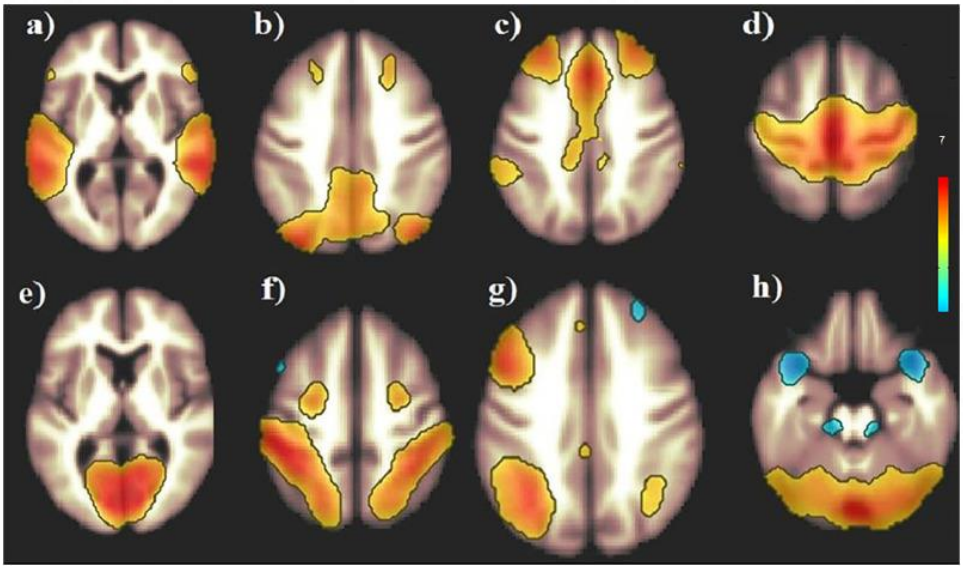


Figure 8: Identified RSNs after ICA analysis a) Language b) DMN c) Salience d) Sensorimotor e) Visual f) Dorsal attention g) Frontal-parietal h) Cerebellar.

Processing data

For processing and analysis of fMRI stationary data, a number of programmes exist. Some programmes are SPM, AFNI, FSL (ICA Melodic in particular), CONN, C-PAC and the Computing System Connectom (CCS).

Methods of analysis

The acquisition and treatment of rsfMRI data are numerous. Either independent components or regions of correlation are the most frequently used methods of analysis.

1.3 STRUCTURAL MRI

Functional MRI quantifies specific brain functions, where as structural MRI defines brain structure / anatomy and pathology. This produces images that can both be used for clinically assessing brain damage and detailed analysis radiological reporting. These scans help in visualization and analysis of anatomical properties of the brain. Structural approaches are particularly useful for detecting brain damage and abnormalities

These MRI scans are intended for brain's volumetry assessments i.e to calculate tissue volumes, to examine diffuse changes in grey/white matter and to evaluate localised lesions. These scans can be rebuilt on any level as well. The volumes of regional grey and white matter (including the brain) vary greatly during the childhood and adolescence, and may get change again in old age and volume alterations are also noticed associated with various neurodegenerative disorders.

In AD-related studies, magnetic resonance imaging is more common due to its noninvasiveness and the absence of pain in patients. Furthermore, MRI offers excellent spatial resolution and excellent contrast. Several studies have therefore used structural biomarkers based on MRI (sMRI-) to classify the AD, describing atrophy of the brain and changes in brain tissue sizes. Literature suffices to show that brain structure atrophy or neurodegeneration is the closest cognitive impairment substratum in AD.

sMRI measures brain morphometry and can therefore capture grey matter atrophy related to neuronal losses, synapses and dendritic de-arborization at a microscopical stage in AD. The atrophy of the white matter is associated with loss of white matter structural integrity, presumably due to demyelination and dying back of axonal processes.

The added value of the MRI based clinical assessment is that it is the non-invasive independent neuronal loss measurement that therefore provides an additional measure based on anatomy only, whereas a clinical testing and neuropsychological testing provide the basis for clinical diagnosis. Many studies have now shown that sMRI is a stable AD progression biomarker. SMRI data published from multicenter studies such as ADNI also showed that sMRI scans from multicenter studies can be combined without great punishment. Besides the diagnostic and predictive value, sMRI can play several roles.

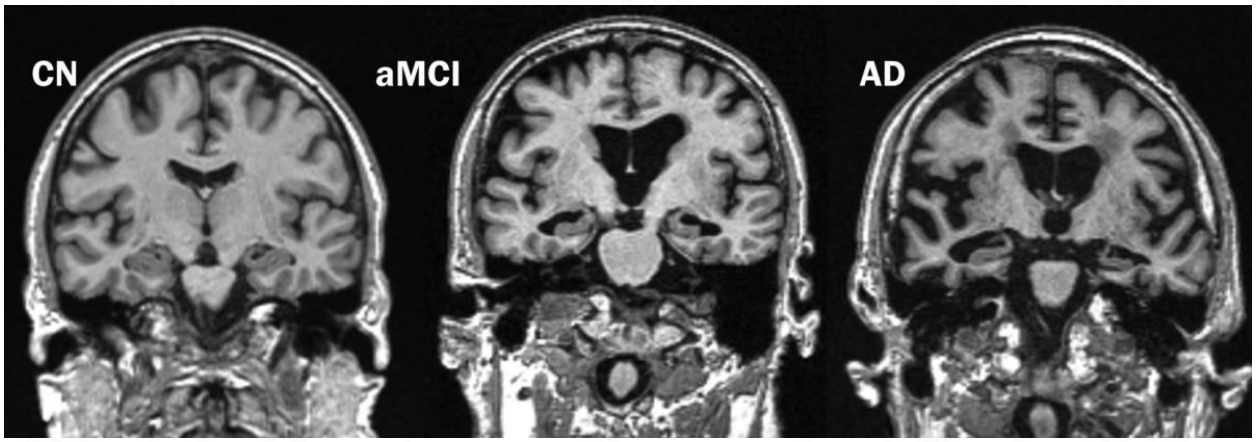


Figure 9:Progressive atrophy (medial temporal lobes) in an older cognitively normal (CN) subject, an amnestic mild cognitive impairment (aMCI) subject, and an Alzheimer's disease (AD) subject (Vemuri P. et al, 2010)

1.4 CONN TOOLBOX:

CONN is a cross-platform Matlab-based programme for fMRI functional connectivity computation, display and analysis (fcMRI). Single-to-voxel connectivity maps, connectivity ROI-to-ROI matrices, connectivity network graph characteristic and voxel-to-voxel measures are the

connectivity measures (intrinsic connectivity, local correlation maps, and others). This tool is compatible with the rsfmri and task-related data. It covers the complete pipeline from the raw fMRI data to make hypothesison testing, includes:

- spatial coregistration,
- ART-based scrubbing,
- a*CompCor*strategy for control of physiological and movement confounds,
- first-level General Linear Model for correlation and regression connectivity estimation, and
- second-level random-effect analyses

To install this toolbox, one must have to download the zip file online and should also have SPM5 and above. Once downloading complete, connect CONN toolbox with MATLAB.

Basically the working of this toolbox divides into 4 steps:

1.**Setup**: Defines basic experiment information, data places, seed regions, time covariates and second-level models.

Structural and functional mri scans can be imported for each subject(patient) here. Default files for rois are in the toolbox, but roi can be uploaded according to your needs. Also, even after the preprocess stage is completed, the first and second covariates can be changed at any time.

2. **Preprocessing**: Defining, exploring, and removing potential BOLD confusion.

3. **Analyses**: Perform first-level analyses. Define the seeds of interest and explore functional connectivity of different sources separately for each subject

4. **Results**: Perform second-level analyses. Define and explore within-and between-subject contrasts of interest.

Typically, all of the structural, functional, and ROI files should be co-registered with one another after selecting all of them in the setup stage (in the same space, e.g., MNI space), and after applying any additional spatial preprocessing if need be. For example, when using the initial CONN spatial preprocessing (defaultMNI) pipelines, functional and structural files are located in MNI space(note

that all the ROIs in the conn/rois folder toolbox are defined in MNI space). This is also done in MNI space.

For my studies, we are going to use **ROI-ROI analysis** and rest condition.

Note 1: One can skip many of the above steps only if you have done all of these steps in SPM and enter one file of SPM.mat for each subject by clicking on Import (right after entering the number of subjects in Basic Setup). This programme will extract from these SPM.mat files the location of the functional data, the number of conditions per subject, the start/duration of conditions of interest, and all covariates specified at the initial level.

In Denoising, in each of the possible confounding sources (for selected subject/session data) the window "Preview results" in the right panel shows the overall variation (r-square). The histogram plot below shows a connectivity between voxel and voxel (r values) prior and after the removal of confusion. In conjunctivity measures, confounds typically introduce a positive bias so that a histogram of the original connectivity values may "shift" to the right. The distribution of connectivity values appears approximately centred after deletion of confusion.

The first level analysis has the option of using a file uploaded during SETUP to choose the desired ROIs. Analyzes are carried out in realtime (any modifications of the definitions "Define sources" affect the result shown in the "Preview" window directly). The measures shown in the brain image 'Preview results' correspond to the selected connectivity measurement (if correlation is selected, or if beta regression is selected). The value of the threshold is defined in the same units (voxels with coefficients greater than 0.25 will be shown in the real-time figure).

The type of first-level model to be used can be selected in analysis type. A weighted general linear model for a weighted regression/correlation measurement of a condition-specific association from the seed/source time series BOLD to each ROI BOLD time series is the default behaviour. This model is suitable for both rest and task-related designs.

Analyzes at secondary level can be performed using voxel-voxel analysis, roi-roi, seed-voxel analysis and the analysis of graph-theory. My study shows that we will primarily conduct royal connectivity and analysis of graph theory.

Analyzes of voxel-to-voxel are analysing the whole matrix of voxel-to-voxel values. These are useful if you do not want to limit analysis to one or more seeds/ROIs and if you want to investigate possible differences in connectivity across the whole brain.

The brain display on the right displays an axial view of the second-tier analysis results estimated on the selected second-level models and contrasts in real time when you are selecting ROI-to-ROI analysis (the left tab in the figure). These results can be threshed with an uncorrected p-value or p-values corrected with FDR and one- or two-side values at the desired p-value threshold.

1.5 GRAPH THEORY AND IT's VARIABLES:

Graph theory is a way to describe the relationship between two or more objects in an intelligent manner. Graph theory may model any number of objects in mathematics – neurons, people, towns, etc. We will focus on graph theory for neuroimaging data and in particular the restorative state data for our purposes. Individual voxels or voxel groupings in this scenario are the pairs of objects we want to model. The graphic theory can provide another insight into the way these voxels are connected and inform us about the organisation of the brain. (Farahani FV et al, 2019)

The advancement in graph theory and network neuroscience(i.e., the study of the function or structure of the nervous system) provides an opportunity to understand the details of the complex network of human brain and its modeling. In human neuroscience, graph theory technique is generally applied to either functional or effective connectivity. However, most of the studies are done till now in the context of functional connectivity. (Farahan F. V., 2019)

We look for an efficient understanding of the mechanisms underlying human awareness compared to traditional methods by recognising the brain connectivity properties by graph theory (as measured by fMRI). We look for an effective understanding of the mechanisms of human

knowledge in relation to traditional approaches when it comes to identifying brain-connectivity properties by graph theory (as measured by fMRI).

We used the graph theory option of CONN Toolbox for my study. The ROIs are either used as nodes with the input correlation maps and the correlation values among the nodes are the edges. As with any network dataset, only those values that are strongest or most robust can be shown by the correlation values.

Some of the parameters we got from this analysis are:

Degree & Cost

At each node, degrees and costs are defined as the number (degree) or proportion (cost) of the edges from and to each node. Likewise, Network Grade/Cost is the average or grading/cost for all graph nodes.

$$d_i = \sum_j A_{i,j}$$

$$d = \frac{\sum_i d_i}{N}$$

$$c_i = \frac{\sum_j A_{i,j}}{N-1}$$

$$c = \frac{\sum_i c_i}{N}$$

where ***A*****=** adjacency matrix, ***N*** = total number of nodes in a graph, ***c*****=** the cost of a graph (of each individual node/ROI), ***d*** =the degree of a graph (and of each individual node/ROI)

The degree and cost of each node/ROI are network centrality measures, which characterise the degree of local connectivity of the individual ROI in a graph. Adjacence matrix thresholds are typically applied using a set level of network costs (e.g. keeping the 10% of connections strongest), in order for other graphical measures of interest to be sensitive to be compared between networks.

Average path distance

The distance between each node pair in a graph is defined as the minimum number of rims crossed in an optimal way. The mean path distance of each node in the connected nodes subgraph is defined as the average path distance between this node and all other nodes:

$$L_i = \frac{\sum_{j \in \Omega_i} D_{i,j}}{N_i - 1}$$

$$L = \frac{\sum_i L_i}{N}$$

where ***D***= shortest-path distance matrix, ***N***= the total number of nodes in a graph, and ***L***= the averages path distance of a graph (and/or of each individual node/ROI)

Average path distance represents a measurement of node centrality within a network, describing the degree of global connectivity of each ROI within a graph.

Clustering Coefficient

Clustering coefficient shall be defined in the local nearby subgraph for each node/ROI as the proportion of connected edges.

$$CC_i = \frac{\sum_{j,k \in \Gamma_i} A^{(i)}_{j,k}}{d_i(d_i - 1)}$$

$$CC = \frac{\sum_i CC_i}{N}$$

where, d= the degree of each node, A = the adjacency matrix within the neighboring sub-graph at each node, characterized by all the nodes neighboring this node and all existing edges among them, and CC= the clustering coefficient of a graph (and/or of each individual node/ROI)

Clustering coefficient represents a measure of the local integration, characterizing the degree of inter-connectedness among all nodes within a node neighboring sub-graph.

Global Efficiency

Global Efficiency at a node is defined as the average of inverse-distances between this node and all other nodes in the same graph:

$$GE_i = \frac{\sum_{j \neq i} 1/D_{i,j}}{N-1}$$

$$GE = \frac{\sum_i GE_i}{N}$$

where ***D***= shortest-path distance matrix, ***N***= number of nodes in a graph, and ***GE*** = Global Efficiency of a graph (and/or of each individual node/ROI).

Global efficiency at a node shows a measure of this node centrality within the network, describing the degree of the global connectedness of each ROI(node).

Local Efficiency

Local Efficiency at each node is defined as the Global efficiency of its neighboring sub-graph

$$LE_i = \frac{\sum_{j \neq k \in \Gamma_i} 1/D^{(i)}_{j,k}}{d_i(d_i-1)}$$

$$LE = \frac{\sum_i LE_i}{N}$$

where ***d*** = degree of each node, ***D*** = shortest-path distance matrix within the neighboring sub-graph at each node, characterized by all the other nodes neighboring this node and all existing edges among them, and ***LE***= Local Efficiency of a graph (and of each individual node/ROI).

Local efficiency represents a measurement of local integration or coherence, characterizing the degree of inter-connectedness among all nodes within a node neighboring sub-graph.

Betweenness Centrality

Betweenness centrality represents an alternative method to measure node centrality within a chosen graph. It is defined as the number of times that a specific node is the part of a shortest-path between any two other pairs of nodes within the same chosen graph.

$$BC_i = \frac{\sum_{j,k \neq i} [i \in P_{j,k}]}{(N-1)(N-2)}$$

$$BC = \frac{\sum_i BC_i}{N}$$

where ***P***= set of nodes in shortest-path between each pair of nodes, ***N*** = number of nodes in a graph, and ***BC*** = Betweenness Centrality of a graph (and of each individual node/ROI).

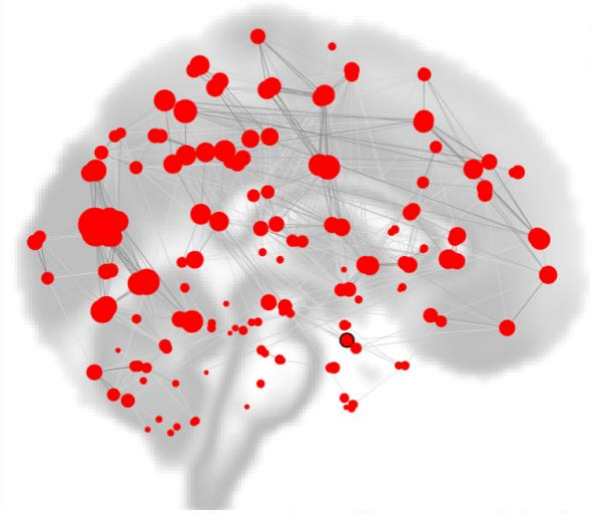


Figure 10:Showing the CONN Results window, where nodes are depicted as red circles, along with the size of the circle depicted as the strength of the currently selected graph theory metric. Edges between the nodes are depicted as blacklines.(Source: andysbrainbook.readthedocs.io)

1.6 NEUROPSYCHIATRIC TEST AND SELECTED VARIABLES FOR STUDY :

Neuropsychological assessments are designed to identify the scale and severity of a cognitive and behavioural impairment of the patient. They enable us to establish a pattern of relative cognitive strengths and weaknesses that provides information on the functional and structural integrity of a patient's brain indirectly. Normed tests are used by neuropsychologists to evaluate cognitive abilities such as attention, memory, language, speed of processing, visual spatious and executive functions. Typically it takes time, but most neurological practitioners prefer a short cognitive examination to quickly assess the cognitive function of the patient.

Because AD is an age-related neurological illness, especially in the memory field, with early cognisance and behavioural problems. Neuropsychological test batteries are commonly used to diagnose AD by relaying the patient scores on each test to an arbitrary criterion which shows a disability below the mean score of a normative reference group. This is traditionally done by related.

Multivariate methods for analyzing neuropsychological test batteries have been explored by others, examined the NINCDS-ADRDA criteria through a series of neuropsychological tests administered to only AD patients to determine how many factors best represent AD. They come up with six factor model which includes:

- General memory and intelligence
- Attention and concentration
- Visuospatial skills and,
- Verbal abilities
- Motor and sensory skills
- Reasoning and problem solving

And it has also been seen that this six-factor model works better as compared to one-factor model for AD participants.

This research has led to greater knowledge of the specific cognitive deficiencies in the earliest stages of AD and has strengthened the possibility of early clinical diagnosis of the disease. There

were also descriptions of the impact of ageing on the ability to detect AD and subtle cognitive modifications which could anticipate the development of dementia in those with preclinical DA.

A number of features that are quite effective in distinguishing between mildly depressed AD sufferers and normal older adults have been found in studies of the clinical utility of early detecting AD episodical memory measures:

First, Absolute delayed recall scores**,** It is calculated by the Amount recalled after the delay divided by the amount recalled on the immediate learning trial. This can differentiate mildly demented AD patients from healthy elderly controls with approximately 85% to 90% accuracy.

Second, the retrieval demands reduced by the use of the recognition test are not accessible at a later date.

The **third** point is that patients with AD exhibit an anomalous serial position effect that attenuates the primacy effect (i.e. the reminiscent of words from the beginning of a list) that suggests that information from primary to secondary memory cannot be effectively transferred.

Fourth, semantic encoding is less effective than normal elderly people in increasing the episodic memory performance of AD patients.

Fifth, AD patients are more likely to produce intrusion errors on verbal and nonverbal memory tests, which are probably due to increased interference sensitivities and/or decreasing inhibitory procedures (e.g., if information previously learned during the attempt to recall the new materials).

(Chapman RM et al, 2010)

The assessment of these memory defect characteristics of AD is incorporated in various memory tests that are effective in early disease detection and developed in clinical algorithms to distinguish AD from other types of dementia. Some of the tests we included in our study include:

<u>MMSE (Mini-mental state examination)</u>

During the MMSE, a patient is asked by a health care provider a number of questions to test various mental skills every day. The maximum score for MMSE is 30. Score between 20 and 24 shows mild dementia, between 13 and 20 indicate moderate dementia, and less than 12 show severe

dementia. A person with Alzheimer's MMSE score decreases annually on average by two or four points.

During the Mini-Cog (a 3-minute instrument that can increase detection of cognitive impairment in older adults), a person will be asked to complete two tasks:

- To remember the minute details and after a few minutes repeat the name of three common objects.
- Drawing a face of a clock, which show all 12 numbers in the right places and a time specified by the examiner.

ACE (Addenbrooke's Cognitive Examination)-

It was also developed in order to overcome the neuropsychological defects in the Mini Mental State (MMSE). It takes 15-20 minutes to detect a brief cognitive tool and includes attention, guidance, memory, language, visual perception and visuospatial abilities. The breakdown of scores is supposed to be:

Attention- 18points, Memory- 26points, Fluency- 14 points, Lamguage-26points, Visuospatial-16 points. (Bruno D. et al, 2019)

RAVLT (Rey's Auditory Verbal Learning Test)-

It's a well-proven, widely used wordlist memory test used to evaluate the learning curve, the memory strength after the interference task and learning pattern (serial positioning effects), as well as to measure memory recognition.

We divided the whole list of 15 words into five parts of three words each — the first three words of primacy, the middle three words of intermediate, and the final three words of recital. Total score will be out of 25. (Ranjith N. et al, 2010)

Delayed RAVLT(after 20 minutes):

After a 20-minute delay during which a non-verbal task occurs (including the Digit Modalities Test and the Trail Making Test), free reminder (i.e. the Delayed Free Recall) and category reminder is tested. In the end, a recognition test is performed which includes 12 (semantically associated) target words and 12 distract words. (Zhao Q. et al, 2012). Scoring will be out of 15.

1.7 PURPOSE OF STUDY:

The studies have shown that it was difficult to treat Alzheimer's disease as it was diagnosed late, or it is difficult to interpret its progression. Additionally, before structural changes in the brain, functional modifications following deposition of the amyloid plaque have been studied. Using these findings, relevant biomarkers can be discovered both biologically and in imaging, making the clinicians feasible to identify patients with mild cognitive impairment. Thus, helping them to provide effective therapy to stop/delay the progression of Mild Cognitive Impaired (MCI) patients to Alzheimer's patients.

There are relevant imaging technologies available to study the functional activity of the brain, such as fMRI, DTI, PET, etc. To understand neurodegenerative disorders at a whole-brain connectivity level, advanced network analysis techniques employing resting-state functional Magnetic Resonance Imaging (rs-fMRI), which is also referred to as 'task-free fMRI, has been extensively used. This study will focus on the two modalities, rs-fMRI, and sMRI, to study the brain structurally and functionally in the Normal Controlled (NC), MCI, and AD patients diagnosis can be prominent and directionally. Also, besides the imaging method, the other form of assessment is chosen by the clinician is neuropsychiatric tests; we involved it in our study to find the role of each parameter in cognitive functioning.

By pairing and comparing, we can realize which combination of methods can give us a better diagnosis of elderly patients with cognitive impairment or help determine the progression of AD.

The following OBJECTIVES which are going to target are:

1. Univariate study of Graph theory connectivity study among the three pairs of diagnostic groups in the chosen ROIs.
2. Correlation between Functional connectivity (graph theory measures) and Cognitive Functioning (neuropsychiatric test scores). Also, find a correlation between functional connectivity(Graph theory) and Structural feature (grey matter volume decay) of the selected ROIs.
3. Multivariate analysis of selected parameters using the classification model

The three groups chosen are Control, Mild Cognitive Impaired(MCI), and Alzheimer's disease(AD).

Some of the assumptions of our study are:

- There is a significant increase in functional connectivity in the brain region.
- The increase in functional connectivity must be accompanied by structural atrophy (gVol reduction).
- There should be a positive correlation of connectivity with cognitive performance and a negative correlation between grey matter volume & functional connectivity in either group of patients to delineate the compensatory mechanism.

Since AD is a significant cause of dementia, especially in India due to lack of awareness, early diagnosis of this disease becomes necessary. The early appearance of pathological lesions and the progressive nature of cognitive deterioration in AD indicate a great need for developing imaging-based markers of disease that are sensitive to the brain changes that are expected to occur decades prior to the onset of clinical symptoms. Ideally, a biological measure would be predictive of AD in pre-symptomatic individuals but not a good option to track progression. Rs-fMRI is a flexible way of identifying patients with various cognitive disorders with neuropathology. In the near future, Rs-fMRI can be an option to provide valuable diagnostic data for the disorder's early stages.

CHAPTER 2: LITERATURE REVIEW

2.1 EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE:

The aggregation of large Aβ fibrils has been seen for many years as the key occurrence in AD disease and the major determinant of neuronal degeneration.

The NFT stage and severity of the clinical status in AD are associated. NFTs originate from the MTL, which plays a critical role in memory neural control, and then begin to interrupt episodic and autobiographical memory in the adjacent posterior cingulate (PCC) and temporal cortex in mild AD. Also, In moderate to severe dementia, NFTs develop cortices, which are included in neural control of attention, language and perception in the parieto-temporal and prefrontal association. Aβ depositions are also found in MTL at the later stages of disease, despite an initial preference for neocortex. (Braak H. et.al, 1996)

Several forms of early diagnostic tools for AD are promising to build. The measures included: MRI, positron emission tomography (PET), MTL, PET, and cerebrospinal (CSF) biomarkers for tauopathy and Aβ. This includes magnetic resonance imaging measurements. None of these is currently recommended as they have not yet been validated in large prospective studies in any consensus guidelines on the diagnosis of ND. (Mistur R. et. al., 2009)

The overview of the application of these methods performed by many clinicians are:

(Cheng et al., 2018) examined the effectiveness of some **plasma proteins** in plasma patients and 101 healthy controls at the Wuxi and Shanghai Mental Health Centers as potential diagnostic biomarkers for AD using plasma sampling. They found that a combination of BDNF, AGT, IGFBP-2, OPN, cathepsin D, SAP, C4 supplement, and TTR plasma protein can provide the Chinese population with diagnostic AD biomarkers. While the sampling size of the conducted study was small and several factors could modify protein levels in plasma, the AD population could be solved in the future with this plasma panel.

Ashraf and Baeesa identified a potentially strong galectin-3 effect as a promising biomarker for these chronic diseases in **serum and cerebrospinal fluid samples** and in lateral amyo transpinal sclerosis patients. A comparative analysis of galectin-3 expression patterns was conducted. It also conducted a number of neuropsychological evaluations that demonstrated a strong correlation between the levels of galectin-3 and cognitive decline in patients with AD, and inflammation and apoptosis activation.

(Laptinskaya et al., 2018) used the low cost technique and a novel and innovative **neuropsychological assessment** approach for seniors using non-invasive electroencephalography. In this paper, 59 participants were classified as subjective memory impairment, 19 as naMCI, and 24 as aMCI, and the authors created an extended version of the MMN (mismatch negativity), which was highly linked with baseline episodic memory, as a potential biomarker for early diagnosis and AD monitoring.

(Luo et al., 2018) analysed data on **neuroimaging** of patients with single domain amnestic (sd-aMCI) and multiple domain amnestic MCI(md-aMCIs), including fluorine 18 positron emission tomography, which led them to conclude that there are interconnection patterns in samples. In particular, the authors assessed 49 controls 32 SD-aMCI and 32 MD-aMCI patients with very promising and clear evidence that the inter-hemispheric connectivity as potential biomarker for controlling the disease progression in AMCI patients can contribute to the development of distinctive clinical symptoms in sd—aMCI and md-aMCI patients.

The **Random Neural Network cluster**, which contains the multiple neural networks, offers a new method for enhancing effective discrimination of AD patients through healthy checks on fMRI data from (Bi et al., 2018). In order to provide exact AD diagnosis in large samples and in large-dimensional data, the authors used the random Elman network cluster of 25 AD patients and 36 healthy checks imported from the data package for the AD Neuroimaging Initiative to identify significant abnormal brain regions. With a diagnosis of AD by 23 abnormal regions, like precentral Gyrus, the frontal gyrus, and the supplementary motor area, compared to the healthy controls authors provide with a precision of >90 per cent.

Although functional MRI is an established diagnostic method for all stages of AD development, the importance of different **graph construction** methods and analysis of data on fMRI is

emphasised by Bachmann et al, 2018. In the study, a sample of 26 healthy controls, 16 mild cognitive impaired subjects (MCI) and 14 with AD were evaluated based on several methods such as the Ward clustering, the Atlas-based clustering, the region of growth and the selection algorithm. The results were evaluated on the statistic significance of the mean difference in graphical properties, the result's sensitivity to model parameter choices, and the relative diagnostic power based on a statistical model (such as support vector machine), which resulted in differences between techniques and their biological understandings.

The most exact reference standard would be the use of **Alzheimer's brain biopsy** to combat the current lack of a "gold standard" antimortem test. In the live patient, however, this is purely unethical for research purposes. (Mason S.E., 2010)

Progress from normal to sporadic AD ageing. These studies is limited because the clinical change of a low incidence of decline is intrinsically difficult to observe (1 to 3 percent/year) and the cognitive deterioration is slow. 67 After normal cognitive individuals in time, before they develop dementia, large samples, long intervals and high costs are required. (Petersen RC, et. al., 1999)

2.2 CHALLENGES IN THERAPEUTIC MODELING FOR AD:

The problem of the unsuccessful design of drug studies due to the usual late diagnosis of AD and associated irreversible brain modifications was recognised and discussed in some of these papers.

Instead of a single biomarker, Khan T.K., 2018 discussed the need to implement complex models for diagnosing AD. The authors proposed a theoretical algorithm that combined heterogeneous evidence such as neuroimaging data, CSF biomarkers and genetic markers. In addition to this, the authors have highlighted diagnostic accuracy, the selection of patients in clinical trials, universal standardisation of diagnostic protocols, the high cost of diagnosis and possible ethical challenges to address in the future.

The effect of non-drug interventions among dementia groups, including physical exercise with music and cognitive stimulation tasks, was compared by Tabei et al., 2018. The authors enrolled 46 patients with mild to moderate dementia, 25 were physical music exercise and 21 were cognitive stimulation subjects. However, the results might be influenced by the study's limitations, such as

not incorporating healthy controls or the rapid period of study intervention. The authors also conclude that the importance of individualized non pharmacological interventions has not been improved in patients with mild-to-moderate dementia, accompanied by cognitive decline and extensive cortical atrophy.

The role of MCI evaluation as a crucial AD control point has been discussed in long et al., 2018 69 MCI patients and 63 controls were enrolled in this study. None of the MCI patients took medicines that might interfere with cognitive function. The authors proposed and examined the exponent from Hurst of the fMRI from various brain regions including the left middle frontal gyrus, the left cingulate gyrus, the right hippocampus, the bilateral para hippocampus gyrus, the bilateral amygdala, the left insular gyrus, the left midgyrus gyrus, the left orbital gyrus, left superior parietal gyrus and the left basal ganglia, which were evidence of MCI identification.

The importance of an effective diagnosis of early AD has been discussed by El Gamal et al., 2018 The authors developed and programmed a computer-assisted diagnostic system to analyse Pittsburgh Component B-Positron Emission Tomography and to analyse the brain areas for individualised diagnostics. The system was validated using a dataset of 19 standard controls and 65 MCI subjects from an Alzheimer's Disease Neuroimaging Initiative dataset.

2.3 BRAIN REGIONS STUDIED IN THE CLASSIFICATION OF MCI and AD:

Based on the various studies, we selected the following ROIs(Region of Interests):

Chen J. et al., study provides a new insight into the heterogeneity of its broad connectivity within **MTL(Middle Temporal Lobe)** sub-regions that are at the root of aMCI's memory deficits. It also suggests that functional changes in the right HIP-ERC-PRC (Hippocampus- Entorhinal Cortex-Perirhinal Cortex) temporary circuit may constitute an early indicator of early changes in the disease and its progression. Their results showed that the anterior to posterior axis PHG and HIP were distinguished from NC(control) by differently abnormal connectivity patterns and the asymmetries of damaged degrees in an MCI from left and right hemispheres.

Son S.J et al., 2017 have studied the group- wise difference using Structural volume and functional connectivity relation. They found significant volume reduction between NC and MCI in **Right**

hippocampus, also significant volume reduction is seen between NC and AD in **hippocampus L/R** and **amygdala L**. They also mentioned that structural change does not necessarily impacts change in connectivity of the regions **hippocampus R** and **amygdala L/R.** They found the greater functional connectivity in caudate while decrease connectivity in amygdala and **default mode network.**

According to Zhang X. et al., 2021 study, AD subjects showed decrease in BC (Betweenness Centrality) in the brain regions of right hippocampus, **left posterior cingulate gyrus** and left amygdala as compare to NC patients, while BC increase in the regions of **left middle frontal**, **right middle frontal**, and right amygdala have noticed. Compared with NC, MCI subjects showed BC decreases in the brain regions of the **right posterior cingulate gyrus**, the **left middle temporal**, the **right middle temporal**, and the left hippocampus, while the BC increases were in the brain.

In medial parietal (i.e. cortex retrosplenial, posterior cingulate, and **precuneus**) and cortex parietal association, mild AD patients show less brain volume. The global cortical volume of AD patients are also lower than MCI ones (Wolf H et al., 2004).

Study done by Moretti D.V.,2015 found that there is significant memory reduction correlation seen between AVLT delayed recall and supramarginal gyrus in the alpha3/alpha2 frequency group. Also MCI group shows greater atrophy in the two specific brain regions: the **precuneus** and the **supramarginal gyrus** (a brain area belonging to the inferior parietal lobule), on both the left and right hemispheres.

In patients who have AD, MCI, and age-related healing patients, Liu and colleagues examined the topological properties of whole brain networks by using functional MRI. The MCI patients showed lower central position in the right pars triangularis, right **superior parietal cortex** and left hippocampus, in comparison with NC. Also reduction in cortical thickness has been observed in the following areas: pericalcarine cortex, **supramarginal gyrus**, cuneus cortex, lateral occipital cortex, **precuneus cortex**, paracentral lobule, fusiform gyrus, **superior frontal gyrus**, lateral occipital cortex, entorhinal cortex, inferior parietal cortex, isthmus-cingulate cortex, postcentral gyrus, superior parietal cortex, caudal middle frontal gyrus, insula cortex and precentral gyrus by Yang H. et al., 2019.

2.4 sMRI AS A POTENTIAL BIOMARKER:

Early diagnosis of AD and MCI:

The reduction in medial temporal lobe and hippocampal volume from MCI (10-15% reduction) and AD(20-25% reduction) will be significant biomarker for the early diagnosis (Shi F et al, 2009). And there is a proposal to add quantitative atrophy data from sMRI scans along with the clinical and psychometric tests.

Tracking Disease progression:

Monitoring structural changes in the brain is important, which can be done by charting the data over time. Since atrophy rates can help to predict subsequent clinical progression, especially in the MCI and cognitively NC patients, so it is important to track in these groups (Fox NC et al., 1997). Irrespective of cross-sectional measures, the measures of increase in ventricular volume and decrease in brain volume are more sensitive.

Predicting the risk of progression:

It has been recorded that annually on an average 10-15% of MCI patients progress to AD, one reason could be there is pathological changes occur before the beginning of clinical symptoms. The efficiency of biomarkers is quite high in the prediction of this progression, one study (Yuan Y et al., 2009) showed that hippocampal volume can be helpful detecting an average of 73% of MCI patients, who get progress to AD. Thus, quantitative study of sMRI can help us to measure this risk.

Mechanistic inferences into the disease process:

The application of sMRI as an independent biomarker for neurodegeneration helps to understand cognition-degeneration relationships in AD. This led to insights into the mechanisms of disease in AD. The result from several sMRI studies in the model shown in Figure 11 from Jack and

colleagues(2010) conclude that the neurodegeneration is associated more closely with cognitive decline.

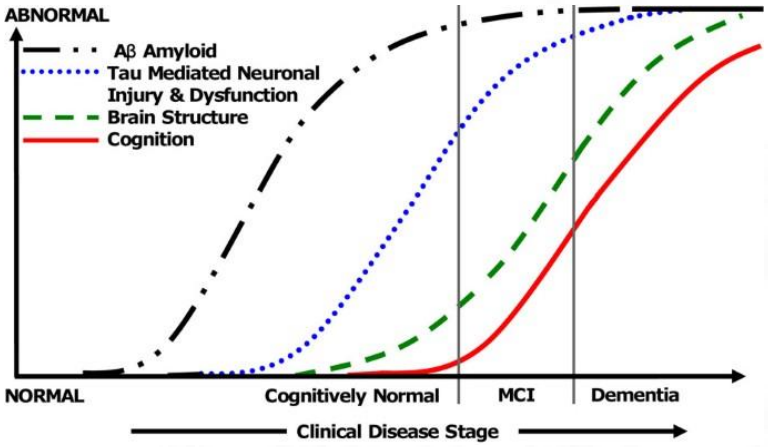


Figure 11: Proposed Alzheimer's disease pathological cascade based on biomarkers. MCI, mild cognitive impairment. Modified by (Vemuri P 2010)

2.5 TECHNIQUES OF FUNCTIONAL CONNECTIVITY IN rs-FMRI:

The rsfMRI is a potential tool for macro-scale connectomic analyses to characterise the intrinsic function of the brain, although the main limitations of preprocessing data are brain correlation and head motion correction. The entire brain correlation causes negative correlations that are irrelevant to physiological signals. Inadequate head motion correction can result in false positive data processing results. (Anderson J S et al, 2011), (Power J D et al, 2012). Thus, fMRI requires highly skilled personnel for aquisition of MR image, pre and post- image processing methods, and also require a unique method analyse image for optimal results.

To remove these errors pre-processing is required which can be attained by using the following available tools:

- Statistical Parametric Mapping (SPM),
- Analysis of Functional Neuro Images (AFNI),
- Functional Connectivity Toolbox(CONN)
- Functional MRI of the Brain Software Library (FSL-specifically) for Multivariate Exploratory Linear Optimized Decomposition into Independent Components(MELODIC), and

- Configurable Pipeline for the Analysis of Connectomes (CPAC)

Once the pre-processing is done, rsfMRI will be used for post-processing. The post-processing involves various techniques according to the study involved. These are:

1. Seed-based Correlation Analysis
2. Independent Component Analysis
3. Parcellation
4. Graph Theory method

Each of these methods are widely used in post processing of rsfMRI data in the Alzheimer's disease studies. The main purpose of these techniques is to evaluate the brain's functional network in terms of its strength and orientation within the brain functional connectivity network either qualitatively or quantitatively. Impulsive variations of the seed region/ROI BOLD signal are correlated with fluctuations in BOLD signal from all other voxels in the task-free brain in an effort to create a resting state connectivity map.

1. Seed Based ROI data processing:

   The seed analysis, which is straight forward and provides large results, is a traditional and most popular method. The seed-based ROI method consists of selecting the ROI and correlating the spatio-temporal BOLD signals within the ROI and other regions of the brain. This procedure requires an advance understanding of ROI selection. The seed-based approach to analysing the functional connectivity of the rs-fMRI BOLD signal is beneficial because the results are focused on special ROIs, so neuropathological pathology could be easier to understand.

   Wang et al. assessed functional connectivity in age-coordinated controls between the hippocampus and other brain areas and AD patients. In the hippocampus region, a seed-based ROI selection was used and the connectivity to other brain areas was evaluated. Their activity in AD was reduced and hippocampus connectivity to certain regions was disrupted, which indicated that cortical networks in AD were less integral. Zhang et al have tested RS fMRI for PCC connectivity to the rest of the brain and found disruptive and offset functionally connectivity. An evaluation of functional connectivity by Zarei M et al., in three different functional segments of the hippocampus region (Hippocampal head, body

and tail) to the PFC(Pre Frontal Cortex), thalamus and PCC(Posterior Cingulate Cortex) showed stronger links to PFC in AD cohort and correlated well with Mini Mental State Examination (MMSE) results, and a weaker functional connectivity to the PCC. PCC is at risk for separation from the remainder of the AD brain.

Another study by Zhang et al 2010 has demonstrated that the progression of AD has increased connectivity disruption and that changes in DMN. At the early stage of AD, amygdala structural disruption of connectivity is demonstrated and the results related to the MMSE score. In opposition to the above findings, Kenny et al in the AD cohort observed greater connectivity in the hippocampus (n=16).

The advantage of SCA (seed-based correlation analysis) is that it provides the network of regions most functionally linked to the region of interest. This SCA is easy to understand and attracted a lot of researchers. But the major limitation of the technique is the noise caused by the influence of other structural spatial rest networks resulting from head motion or scanner-induced devices. The preprocessing techniques, such as temporal filtration, can remove these artefacts. The final outcome depends on the choice of the seed size, the location and the spatial normalization. Hence a careful selection of ROI plays a vital role in this method.

2. Independent Component Analysis (ICA) data analysis:

ICA is another widely used approach that is a mathematical technique that makes the statistical choice between its constituents the most useful. ICA can be used to rank distinctive RSNs from rsfMRI information in a spatial way. ICA benefits a priori from assumptions, but it requires the manual selection of significant components and discriminatory signals originating from the physiological origin's low-frequency fluctuations. Functional ICA technological connectivity measurements decompose the 2D data (voxel and time viz) into a set of timelines and produce related spatial maps of the hidden signals. (McKeown M J et al., 1998). ICA has some benefits compared to seed-based methods, like automated parts identifying RSNs, but both of these techniques produce similar results when used in a group of healthy subjects. (Rosazza C., 2012). In a follow-up study, Binnewijzend et al reported a decline in network functionality in AD in default mode. Another two-state study by Schwindt et al. (rest and task-based) showed that

interrupted DMN connectivity is a non-durable phenomenon and that the degree of apparent changes in FC in DMN can clearly predict the cognitive state.

Another study (Pasquini L et al, 2015) found a rise in local (hippocampus) and a decrease in global connectivity (DMN) in AD. Default mode networks have shown enhanced functional AD connectivity. In comparison with late onset patients, functional connectivity in DMNs was further disturbed at an early start of AD. The abnormal reduction in PCC and right hemisphere functionality in DMNs of AD cohorts in relation to CN has also been confirmed by a different study. (Castellazi Z et al., 2014)

In another study by Dai Z et al., 2015, structural MRI and resting state functional MRI parameters were used to discriminate against NC & AD patients. Zhou et al used ICA to assess the functionality of the salience network and DMN for another interesting study. In which, in patients with frontotemporal dementia decreased connectivity was documented on the salience network and increased connectivity in DMN; while in the case of AD, greater connectivity and reduced DMN connectivity was noted.

3. Graph Theory Data Analysis:

The ball and stick model is used by the graph method to connect RSNs as a nodal (ROI) group with "edges" (correlation between ROIs). This is a unique method and can also be an alternative seed analysis and ICA method. The region of interest corresponds to the nodes in this method and the relation between the ROIs corresponding to the connectivity of the edges. This graph theory is used to quantify these connectivity parameters. The average path length, global efficiency (average length of shortest connection for all node couples), local efficiency (average global efficiency of subgraphs for each node of adjacent nodes) are few of the parameters. Sanz-Arigita et al used graph theory in their study and found out alterations in global distant functional connectivity among AD. In one of the study conducted by Supekar et al., Clustering coefficients were significantly decreased in patients as compared to controls in the hippocampus region of brain. Toussaint P J et al., 2014 showed how a decreased anterior-posterior and increase in local clustering in frontal and parietal system can be defined by graph theory quantified functional in the four DMN subsystem.

4. Parcellation Data Analysis:

   A brain atlas/parcellation is a voxel-based labelling in "structural and functional units" of the data. In a parcellation scheme a numerical (integer) label is assigned to each voxel corresponding to the structural / functional unit, according to certain criteria. The averaging of voxel is done in order to avoid:

   - Effects of random noise in the rs-fMRI.
   - Constructive framework can be designed by using this method.
   - Reducing Type I errors by limiting the number of statistical tests and preserving the originality of the statistical data.
   - An easier way to visualize the data, one might have 17 or upto 200 data points rather than 40*40*40= 6400 data points, which in itself an amazing approach of reducing statistical data.

In the study done by Wu Z. et al., 2019, Structural connectivity done by using different parcellation model techniques and then compare the progression from NC to AD. They found extensive disruption exist in MCI and AD and this topological characterization can provide significant biomarkers for AD.

Table 1: Comparative study among all the techniques: (Rajamanickam K., 2020)

Methods	Purposes	Strengths	Limitations
Seed-based Data analysis	To Estimate the correlations between predefined voxel and rest of the brain voxels	i) It is easy to understand and compute also.	i) It requires a prior selection of ROI, which might also require skill and thus lead to potential biases
Independent Component Analysis	It identifies noise within BOLD signal by separate out distinct resting-state networks which are spatially or temporally not depending on each other	i)It can generate both temporally and spatial distributed patterns of default mode network functional connectivity keeping some prior	i)While composing such network, It is less sensitive to interindividual variation. If network presents across multiple components then chances of errors

		assumptions	at the group level might occur
Graph Theory Analysis	This explains the functional brain networks topology via calculating connectional characteristics and generating matrixes of the graph involves nodes (voxels) and edges (connections between those voxels)	i)It can directly delineate and compare different brain networks by using topological parameters	i)quite difficult to interpret, since involves statistic parameters
Parcellation Data Analysis	the connectivity patterns between a set of predefined brain regions (eg, functional parcellations) are represented as a matrix and subject to statistical analysis	i)Less statistical data to deal with.	i) Lack of Universally-accepted parcellation templates which can be use as a reference.

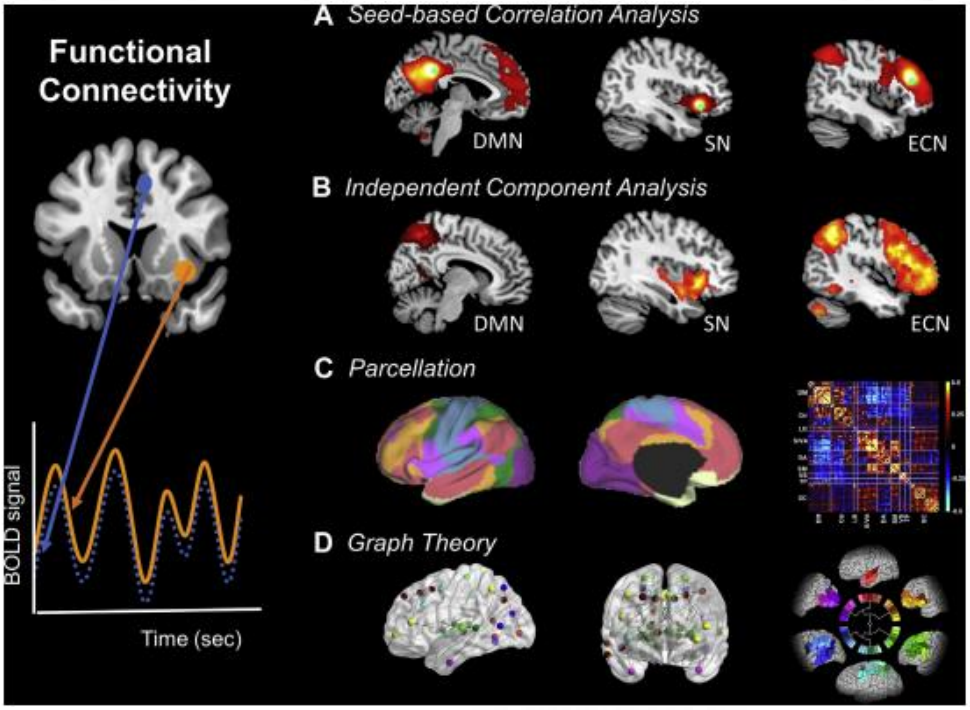


Figure 12: Summary of common techniques to derive functional connectivity from rsfMR imaging data(Zhou J. et al., 2017)

2.6 GRAPH THEORY ANALYSIS IN THE CLASSIFICATION OF MCI and AD:

The distinctive features of the regions composing the DMN (nodes) and also the connections or interactions between these regions (links) were examined. Correlations between regions (or nodes) are symmetric relations that may be summarised just by an undirected graph. Graphs are sets of vertices (or nodes) with corresponding sets of edges (links) that may be accustomed represent networks (Stam and Reijneveld, 2007). A link connecting two nodes will be interpreted as presence of interaction or connection between them. Information about the connectivity structure of a graph is contained into the adjacency matrix A, whose elements Aij = 1 when there is an edge exists between nodes i and j, and Aij = 0 otherwise. Human's brain network also can be described abstractly by a graph at the microscopic scale, with nodes defining as neurons and edges representing as axonal connections. At macroscopic scale of neuroimaging data, the chosen nodes represent regions or voxels, and also the edges are some statistical measure of association like correlation or mutual information (functional connectivity or synchronisation levels) between regions. The above calculated matrix of correlation was thresholded (from 0.1 to 0.9, with steps of 0.05) and accustomed to approximate the adjacency matrix. for every threshold value, three indices were calculated to get representative values of the amount and strength of connections as a function of correlation threshold, so as to characterise functional connectivity within the network: the degree k, which is that the number of edges (connections) arriving at or leaving a given node (region) ki = $\sum j \in N aij$— with N = 1,…,n the amount of nodes, aij = 1 if nodes j and that i are connected to each-other, and aij = 0 otherwise (the higher the degree of a given node, the more interactions it has with other nodes within the network); the mean characteristic path length L, which is that the average distance between all node pairs — where, Li denotes the common distance between node i and every one the opposite nodes, and smaller L suggests faster information transfer; and therefore the mean clustering coefficient C, which is defined because the ratio of number of links existing to the amount of possible links between neighbours of a given node , with higher C indicating a highly specialised functional network (Watts and Strogatz, 1998). Metrics were calculated for the weighted original graph (with weight wij = 1 − rij) moreover as 1000 completely random graphs.

Wang J. et al., studied the topological architecture of the functional connectome in patients having aMCI. They analysed abnormal connectome organization within the aMCI patients and reported results as: 1) impaired functional connectivity between different functional modules, 2) decrease nodal strength in default network and 3) increase in characteristic path length. Abnormal network metrics and cognitive performance is correlated in their study using quantitative analysis. They also noted that in aMCI classification connectivity strength outperforms nodal and global network.

Cognition-related ROIs are summarized by Behfar Q. et al., 2020 to indicate the compensatory characteristics during a resting-state fMRI. They reported a number of the compensatory regions in MCI patients, those regions are right superior parietal gyrus , the right and also the left precentral gyri, and the right middle frontal gyrus. In MCI patients, they correlated Centrality of the compensatory ROIs with cognitive performance, on contrary this correlation isn't relevant in senior healthy controls. This increase in connectivity mechanism might be consider as brain mechanism of cognitive decline performance.

In the study by Liu J. et al., 2019, they performed multi task feature selection method to spot EMCI and by using both imaging and non- imaging phenotypic measures constructed a subject graph. And to perform this they concluded a GCN model as a preferred one.

2.7 CORRELATION AND INTEGRATION MEASURES:

Statistical characterisation of the chosen regions was administered by measuring correlations and integration between regions composing the network. The mean activity within a given brain region i will be defined by its average time-course yi. for 2 regions i and j, the correlation coefficient rij calculated between their mean temporal activities yi and yj is classically interpreted as a functional dependency, or connectivity, between them (Friston et al., 1994):

$$\sigma_{ij} = \frac{1}{T-1}\Sigma_T \mathbf{y}_i(t)\mathbf{y}_j(t)$$

where σij – the covariance of time series yi and yj – is calculated as: .

$$r_{ij} = \frac{\sigma_{ij}}{\sigma_{ii}\cdot\sigma_{jj}},$$

When there are over two regions involved, functional connectivity within the network can thus be fully characterised by correlation coefficients (r) calculated with all possible combinations of the N regions (or nodes) composing the network, creating a N × N matrix R. Hierarchical integration (I) was employed in order to quantify the world changes in functional connectivity induced by normal ageing or AD. This measure comes from mutual information (entropy) that informs on the world integration within a network of regions (Marrelec et al., 2008, Tononi et al., 1994). For a given network defined by a group of regional time courses Y, global integration I(Y) is defined as I(Y) = ∑ = 1H(yi) − H(Y), with H(Y) the entropy of the system. For signal averaged over tens of voxels, data will be approximated to having Gaussian distribution with mean μ and covariance matrix

$$\Sigma, I(\mathbf{Y}) = \tfrac{1}{2} \ln \left[\frac{\prod_{i=1}^{N} \Sigma_{ii}}{|\Sigma|} \right],$$

with Σii the diagonal elements, and | · | the matrix determinant. If Y consists of K sub-systems Sk (k ∈ [1, …, K]) of the N regions, such that, , then integration are often decomposed into a sum of terms representing integration between sub-systems (Iinter), and within each sub-system (Iintra) as: I(Y) = ∑ Iintra + Iinter, where Iintra= ∑kI(Ysk) and Iinter= I(Ys1…Ysk) = ∑kH(Ysk)-H(Ys1,….,Ysk). In terms of the covariance, integration between sub-systems is written:

Iinter=1/2 $\ln \left[\frac{\prod_{k-1}^{K} |\Sigma_{S_k S_k}|}{|\Sigma|} \right]$,

where $|\Sigma_{S_k S_k}|$ represents the portion of Σ corresponding to regions of Sk. Integration within a sub-system becomes:

$$I_{intra} = \tfrac{1}{2} \ln \left[\frac{\prod_{n \in S_k} |\Sigma_{nn}|}{|\Sigma_{S_k S_k}|} \right].$$

The covariance matrix may also be expressed as a function of the correlation matrix R of signals of Y as: ∑ = sqrt[diag(∑)R] sqrt[diag(∑)],

where diag is diagonal, and R is matrix of correlation coefficients (rij) calculated between the mean regional time courses. Total integration thus becomes: I= ½ ln|R|, which shows that integration

derives from functional connectivity, summarizing the N × (N − 1)/2 correlation coefficients into one number which is zero if there's no functional connectivity, and positive otherwise. the entire integration of the default mode network was calculated as IDMN= ∑ IintraDMN + IinterDMN.

Bayesian inference was utilised to approximate the posterior probability distribution of the parameters of interest, and infer on the worth of the covariance matrix (Σ) of the Gaussian model (Marrelec et al., 2006). Given the regional time courses Y, the posterior probability p(A|Y) of an assertion A associated with any pairwise comparison of integration measure (total, inter- or intra-system) was approximated with the statistics obtained from the Bayesian sampling scheme. Correlation matrices and integration values were computed from 1000 samples of covariance matrices approximating the posterior distribution of the parameters of interest in a very group analysis. The correlation matrices were thresholded at value chosen from a correlation table consistent with the degrees of freedom, which corresponds to the quantity of acquired volumes (119 within the present case), for a probability $p < 0.05$ (95% significance).

The validity of the pairwise comparison of integration measures was tested using the logarithm of the odds ratio or evidence, a measure of integration significance, as:

$$e(A|\mathbf{Y}) = 10 \cdot \log_{10}\left(\frac{p(A|\mathbf{Y})}{1-p(A|\mathbf{Y})}\right)$$

(in decibels, dB) (Jaynes, 2003). Two values of integration are going to be considered significantly different when |e(A|Y)| > 10 dB, equivalent to a $\left(\frac{p(A|\mathbf{Y})}{1-p(A|\mathbf{Y})}\right)$ ratio greater than 10:1.

2.8 CLASSIFICATION MODEL USING GRAPH THEORY STUDY:

Since there is range of biomarkers studied for the classification or earlier diagnosis of the progression of the MCI and NC to AD. The study by Wang J. et al., 2013 showed the significance of graph based metrics in the diagnosis of aMCI by plotting the receiver operating characteristic curves (ROC) and found the sensitivity of 86.5% and a specificity of 85.1% to distinguish aMCI from NC on the basis of network-based statistics (NBS) functional connectivity. Liu et al., 2020

have also verified the effectiveness of graph convoluted networks(GCN) in the diagnosis of the early mild cognitive impairment(EMCI), they find out the classification accuracy between the two models, one involves only imaging features while other involves both imaging and non-imaging features. The classification performance of the graph formed from both imaging and non-imaging features is comparatively high (i.e. 84.1%) than the graph formed from selected features (Accuracy= 80%) and from original features (ACC<70%). The accuracy of the GCN-EMCI model is even greater than the already existing model of Tripathi et al, 2017 (ACC= 75.8%) and Jie et al., 2018 (79.5%).

In the recent study by Lama R. K. et al., 2021, they included the ADNI data of MCI & AD in their studies of finding out which classification model works best between linear SVM, and RELM classifier. From the total ADNI dataset 90% was used as training data while 10% was used as test data, for each training and test data separate feature selection methods were done to avoid bias and then calculated mean accuracy and standard deviation of each selection method against both the classifier. They found out in almost all the cases LASSO gives the maximum accuracy, specificity and best sensitivity for feature selection. They have also compared the accuracies of other classification model represented in other studies and concludes that linear SVM along with LASSO works best for the classification with graph features of connectivity.

2.9 SIGNIFICANCE OF OUR STUDY:

The study we are targeting for diagnosis involves the quantitative analysis of structural and resting-state functional MRI in the three diagnostic groups which are NC, MCI, and AD. Also, Most of the previous studies did analysis considering age difference (Toussaint P.J. et al, 2014), but we chose to control age, sex and education factors, so that we can be more focused on structure and connectivity changes. To have a quantitative analysis of functional connectivity we also took the help of graph theory parameters in the selected regions from AAL atlas and neurophometric atlas, unlike in other studies they are more focused in DMN (Toussaint P.J. et al, 2014; Wang J. et al, 2013). Later we assumed to do bivariate and multivariate statistical analysis and doing logistic regression for better classification and to check which independent parameters can give us maximum accuracy for a diagnostic purpose. Also, we are assuming to be able to justify compensatory mechanism in AD and MCI patients while using our MR imaging biomarkers. Since many studies have used combination of biomarkers for diagnosis of the progression of AD, but from our best of knowledge none have used the biomarker of structural atrophy explained by the reduction of grey matter volume and another is functional connectivity among ROIs explained by using the graph theory analysis. We tried to keep our biomarkers limited and with simple technique of MRI machine, so that the gained diagnosis models can be implicate at most of the places for the purpose of having a keen check on the progression of AD continuum.

CHAPTER 3- METHODOLOGY

3.1 SUBJECTS SELECTION

A case control study design was followed to meet the objectives of the study. Patients were selected from the decade old well-established Dementia Clinic in the Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Thiruvananthapuram, Kerala for period of 2010- 2014. The patients were undergone a detailed historic and neurological examination, neuropsychological evaluation, behavioural assessment and a high quality MRI scan for their respective diagnosis. All these evaluations were repeated at 5 year follow up. Patients were included if they had a history of progressive change in personality and behavior such as apathy, disinhibition, loss of empathy, lack of insight and foresight, reduced verbal output, poor planning and judgement, perseveration, distractibility and impulsiveness.

Exclusion of Subjects: We excluded patients who had a past history of cerebral ischemic infraction or hemorrhage, head trauma, alcohol abuse, cardio vascular and major psychiatric diseases, past history of depressive illness and epilepsy or other neurological disorders such as dementia, FTD (Fronto Temporal Dementia, fvFTD. Also patients having claustrophobia or having metal implants to avoid accidents while taking scans in MRI. After the screening the subjects were stratified into NC, MCI and AD.

For all the patients, duration of illness was defined as the estimated onset of behavioural symptoms to the date of MR imaging. Assessment were conducted to get information on activities of daily living (self care, using domestic appliances, concentration, communication, orientation, using public transport, participation in social activities, taking medications and handling finances), Age of onset, education and occupational history, family history of dementia or other neurological diseases, and substance abuse from proxy informant.

Normal healthy age matched controls with no past history of neurological and psychiatric diseases, and no contraindications of neuroimaging were recruited from the first degree relatives (mostly spouses) of patients and SCTIMST personnel. The study was performed after taking written

informed consent from all subjects/first degree relatives, which was approved by the Institutional Ethics committee of SCTIMST.

The original data set of current study was included a total of 132 subjects: patients with 38 NC, 51 MCI (non-converters from the 5 years follow-up) and 43AD. Out of these patients, some were excluded due to poor image quality and movement artifacts, while some are removed to avoid data discrepancy or outliers. The chosen data set includes patients from 61 to 79 year old and makes the final set consisted of 23 NC, 34 MCI and 29AD (i.e total= 86 subjects).

3.2NEUROPSYCHOLOGICAL EVALUATION

All patients were administered neuropsychological test battery (Table 2).

Table 2: Neuropsychological tests used in the study, number of subjects tested and brief description of test.

Test	Description of test
Mini Mental State Examination,(MMSE, Folstein, Folstein&Mc Hugh, 1975)	A brief test that measures orientation to time and place, registration of three words, attention and calculation, recall language and visual construction. In normals, scores range from 25-30, 21-24 for mild AD, 14-20 for moderate AD, and less than 13 for severe AD.
Adden Brookes Cognitive Examination	It comprised of component scores to assess six cognitive domains, including orientation, attention, memory, verbal fluency, language, and visuospatial skills
Clinical Dementia Rating scale	A global scale developed to note the disease stage and severity. It mainly comprises six domains: memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care.
Rey auditory Verbal Learning test, Immediate and Delayed(RAVLT)	It has proven its efficiency in evaluating verbal learning and episodic declarative memory, including

	scores for proactive and retroactive inhibition, immediate memory, retention of information after a period of time, and memory recognition (Malloy-Diniz, Lasmar, Gazinelli, Fuentes, & Salgado, 2007; Van der Elst, Van Boxtel, Van Breukelen, &Jolles, 2005).
Trail making Test A,B(Reitan, 1958)	It is a measure of attention, visual searching, mental processing speed, and mental flexibility. It intends to assess shifting, in which part A measures processing speed (to draw lines to connect consecutive numbers) and Part B measures the ability to flexibly shift the course of an ongoing activity (to draw lines alternating between numbers and letters). Part A tests visual searching, numeric sequencing, and motor coordination while Part B tests cognitive task performance which requires motivation, problem solving, visual motor and visual spatial abilities and mental flexibility. Latter is difficult and 56 cm longer with more visually interfering stimuli than former one. The part B is considered as sensitive test for frontal lobe dysfunction. Both are timed and score represents interms of time taken and errors.
Hospital Anxiety and depression scale (Zigmond & Snaith, 1983)	It is a self-report screening scale that intend to measure depression and anxiety in patients within and outside hospital and community settings, avoiding any evident physical problems. Both anxiety and depression consists of 7-item subscale and are rated from 0 to 3.

3.3 IMAGE AQUISITION:

A Discovery MR750w 3.0 T MRI Scanner (GE Healthcare, Milwaukee, USA) with a 32-channel phased array head coil was used to acquire rs-fMRI and structural images from each subject. These were acquired using the following parameters: TR/TE = 2500/30 ms, voxel size = 3.31 × 3.31 × 4 mm3, FOV = 21.2 cm, slice thickness = 3.2 mm, matrix 64 × 64. A high resolution reference axial 3Dbrain volume imaging sequence (3D BRAVO) with TR/TE = 7/2.98 ms, slice thickness = 1 mm, flip angle = 12°, matrix size = 256 × 256,and voxel size = 1 mm × 1 mm × 1 mm was collected for anatomical reference. The number of volumes acquired was 132. All participants were instructed to close their eyes and relax while inside the scanner while conducting the 'task-free' fMRI acquisition.

All the MR images were stored in Digital Imaging and Communications in Medicine (DICOM) format.

3.4 IMAGE PROCESSING FOR FUNCTIONAL CONNECTIVITY:

To process the stored DICOM images, nifty format(.nii) images are needed which we converted using a tool "dcm2niigui", this tool can be easily installed from online sources. Once it is converted, images are ready to pre-process and upload in CONN's default MNI pipeline in CONN18.0 toolbox.

SETUP:

This involved preprocessing steps such as: functional realignment and unwarping, slicetiming correction, structural segmentation and normalization, functional normalization, outlier detection, and finally images weresmoothed using a Gaussian kernel of 8 mm FWMH.Since I am working with rs-fMRI scans, thus chosen condition in my setup is 'Resting'. Covariates 1 and Covariates 2 were also chosen in the SETUP:

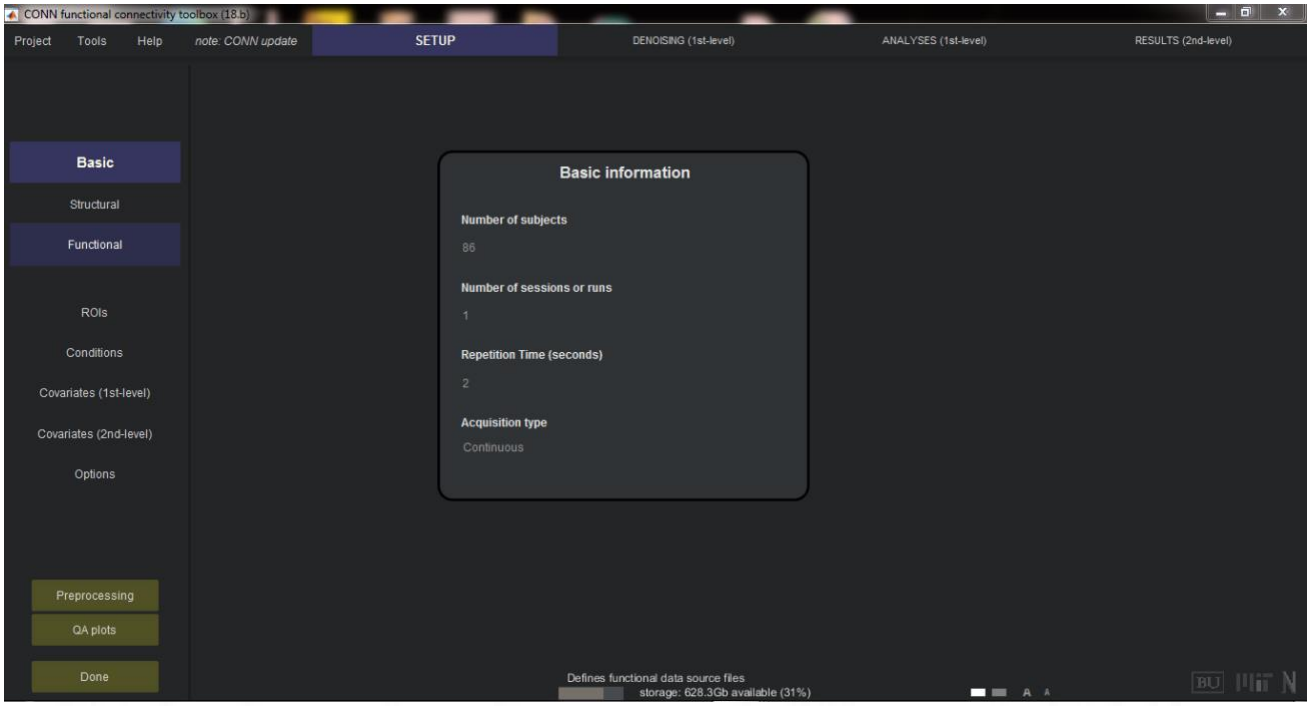
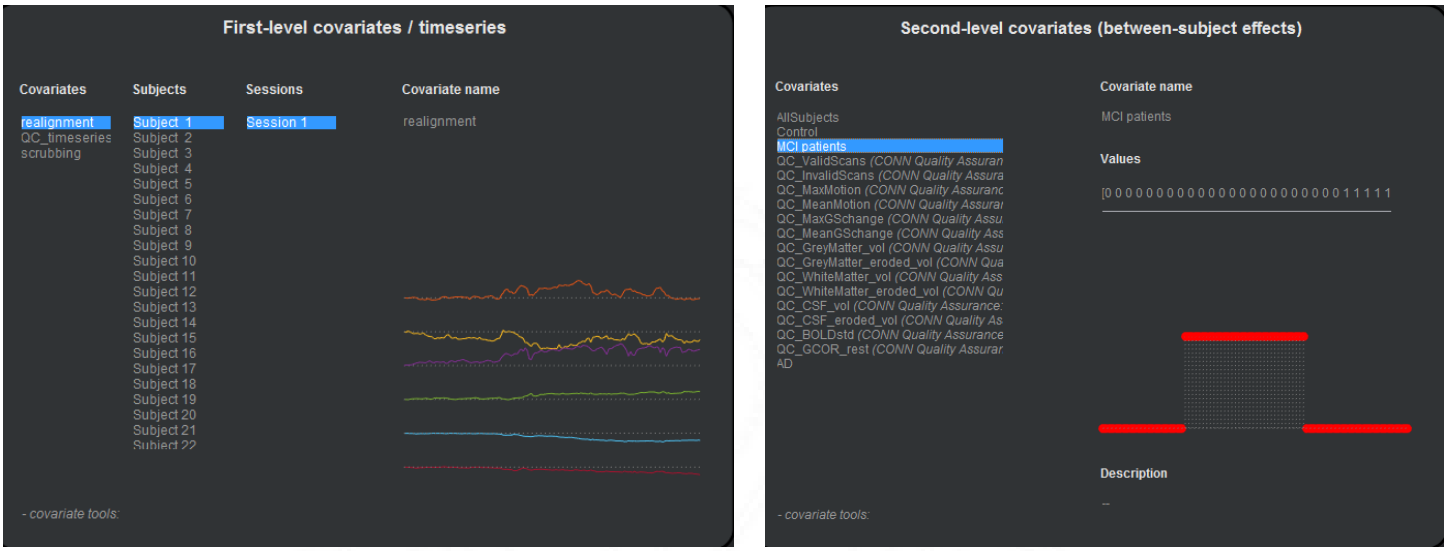


Figure 13: Setup window in CONN18.0 toolbox, showing uploaded number of subjects i.e. 86

Covariates 1 involve the parameters within-subject which are realignment, QC timeseries and scrubbing. Scrubbing means to remove outliers with respect to basic time-series scans.

Covariates 2 involve the parameters between-subject where I have defined my groups as NC, MCI and AD using '0' and '1' in contrast with the appropriate subject number. This will later help to have a study at group level analysis.



a) b)

Figure 14: a) showing the default covariate1 settings, b) shows default and addition covariate2 settings.

Once the setup is done, I have conn_*.mat file saved with me, which can be updated later as per my requirement.

Denoising:

Linear de-spiking was performed on the fMRI data to regress the confounding effects. Potential confounding effects used in CONN's default de-noising pipeline such as noise components from cerebral white matter and cerebrospinal fluid areas, an estimated subject-motion parameters, also identified outlier scans (or scrubbing) were used in this study and these were removed separately for each voxel and for each subject using Ordinary Least Squares regression. The threshold used to detect artefactual time points is z = 0.25. The artefactual time points were removed by default before principal component analysis estimation. Component base noise reduction method (CompCor) in CONN was used to remove principal components from noise regions of interest. The motion parameters were used as first level covariates for the analysis.

This will help to remove all the confounds from each voxel and each ROI, distribution of correlation coefficients can be visualized at the right side of the screen in BOLD% variance

pre/post denoising. Percentage BOLD variance response shows the contribution of each confound on each subject.

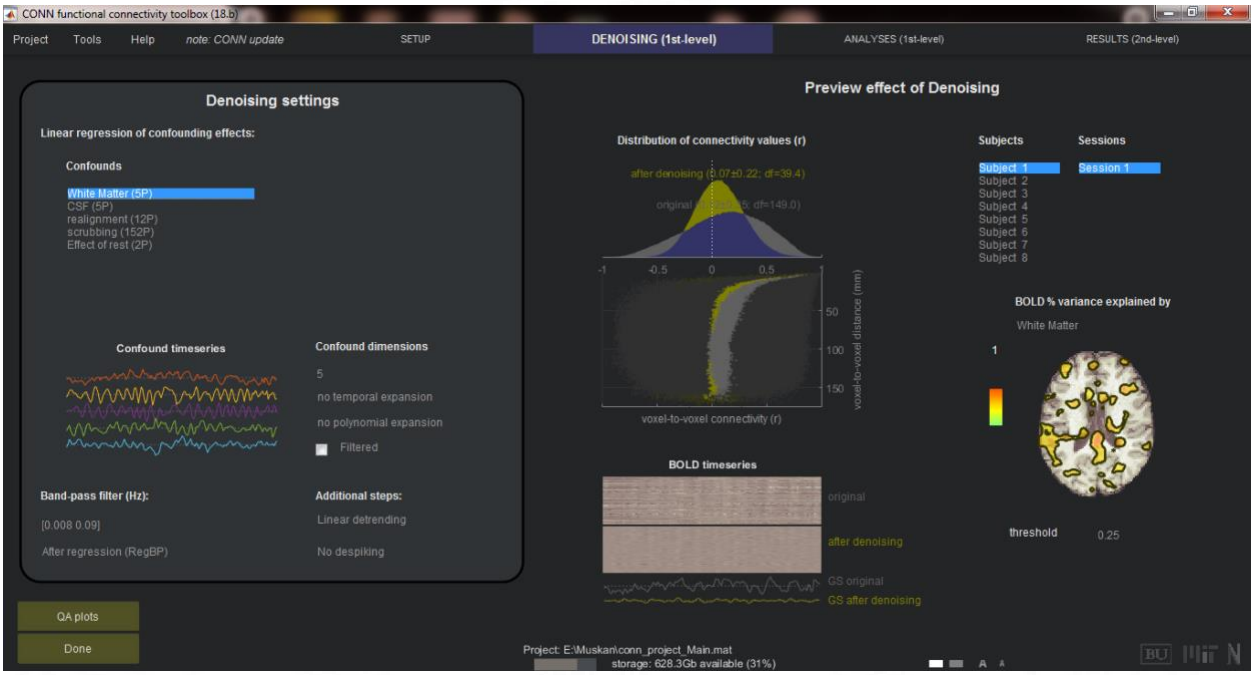
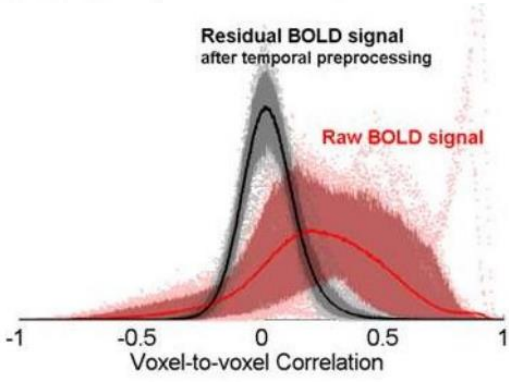


Figure 15: shows denoising window, which has one side settings tab and another side real-time preview tab.



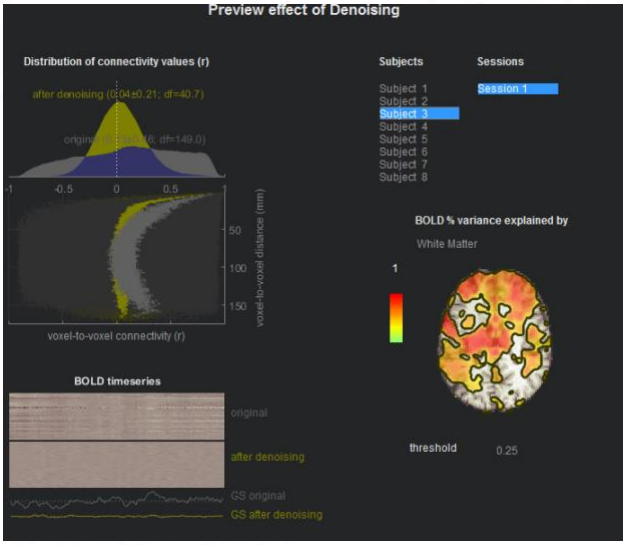


Figure 16: Effects of noise reduction on correlation distribution of covariates, the side bar near brain image shows

Here, Band-pass filter, Addition steps (such as detrending/despiking), or dependency of effects of regressors on frequency can be set. I kept all these factors as default.

Figure 17: Default settings of Band-pass filter(the value at which effect of confounds can be further reduced and to limit the subsequent connectivity), RegBP(assuming that the effects of regressors are independent of frequency), with linear detrending and no despiking.

First-level Analyses:

Different ROIs for the functional connectivity analyses were selected here, I choose the regions as per the default availability of ROI in CONN toolbox (the atlas includes cortical and subcortical ROIs from the FSL Harvard-Oxford atlas, as well as cerebellar ROIs from the AAL Atlas) and on the basis of prior research.

Figure 18: First-level Analysis window; Setting for seed and ROIs done in left tab, while right-side tab shows connectivity of a particular selected ROI with other ROIs and voxels in each subject

Functional connectivity analyses can be performed based on regression (beta values) or correlation (Fisher transformed) measures. In addition when multiple sources are used one can define whether the analyses should focus on bivariate or semipartial correlation measures (or bivariate or multivariate regression).

Bivariate regression $b = (\mathbf{x}^t \cdot \mathbf{x})^{-1} \cdot (\mathbf{x}^t \cdot \mathbf{y})$

Bivariate correlation $r = (\mathbf{x}^t \cdot \mathbf{x})^{1/2} \cdot b \cdot (\mathbf{y}^t \cdot \mathbf{y})^{-1/2}$

Multivariate regression $\mathbf{B} = (\mathbf{X}^t \cdot \mathbf{X})^{-1} \cdot (\mathbf{X}^t \cdot \mathbf{Y})$

Semipartial correlation $\mathbf{R} = [(\mathbf{X}^t \cdot \mathbf{X})^{-1}]^{-1/2} \cdot \mathbf{B} \cdot [\mathbf{Y}^t \cdot \mathbf{Y}]^{-1/2}$

As per my study, I chose bivariate correlation measure i.e. to study the pairwise connectivity between every voxel of the brain and each source ROI separately (effect sizes represent correlation coefficients) by using bivariate regression (models predicting each voxel BOLD signal in terms of the BOLD signal from each of the ROIs separately).

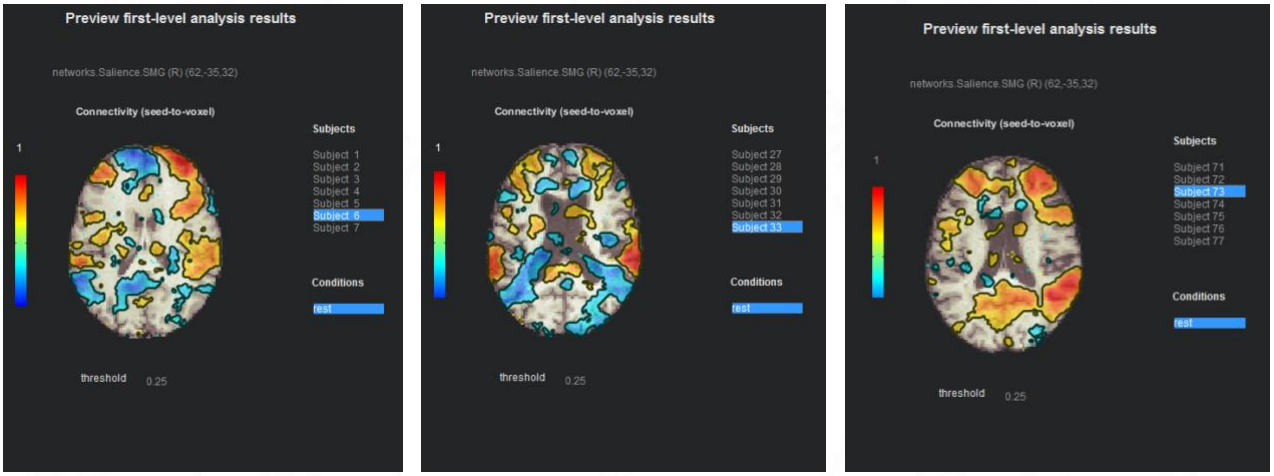
Table 3: Selected ROIs during first-level analyses in CONN18 toolbox

Alphabet code	Selected ROIs	Function/Role
A	networks.Salience.AInsula(L) (-44;13;1)	social affect, such as: empathy, to differentiate primordial emotions like fear, disgust and happiness.
B	networks.Salience.AInsula (R) (47;14;0)	ability to feel own heartbeat, or to show empathize with the pain of others, introspective awareness.
C	networks.Salience.SMG (L) (-60;-39;31)	In verbal memory tasks i.e. shows memory on pitch based

D	networks.Salience.SMG (R) (Superior Marginal Gyrus) (62;-35;32)	Involves in rhythmic memory and perceiving emotions of others
E	networks.DefaultMode.PCC (1;-61;38)	Active during memory retrieval, spatial memory, emotional stimuli (irrespective to positive or negative)
F	atlas.Hippocampus(Hippo)(R)	Spatial navigation, memory
G	atlas.Hippocampus l(Hippo)(L)	
H	atlas.Amygdala(Amy) r	Activate from negative emotion (fear) stimuli, episodic memory
I	atlas.Amygdala(Amy) l	In brain's reward system, able to induce either positive or negative emotions
J	atlas.SPL r (Superior Parietal Lobule Right)	Spatial orientation, receives input from hand and eyes
K	atlas.SPL l (Superior Parietal Lobule Left)	
L	atlas.SFG r (Superior Frontal Gyrus Right)	Cognitive functions, working memory, self awareness
M	atlas.SFG l (Superior Frontal Gyrus Left)	
N	atlas.PO r (Parietal Operculum Cortex Right)	Mathematical input, visuospatial cognition & imagery of movement
O	atlas.PO l (Parietal Operculum Cortex Left)	Sensory motor integration
P	atlas.FO r (Frontal Operculum Cortex Right)	Thought, cognition and planning behavior
Q	atlas.FO l (Frontal Operculum Cortex Left)	
R	atlas.Caudate r	Voluntary skeletal movement, planning the execution of movement, in learning,
S	atlas.Caudate l	

		motivation, reward, emotion and memory
T	atlas.MFG r (Middle Frontal Gyrus Right)	Reorienting of attention
U	atlas.MFG l (Middle Frontal Gyrus Left)	
V	atlas.Precuneous (Precuneous Cortex)	Recollection and memory, affective responses to brain, visuospatial, perception of the environment

There is availability of preview window, which helps one to analyse the connectivity in real-time. Threshold (0.25) represents correlation coefficients, or beta values for regression.



NC(Normal Control) MCI(Mild Cognitive Impairment) AD(Alzheimer's Disease)

Figure 19: First-level analysis of SMG(R) in three different diagnostic patients.The colorbar to the left shows the strength of the t-statistic among the regions.

First-level results are also exported as .nii volumes (one perSubject/Condition/Sourcecombination) in the results/firstlevel folder.

Second-level Analyses:

Further ROI-ROI analysis method in CONN 18.0 toolbox was used to compute the effect of FC between different groups and different ROIs. For each participant, the average BOLD time course of each seed was computed from their respective functional images, and was further correlated with the time courses of whole-brain voxels using Pearson correlation analysis. For general linear model (GLM) analysis, Fisher's r to-z transformation of correlation coefficients was implemented. In the second level analysis, to compute group differences in FC for each network, connectivity maps from all subjects were entered into GLM. Statistical significance for all comparisons were thresholded at $p < 0.05$, FDR corrected for ROI level(cluster threshold) and $p < 0.001$, uncorrected for voxel level (height threshold). Corrections for multiple comparisons were done in voxel and ROIs levels.

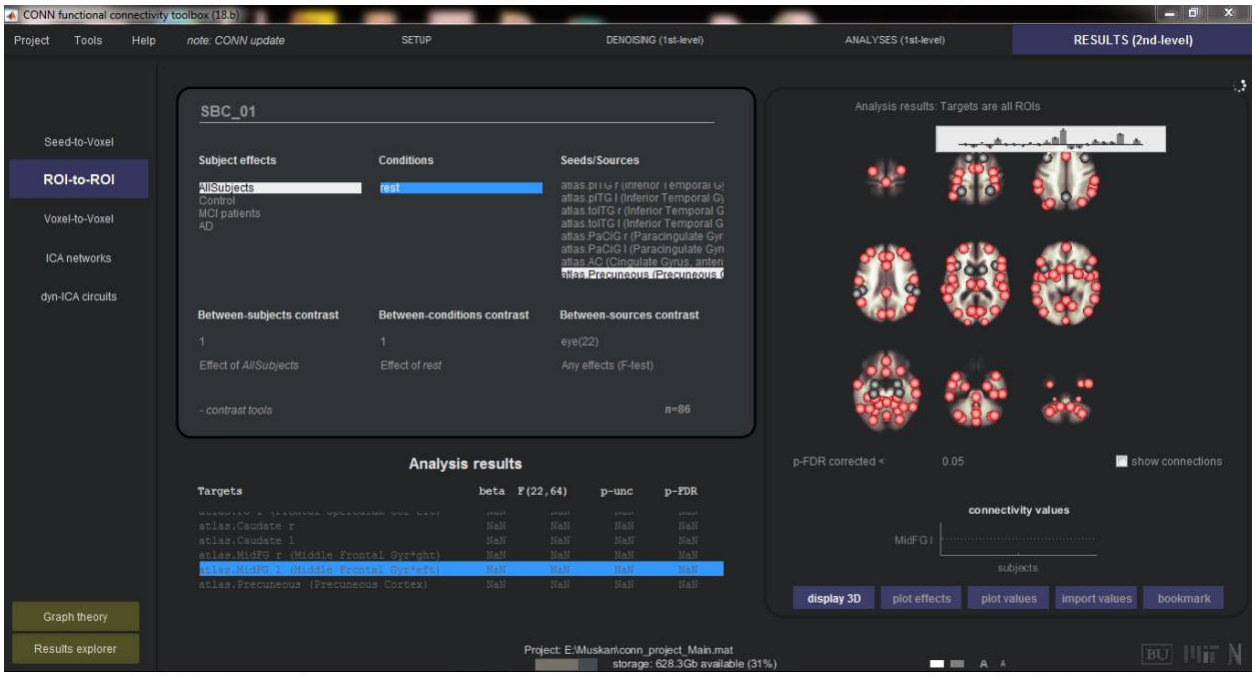


Figure 20: shows 2nd level Results window; Preview windows represents group-level positioning of seeds and connectivity. In the subject effects window, hovering the mouse over the voxels display the p-value and t-statistic for the contrast highlighted, and clicking on a voxel generates a bar plot which show the strength of the contrast estimate.

Graph Theory Analysis was done using CONN 18.0 toolbox, using the selected ROIs as node and connectivity length between the ROIs depict as edges. The output I got through this are: Adjacency matrix (gives correlation value of connectivity of each ROI with other ROI in each subject, with the choice of proper threshold, it converts matrix into binary form; 0= presents no connection, while 1= represent connection) with the threshold value of 0.15 and includes correlation both sided (positive and negative).

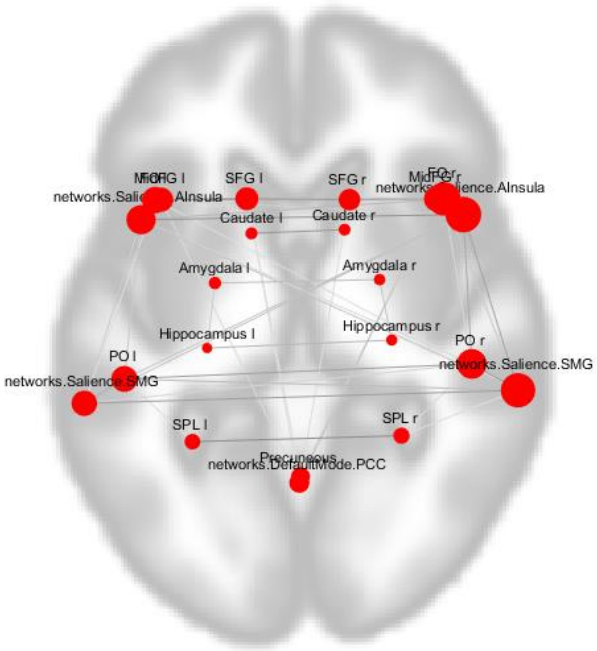


Figure 21: Red Dots are showing the selected ROIs, size of red ball presents the value of F-test or T-test and the lines in between the dots show connectivity edges in the brain (axial-view).

The 95th percentile of each distribution was used as the critical value for a two-tailed test of the null hypothesis with a probability of type I error of 0.05 and FDR corrected applied to provide the significant connectivity between the different ROIs and save into Dataset in the form of .csv format (have correlation value corresponding to the previously described variables of each ROI in each subject).

3.5 IMAGE PROCESSING OF STRUCTURAL MRI SCANS:

Though structural images are already pre-processed using CONN18 but to extract grey matter volume, I have used CAT12 toolbox in SPM12, which helps to do computational analysis. But for my purpose, I have used it's feature of 'Voxel based processing in each scan to extract structural features such as white matter volume, grey matter volume, CSF (cerebral spinal fluid), and thickness of each regions. The attributes are given for the regions according to the available atlas. In the latest CAT12, I followed Neurophometric atlas with 142 ROIs for both left and right hemispheres of the brain.

CAT's processing workflow comprises of two main steps: voxel-based processing and surface-based processing. The 'Voxel-based processing' step can be thought of as one module for tissue segmentation and another one for spatial registration. An optional third module allows for the generation of ROIs and the calculation of ROI-based measures.

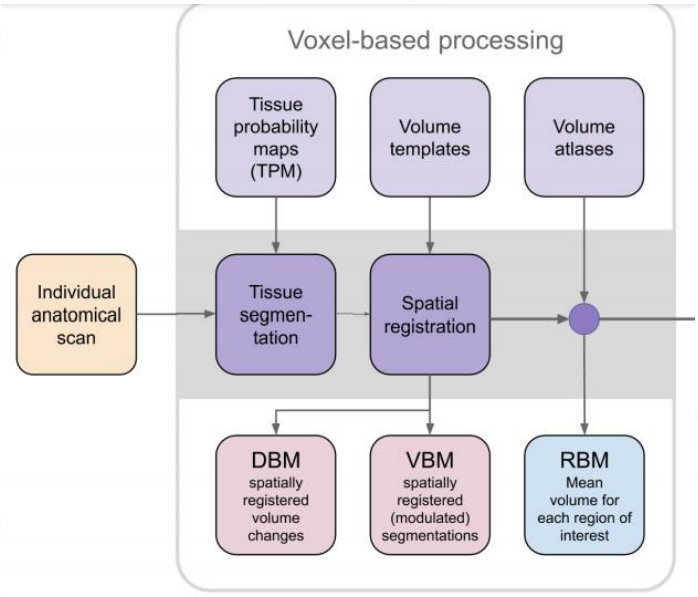


Figure 22: Flowchart of voxel-based processing in CAT toolbox (Source: CAT Manual)

The 'initial voxel-based processing' begins with a spatial adaptive non-local means (SANLM)denoising filter (Manjón et al., 2010), which is followed by internal resampling to properly accommodate low-resolution images and anisotropic spatial resolutions. The data are then bias-corrected and affine-registered (to further improve the outcomes of the following steps)

followed by the standard SPM "unified segmentation" (Ashburner and Friston, 2005). The outcomes of the latter step will provide the starting estimates for the subsequent refined voxel-based processing.

The 'refined voxel-based processing' uses the output from the unified segmentation and proceeds with skull-stripping of the brain. The brain is then parcellated into the left and right hemisphere, subcortical areas, and the cerebellum. Furthermore, local white matter hyperintensities are detected (to be later accounted for during the spatial normalization and cortical thickness estimation). Subsequently, a local intensity transformation of all tissue classes is performed, which is particularly helpful to reduce the effects of higher gray matter intensities in the motor cortex, basal ganglia, or occipital lobe before the final adaptive maximum a posteriori (AMAP) segmentation. This final AMAP segmentation step (Rajapakse et al., 1997), which does not rely on a priori information of the tissue probabilities, is then refined by applying a partial volume estimation (Tohka et al., 2004), which effectively estimates the fractional content for each tissue type per voxel. As a last default step, the tissue segments are spatially normalized to a common reference space using DARTEL (Ashburner, 2007) or Geodesic Shooting (Ashburner and Friston,2011) registrations.

3.6 DATA ANALYSIS AND STATISTICS:

To interpret whether the network parameters are statistically significant across the groups, correlation matrices are developed for the selected regions. For each region, seven graph measures were computed through the CONN18.0 toolbox, among them I choose 4 prime graph features at each ROI, separately for each diagnostic group. To infer the data with multiple variables, I used to work with IBM-SPSS tool in which ANOVA test and correlations are done in the following steps:

Storing data into Excel file:

Since we have three datasets to study, which are; selected graph measures, neuropsychology tests measures (MMSE, ACE-T, RAVLT-T, Delayed RAVLT) and grey- matter volume(gVol) of the selected ROIs. To work in IBM-SPSS, all the datasets must be arranged and stored accordingly in excel sheet.

Segregating and compiling of the dataset:

Then, compile all the required datasets for all the three diagnostic groups(NC=0, MCI=1, AD=2) into 1 excel sheet to make the work efficient. Once it is done, upload the excel sheet into IBM-SPSS for further analysis. Also store each dataset of different test separately in a file with appropriate name.

Analysing Datasets:

To work upon univariate, bivariate and multivariate data analysis, there are certain assumptions need to be considered and for that we have processed our graph theory and volume datasets.It concluded that graph theory parameters are not normally distributed and are multicollinearity in nature i.e. some of the graph measures are closely related such as global efficiency, degree, cost, and Average path length are related among each other while cluster coefficient and local efficiency are related to each other (based on our observation).

One way ANOVA test:

This is done to analyse the significant difference between the groups in respect of each study. This is why I performed one-way ANOVA test with posthoc-Bonferroni correctionseparately for graph theory measures, neuropsychiatric tests measures and total absolute grey matter volume. Record the mean value and the standard deviation of each measure, also comparative between-group study done. When the p-value is <0.05 then it is considered as significant difference between the groups(NC versus MCI, MCI versus AD and AD versus NC) with respect to that particular measure.

T-test:

When there is a study between two groups needed, t-test was performed using IBM-SPSS. This test is used to analyze whether functional connectivity(graph theory technique) shows any significant difference between the groups i.e. NC vs MCI, MCI vs AD, AD vs NC.

Correlation test:

For each correlation analysis, I examined if a higher connectivity was correlated with a better performance or reduction in grey- matter volume, as stated in the objectives. To perform the

Pearson-correlation, all the selected variables from the three analysis will be correlated using the SPSS tool. Correlation matrices are prepared for all the selected 22 ROIs in a way that each graph theory measure correlate with neuropsychiatric measure and grey matter volume separately. Also, the same matrix showed the correlation between grey-matter volume reduction and neuropsychiatric tests measures.

In this way, we can show the interaction between structural-functional, function-cognitive, and structural-cognitive changes occur in each diagnostic groups and how they differ from each other on the basis of these interaction.

In all the analysis, the significance threshold was set at $p<0.05$.

ROC curve for univariate analysis:

ROC (Receiver operating characteristic curve), to check the probability of the functional connectivity-graph theory technique in the classification of different pairs of diagnostic groups (i.e. NC vs MCI, MCI vs AD, AD vs NC). This is a graph between sensitivity (positive rate) and 1-specificity (negative rate).

TPR(True Positive Rate)= TP/TP+FN,

FNR(False Negative Rate)= FP/FP+TN

3.7 CLASSIFICATION MODEL:

After univariate and bivariate analysis, our approach to go for multivariate analysis and to check which combination of parameters classify different diagnostic groups with best accuracy (high specificity and high sensitivity). We decided to chose logistic regression, a statistical classification model, which uses logistic function (a functions that converts log-odds to probability) to model a binary dependent (having two values- '0' and '1' or '1' and '2') variable with multiple independent variables ('predictors'). For selection of our variables, the threshold marked was at $p<0.1$

However, our dependent variable has three categories (NC, MCI, and AD) and to model this options could be multinomial logistic regression or ordinal logistic regression but due to having ordered category NC<MCI<AD and multicollinearity we chose to stick around binary logistic regression.

Descriminant Function Analysis:

Due to earlier limitations, we did analysis with another model with the aim of statistic summary that helped to summarize features (parameters) from group of information which are non-parametric in nature. Once we get selected features, we extract the equations relevant to each model. By reverse test analysis with each equation, we checked the working of model in the classification of diagnostic groups. Then, checked its accuracy in terms of screening and diagnosis of AD from other groups using ROC curve.

We have also exported coordinate values in each ROC curve, to make it feasible for user to choose sensitivity and specificity as per their requirement.

CHAPTER 4- RESULTS

4.1 DEMOGRAPHIC AND CLINICAL FEATURES OF PATIENTS AND CONTROLS

The demographic and clinical characteristics of subjects are summarized in Table 4. The patients with MCI and AD did not differ in terms of age (p=0.446), sex (p=0.483), and education (p=0.456), also there is no significant difference among other pairs of diagnostic groups. The cognitively impaired patients (MCI and AD) were relatively older with more men than control group.

4.2 NEUROPSYCHOLOGICAL TEST PERFORMANCES FOR PATIENTS AND CONTROLS:

The neuropsychological battery administered at the baseline on all patient groups demonstrated findings that stratified both MCI and AD. Some of the patients were not cooperative for the full battery of test because of disease severity. The cognitive screening test data (MMSE and ACE) was not available in 11 out of 51 MCI patients and 13 out of 42 AD patients. The mean MMSE score of the MCI was 27.67 (21-30) whereas that of AD was 22.7 (14-30). The posthoc bonferroni one way ANOVA analysis showed that both patient groups were significantly impaired on ACE(T) ($p<0.001$) in comparison to controls, but MCI patients did not differ on MMSE score ($p=0.18$) as compared to NC.

The subgroups were significantly impaired in their confrontation naming compared to healthy volunteers ($P<0.001$). Comparisons of the RAVLT scores on episodic memory and verbal memory deficit revealed significant impact on the score of patient groups (MCI & AD) ($p<0.001$).

The decline in cognitive functioning in each diagnostic group could be easily depicted from each of the test scores (Figure 23)

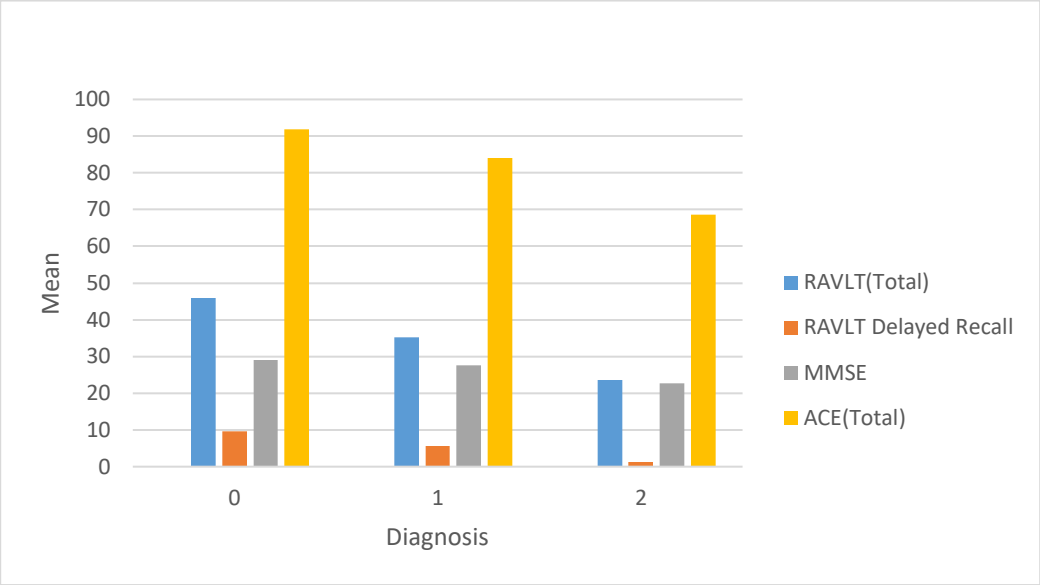


Figure 23: the variation of mean value of each cognitive tests from NC(0)>MCI(1)>AD(2)

4.3 GREY MATTER VOLUME FEATURE FOR PATIENTS AND CONTROLS:

From sMRI, gVol is extracted from each subject's scan. To compare reduction of volume of patients as compared to control, the mean value of absolute grey matter volume of AD patients is clearly lower than the mean value of MCI and normal, while there is slight decline among MCI patients as compared to NC. That's why there is no significant difference between the MCI and NC (p=0.82) in ANOVA test as summarized in Table 4.

Table 4: showing mean values and group difference of demographic variables, neuropsychological performance and grey matter volume among the three groups.

Characteristic	HC(n=23)	MCI(n=34)	AD(n=29)	Corrected P value(ANOVA)		
				MCI versus HC	AD versus HC	AD versus MCI
Sex (M/F)	12/11	23/11	18/11	.247	.483	.65
Age (mean ± SD in years)	68.04 ± 6.66	68.06 ± 4.13	69.24 ± 4.6	.991	.446	.285
Years of formal education(mean ± SD)	14.4 ± 5.0	13.4 ± 3.5	13.2 ± 3.4	0.487	.456	.877
MMSE	29.04 ± 1.94	27.67 ± 2.17	22.7 ± 4.2	.018	<.001	<.001
ACE(TOTAL	91.87± 7.4	84 ± 9.6	68.6 ± 13.9	<.001	<.001	<.001
RAVLT(TOTAL)	46 ± 11.32	35.24 ± 10.3	23.59 ± 7.24	<.001	<.001	<.001
RAVLT 20 min Delayed	9.6 ± 3.4	5.65 ± 4.1	1.28 ± 1.6	<.001	<.001	<.001
Absolute Grey matter volume	556.43 ± 37.85	553.9 ± 42.68	511.79 ± 42.0	.82	<.001	<.001

Post-hoc Bonferroni tests showed no significant group difference among all the three groups in demographic parameters of sex, age and years of education, while there is significant impact seen in neuropsychological parameters and absolute grey matter.

Also, when we checked between-group difference at each ROI's grey matter volume (gVol). We realized that in none of the region there is significant difference between NC and MCI. Also, in the regions of FO(R), SFG(L), SFG(R), SMG(L), SMG(R) have no significant difference between NC and AD. (detailed result)

4.4 GRAPH THEORY MEASURES FOR PATIENTS AND CONTROLS:

Since there are seven graphical features achieved from the inbuilt atlas-based method of graph analysis in CONN18.0 toolbox. Mean value of each feature at 22 ROIs calculated in all three diagnostic groups. Among all, degree shows maximum variation in each group (Table 6), however the values related to each measure are quite close and difficult to interpret on the basis of mean value study (Table 5). So we chose to see the difference from the T-score generated during processing in CONN toolbox on the basis of connectivity matrix. The more the connectivity of a node with other nodes, more will be the T-value.

Table 5: showing cluster coefficient mean values at each ROI and also mean group-difference poshoc Bonferroni significant values.

ROIs	NC	MCI	AD
AInsula (R)	0.66 ±0.04	0.68 ± 0.054	0.66 ±0.078
(L)	0.67± 0.057	0.67 ± 0.066	0.67± 0.085
SMG(R)	0.67 ± 0.056	0.68± .0.05	0.68± 0.067
(L)	0.67± 0.066	0.67± 0.055	0.66± 0.064
PostCingGy	0.68± 0.064	0.68± 0.065	0.68± 0.072
Amygdala(R)	0.65± 0.058	0.65± 0.067	0.65± 0.063
(L)	0.65± 0.059	0.65± 0.07	0.64± 0.76
Hippo(R)	0.65± 0.056	0.66± 0.058	0.65± 0.083
(L)	0.65± 0.053	0.65± 0.077	0.64± 0.082
ParOp(R)	0.66± 0.052	0.67± 0.054	0.67± 0.065
(L)	0.66± 0.059	0.66± 0.062	0.67± 0.069
SupParLob(R)	0.67± 0.064	0.67± 0.058	0.68± 0.062
(L)	0.67± 0.051	0.66± 0.062	0.67± 0.058
FrOp(R)	o.66± 0.063	0.68± 0.05	0.65± 0.076
(L)	0.66± 0.069	0.66± 0.071	0.67± 0.071
SupFrGy(R)	0.66± 0.056	0.66± 0.06	0.66± 0.07

(L)	0.66± 0.054	0.68± 0.067	0.66± 0.072
Caudate(R)	0.65± 0.064	0.67± 0.064	0.66± 0.064
(L)	0.67± 0.07	0.65± 0.055	0.66± 0.065
MidFrGy(R)	0.67± 0.058	0.68± 0.06	0.66± 0.07
(L)	0.67± 0.059	0.68± 0.06	0.67± 0.06
Percuneous	0.66± 0.062	0.68± 0.068	0.67± 0.068

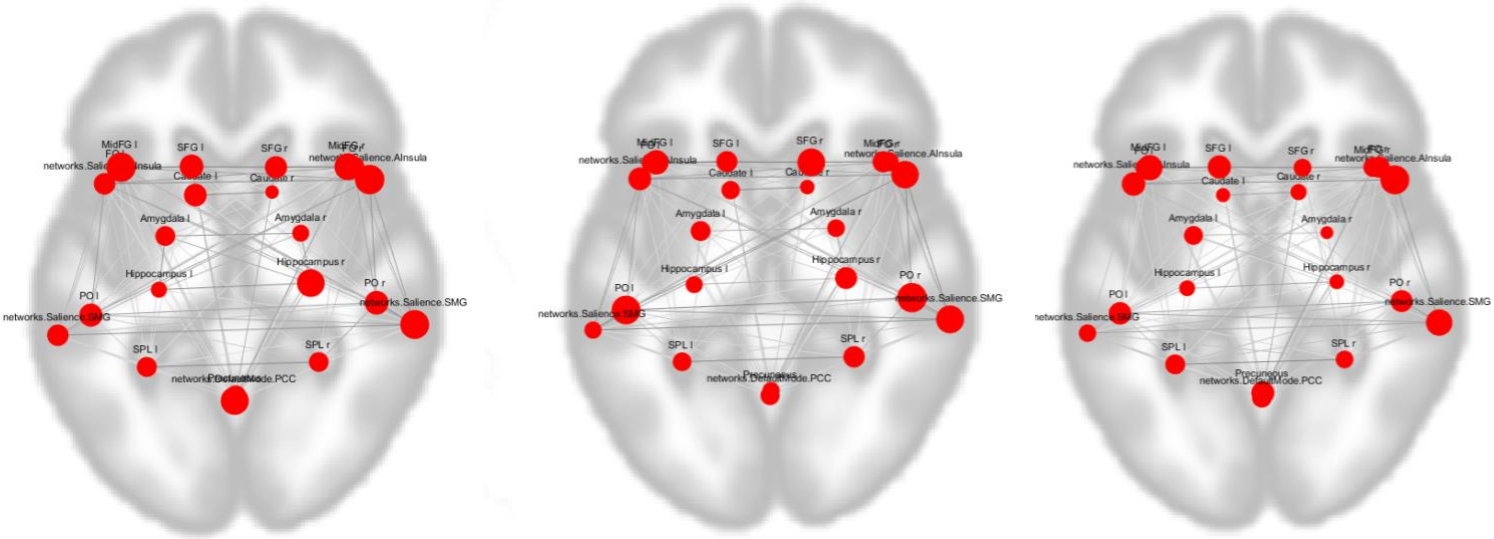
Table 6: mean value of Degree feature of functional connectivity at selected ROIs.

ROIs	NC	MCI	AD
AInsula (R)	46.52 ±5.96	47.38 ±7.32	44.27 ±7.33
(L)	42.22 ± 6.1	45 ± 7.79	42.86± 7.1
SMG(R)	44.7 ± 6.2	48.3 ± 6.46	45 ± 6.35
(L)	45.26± 6.35	45.85± 5.8	44± 6.9
PostCingGy	46± 5.34	43.2 ± 9.25	45.4± 6.5
Amygdala(R)	32.7± 7.08	36.03± 8.46	33.93± 10.84
(L)	37.6± 8.13	32.74± 8.9	34.86± 8.68
Hippo(R)	40.74± 7.6	36.38± 7.6	36.38± 8.2
(L)	37.39± 7.83	35.76± 7.6	37.1± 8.14
ParOp(R)	44.04± 6.65	44.12± 7.77	43.4± 7.12
(L)	42.17± 10.7	44.6± 6.95	42.55± 7.5
SupParLob(R)	42.65± 7.8	44.38± 7.68	46.24± 5.86
(L)	43.65± 6.6	44.9± 6.38	45.79± 5.9
FrOp(R)	41.6± 9.04	42.82± 7.68	42.17± 7.78
(L)	40.78± 9.64	40.35± 10.32	40.03± 8.4
SupFrGy(R)	46.3± 8.06	45.2± 9.56	47± 9.97
(L)	47.6± 8.27	46.4± 7.53	47.76± 7.78

Caudate(R)	35.1± 7.0	38.3± 10.23	39.2± 9.17
(L)	39.6± 9.03	38.47± 9.06	40.86± 9.44
MidFrGy(R)	46.65± 7.14	47.03± 7.05	46.93± 8.6
(L)	48± 5.63	47.9± 61.9	48.5±7.5
Percuneous	46.39± 5.23	43.47± 8.22	45.9± 6.6

To have an idea about global and local connectivity in each ROI within a group, we can compare T-value, thresholded at $p<0.05$ with FDR corrected and both sided. We took 3 variables for this purpose: Degree, BC, and CC.

Betweenness Centrality: It shows the role of each node in close connectivity or it's contribution in shortest path-length connectivity. Among AD, we have higher centrality in most of the regions, while we cannot ignore the regions which shows higher centrality in MCI and NC. In the Table, highlighted value shows the highest in each ROI.

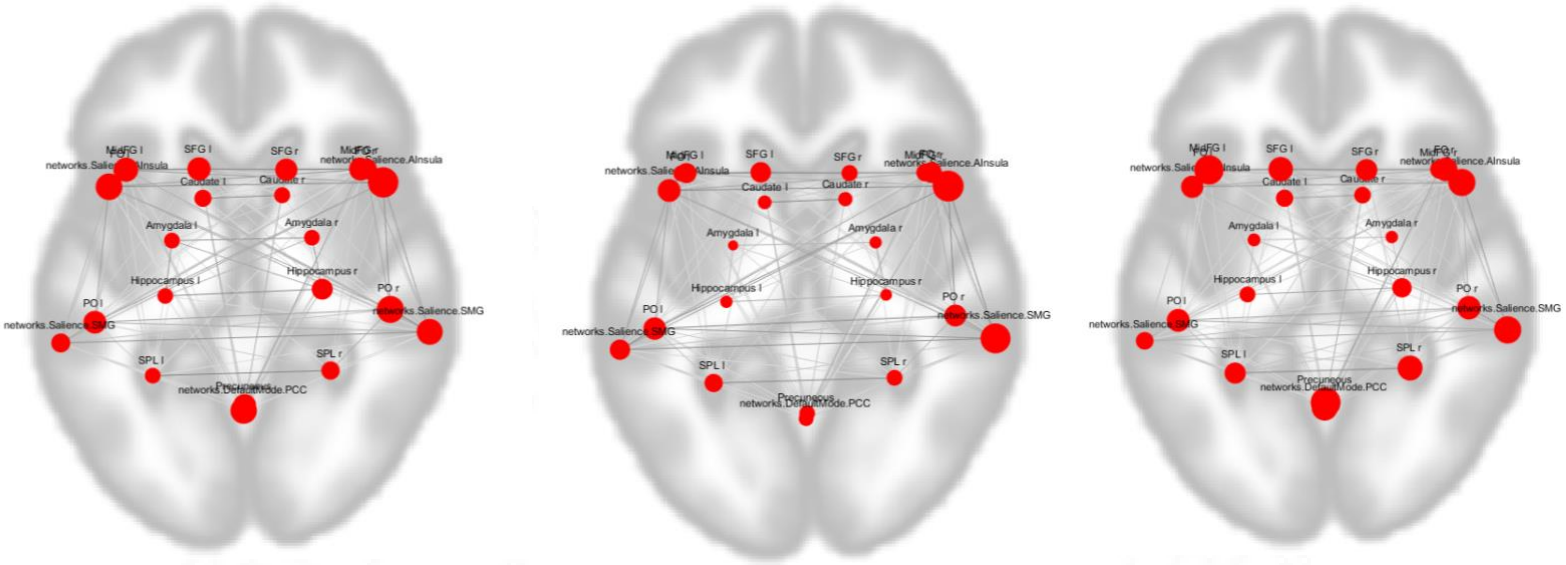


a)NC b)MCI c) AD

Table 7: beta and T-value of BC in each diagnostic group

ROI	NC		MCI		AD	
	Beta	T-value	Beta	T-value	Beta	T-value
AInsula(L)	0.04	4.90	0.05	5.84	0.05	6.64
AInsula(R)	0.06	6.69	0.07	7.02	0.07	8.16
SMG(L)	0.04	4.88	0.06	4.41	0.05	4.93
SMG(R)	0.06	6.58	0.05	7.12	0.04	7.44
PCC	0.03	6.38	0.03	4.89	0.03	5.59
Hippo(R)	0.06	6.31	0.05	5.63	0.04	4.17
Hippo(L)	0.05	3.66	0.04	4.35	0.03	4.47
Amy(R)	0.04	3.87	0.04	4.53	0.02	3.63
Amy(L)	0.05	4.51	0.02	5.08	0.03	5.31
SPL(R)	0.03	4.49	0.04	5.47	0.04	4.98
SPL(L)	0.03	4.56	0.04	4.80	0.04	5.61
SFG(R)	0.05	5.06	0.05	7.07	0.06	5.03
SFG(L)	0.04	5.42	0.06	5.39	0.05	6.54
PO(R)	0.05	5.40	0.03	7.50	0.05	6.19
PO(L)	0.04	5.26	0.06	7.35	0.04	6.34
FO(R)	0.04	4.78	0.05	4.89	0.04	6.01
FO(L)	0.04	2.84	0.03	5.07	0.04	5.46
Caudate(R)	0.03	3.07	0.04	3.71	0.03	4.56
Caudate(L)	0.04	5.18	0.02	4.70	0.03	3.99
MFG(R)	0.05	6.10	0.05	5.44	0.06	5.60
MFG(L)	0.05	6.68	0.05	6.25	0.04	7.16
Precuneous	0.03	4.77	0.03	4.39	0.03	6.49

Degree: It indicates the total number of edges that particular node have, it can be short-range or long-range. Here we have higher degree both in MCI and AD as compared to NC, we can say due to structural atrophy, they started forming more network or have larger radius of connectivity



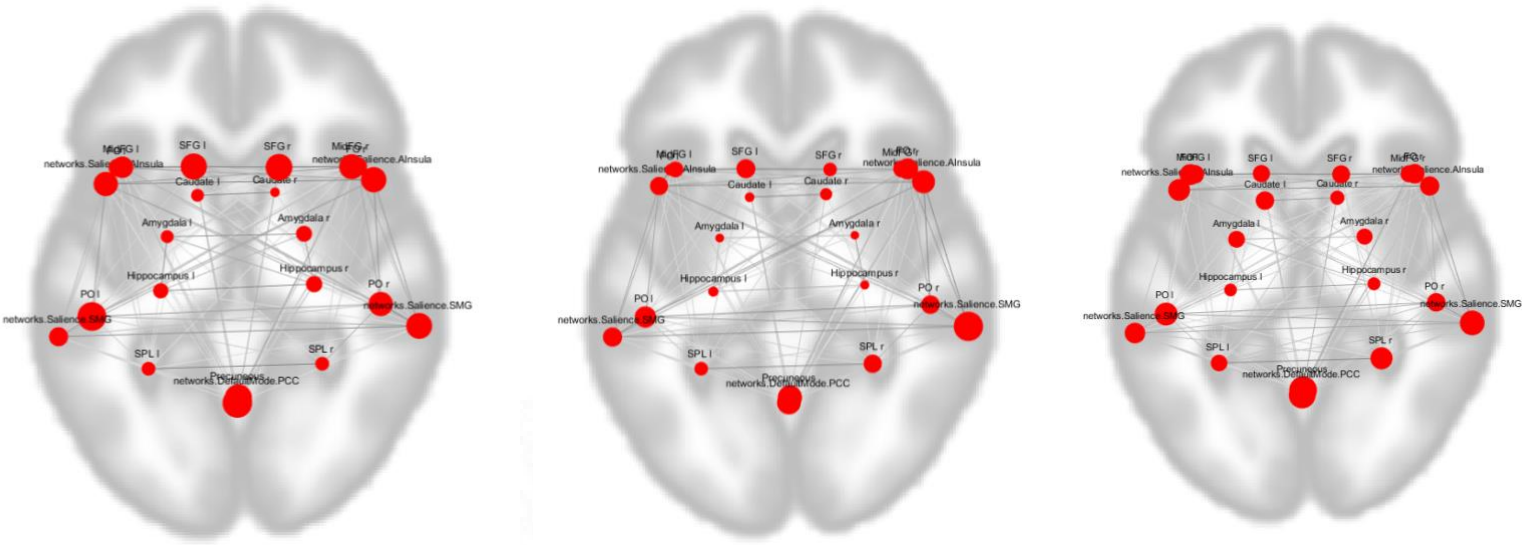
a)NC b)MCI c) AD

Table 8: beta and T-value of Degree in each diagnostic group

ROI	NC		MCI		AD	
	Beta	T-value	Beta	T-value	Beta	T-value
AInsula(L)	7.78	15.86	8.53	18.42	8.00	14.48
AInsula(R)	9.09	18.08	9.71	25.00	8.86	17.62
SMG(L)	7.65	11.32	8.32	16.44	7.21	11.47
SMG(R)	8.83	15.44	9.29	24.23	8.38	17.76
PCC	7.00	15.75	7.15	12.03	7.76	16.99
Hippo(R)	6.30	12.49	4.76	9.44	5.45	12.63
Hippo(L)	5.26	9.34	4.29	10.00	5.21	10.32

Amy(R)	4.96	9.33	4.44	9.93	4.59	8.06
Amy(L)	5.78	9.39	3.88	8.23	5.48	8.38
SPL(R)	5.52	10.90	6.50	12.86	6.93	16.24
SPL(L)	5.57	9.39	5.91	14.81	6.86	13.78
SFG(R)	7.17	13.21	6.97	13.07	7.69	14.08
SFG(L)	7.17	13.87	7.74	16.57	7.79	15.91
PO(R)	7.87	16.12	7.88	17.66	7.52	15.33
PO(L)	7.70	13.56	8.29	18.20	7.03	14.90
FO(R)	7.57	12.23	7.76	16.85	7.48	15.34
FO(L)	6.39	9.29	6.32	13.10	6.28	12.65
Caudate(R)	4.04	9.54	5.24	11.56	5.24	10.86
Caudate(L)	4.87	10.23	4.97	11.15	5.72	11.26
MFG(R)	7.26	13.69	7.12	14.36	7.69	12.36
MFG(L)	7.57	14.40	7.53	16.26	7.52	18.99
Precuneous	6.83	13.61	6.91	12.65	7.45	19.44

Cluster Coefficiency: This describes how stronger is the connectivity of neighbor's nodes among each other and with the selected node, thus more stronger connectivity leads to formation of clusters around each node. Among MCI, we found higher clusters in most of the regions.



a)NC b)MCI c) AD

Table 9: beta and T-value of CC in each diagnostic group

ROI	NC		MCI		AD	
	Beta	T-value	Beta	T-value	Beta	T-value
AInsula(L)	0.60	16.51	0.57	17.55	0.55	15.93
AInsula(R)	0.55	17.20	0.58	22.18	0.51	14.08
SMG(L)	0.59	12.96	0.62	18.58	0.53	15.04
SMG(R)	0.54	17.57	0.61	28.31	0.54	18.06
PCC	0.63	20.16	0.67	23.03	0.61	19.69
Hippo(R)	0.48	11.06	0.42	8.70	0.46	9.51
Hippo(L)	0.63	10.38	0.53	9.84	0.50	9.28
Amy(R)	0.65	10.85	0.45	8.22	0.58	11.79
Amy(L)	0.51	8.77	0.48	8.44	0.51	12.26
SPL(R)	0.58	9.36	0.56	17.60	0.56	16.17
SPL(L)	0.53	9.37	0.53	13.22	0.57	12.15
SFG(R)	0.52	18.16	0.49	13.08	0.50	13.16
SFG(L)	0.54	17.78	0.57	18.34	0.47	12.57
PO(R)	0.57	16.51	0.63	17.85	0.49	13.53
PO(L)	0.62	19.55	0.57	20.61	0.53	16.75

FO(R)	0.56	12.86	0.67	20.27	0.60	14.32
FO(L)	0.68	12.21	0.56	11.42	0.55	15.30
Caudate(R)	0.52	6.51	0.51	11.72	0.52	10.44
Caudate(L)	0.40	8.71	0.56	9.32	0.57	13.55
MFG(R)	0.50	16.99	0.56	16.48	0.43	12.52
MFG(L)	0.56	14.68	0.54	15.22	0.49	13.70
Precuneous	0.64	18.50	0.69	23.40	0.62	20.33

4.5 CORRELATION OF THE TESTS MEASURES IN EACH DIAGNOSTIC GROUP:

Pearson correlation study was done between the set of different features in each group using IBM-SPSS, having the threshold value of $p<0.05$. The significant regions are recorded in each group and then plotted only those having ($r >0.3$), but for better comparison among the groups- if any of the group have matched threshold value for significance and correlation coefficient in any region, then other corresponding groups in the same region have also plotted along with it.

Output of correlation values at each group

Among all the correlation, we have plotted the significant correlations in three classes (0=NC, 1=MCI, 2=AD):

- Graph theory variable with Neuropsychiatric test variable
- Graph theory variable with Grey-matter volume
- Grey-matter volume with Neuropsychiatric test variable

From the above, gVol is correlated with NPVs at most of the regions in AD and then in MCI subjects but not in NC. The correlation of gVol reduction with cognitive performance is more significant from NC<MCI<AD.

While the correlation of functional connectivity and cognitive performance is more significant in MCI as compared to NC and AD.

However, the correlation between gVol and functional connectivity was seen less in all the three groups i.e. only at few regions. Among all the found correlations, AD showed negative correlation was the highlight of this study.

Relation between Graph Theory variable and Neuropsychiatric test variable:

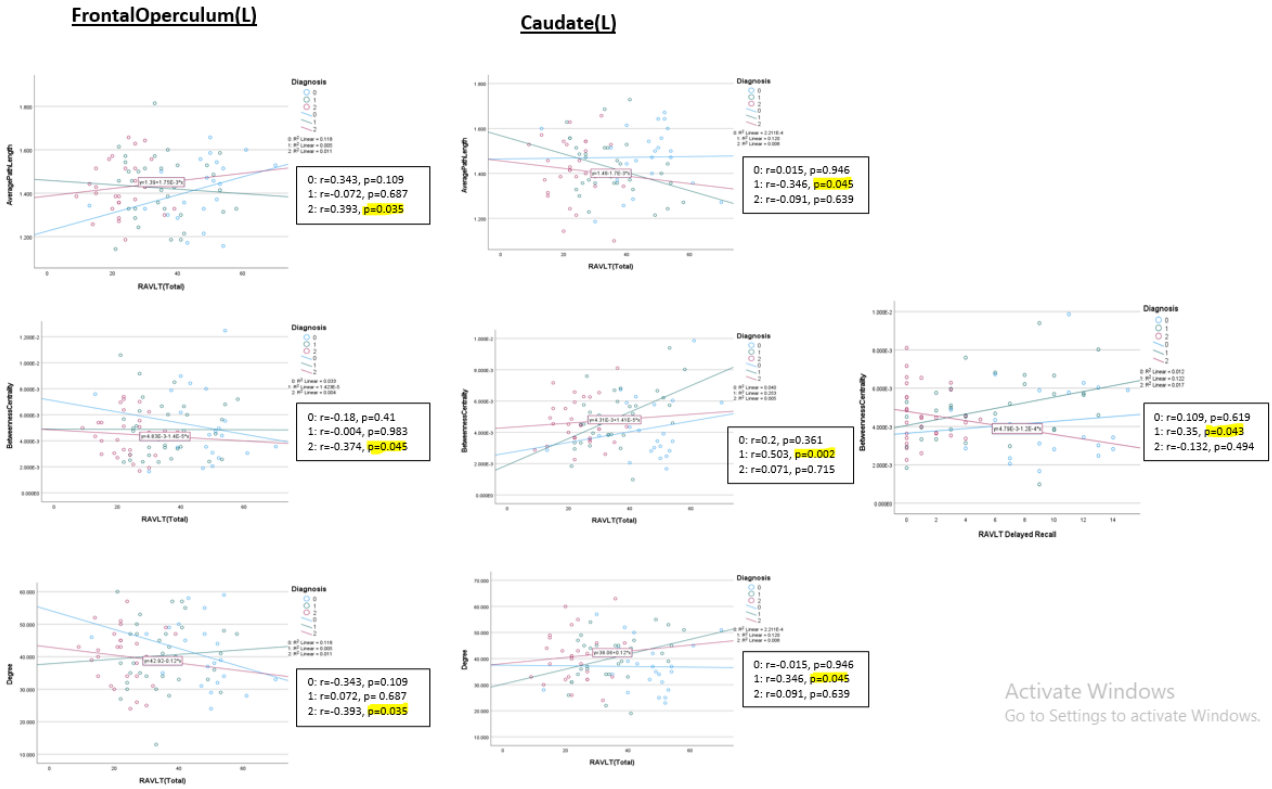


Figure 24: Scatterplot showing relation of NV(RAVLT total and delayed RAVLT) with GT(BC, AvPL, Degree) at FO(L) and Caudate(L) in three diagnostic groups. In FO(L), the classification between MCI and AD can be done on the basis of correlation opposite behavior in each case of AvPL & degree with RAVLT total; which represents in AD patients less involvement of this region in connectivity raises the score of RAVLT while opposite is seen in MCI patients. On contrary, in caudate(L), the involvement of this region in connectivity increases the score of RAVLT and for classification between MCI & AD, relation between BC and RAVLT delayed score can be used.

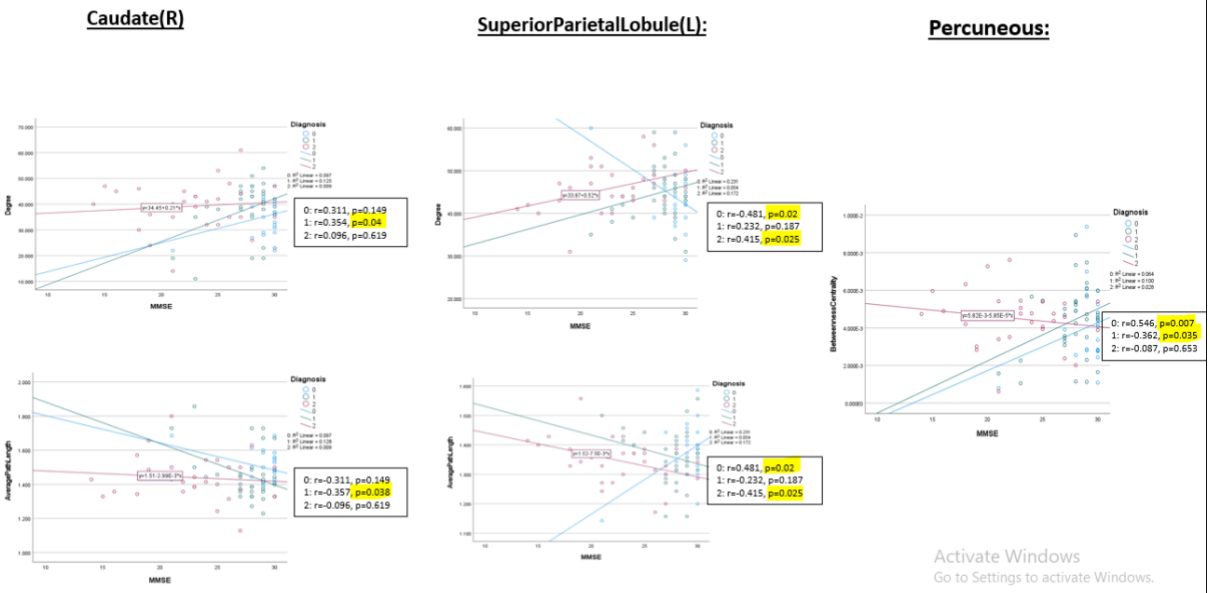


Figure 25: Scatterplot showing relation between MMSE with GT(BC, AvPL, Degree) at Caudate(R), SPL(L), Precuneous in three diagnostic groups. In Caudate(R), the scoring of MMSE increase with the involvement of this region in connectivity but the relation is seen more stronger in MCI patients as compared to normal and AD patients.

While in SPL(L), the positive relation is same but AD and normal patients shows significant result as compared to MCI patients.

However in Precuneous, MCI and AD shows reverse relation whereas MCI is having significant value.

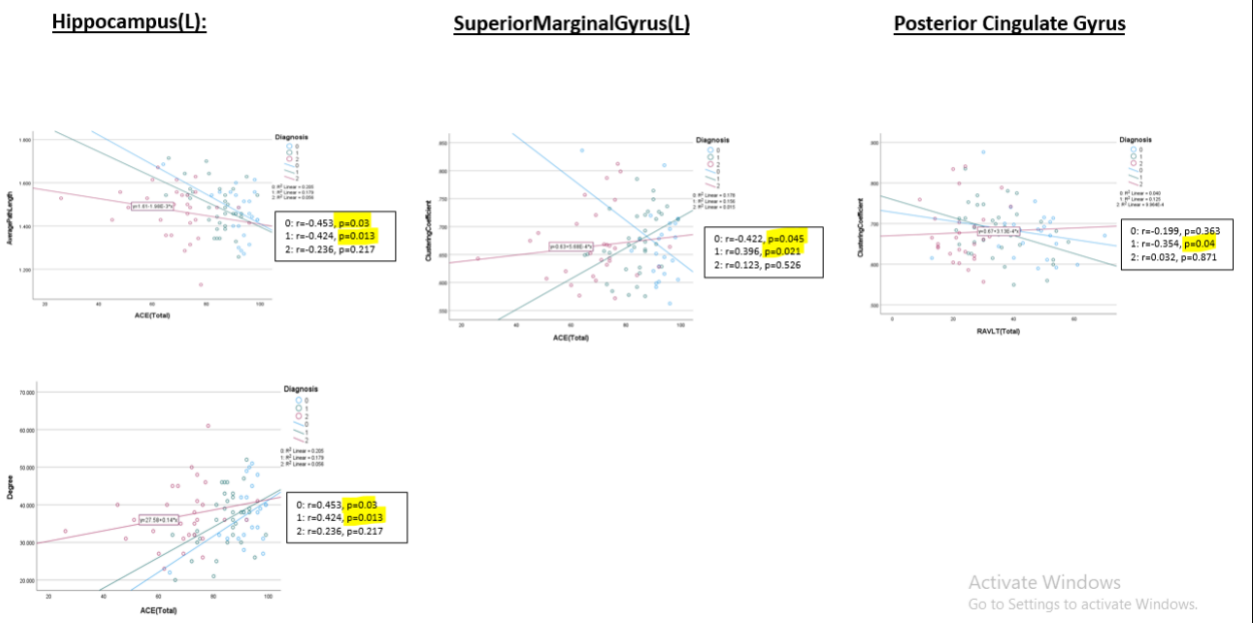


Figure 26: Scatterplot between NV(ACE_total, RAVLT_total) and GT (AvPL, Degree & CC). In Hippo(L), positive relation is seen for all the three diagnostic group but not significant in AD patients. While in SMG(L), classification between NC and MCI can be done since both are showing strong relation but opposite, which means in normal the involvement of SMG(L) is not much in cognitive functioning but this involvement get reverse in MCI patients.

PCG can also be another region to classify AD and MCI patients, since both are showing opposite relation and no significant relation seen in AD patients.

Relation between GT variable and Grey-matter volume atrophy:

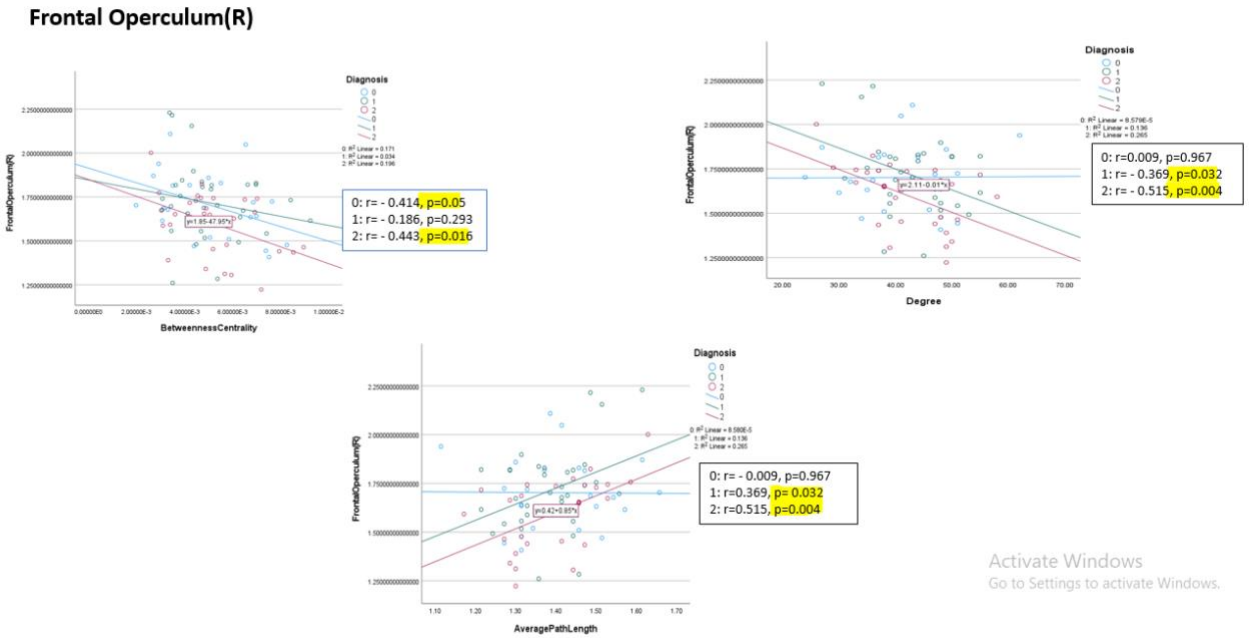


Figure 27: Scatterplot of relation between grey matter volume decay and GT connectivity (BC, AvPL and degree) at FO(L) in three diagnostic groups. It can be seen that more is the decay in the volumeof specific region, more is the connectivity be there. Also, with BC parameter, can classify MCI and AD.

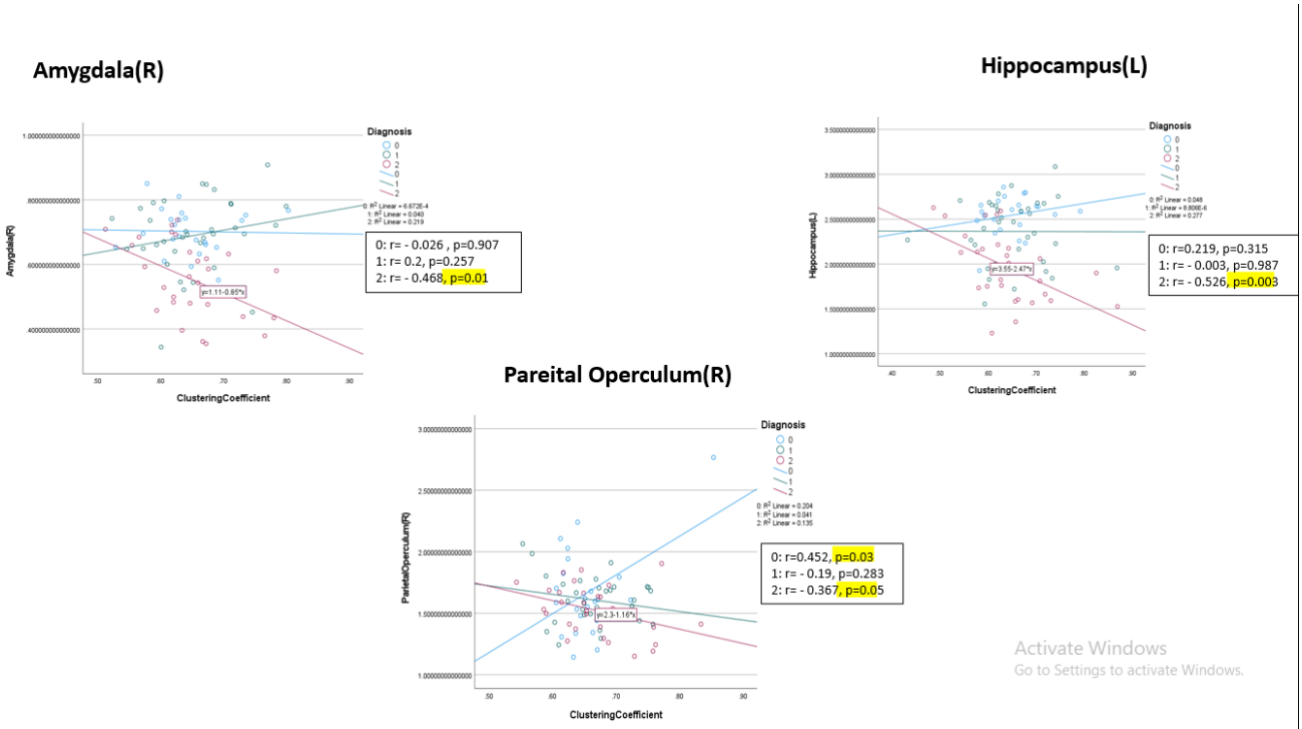
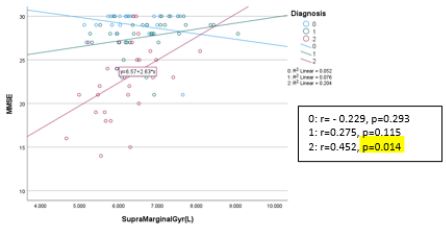


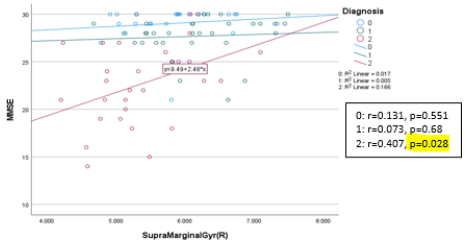
Figure 28: Scatterplot showing relation between cluster coefficient and gVol at i) Amygdala(R), where we can have good classification between MCI and AD, showing opposite relation, MCI patients decrease connectivity with the atrophy of this region. ii) PO(R), here patients and normal can be classified but not MCI and AD. iii) Hippo(L), AD patients showing significant negative relation which means role of this region increases with the decay.

Relation between gVol with NV:

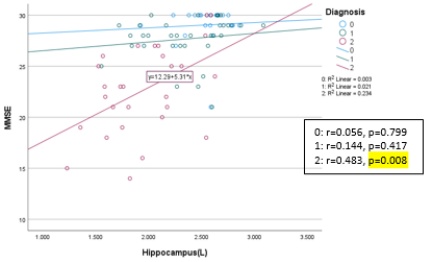
SupMarGy(L)



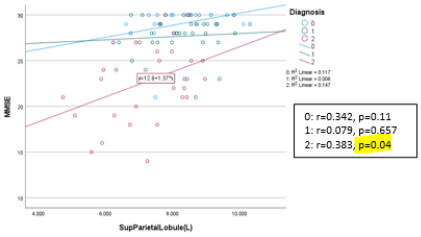
SupMarGy(R)



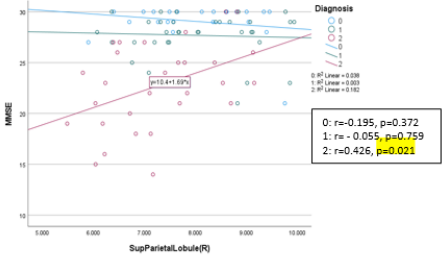
Hippocampus(L):



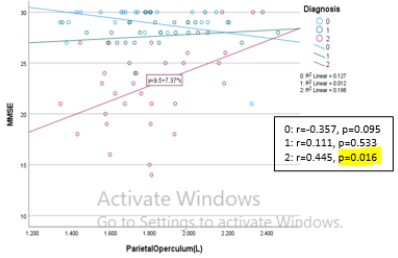
SupParLobule(L):



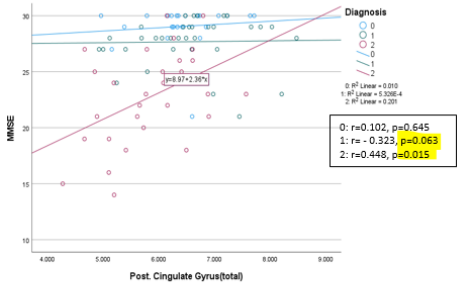
SupParLobule(R):



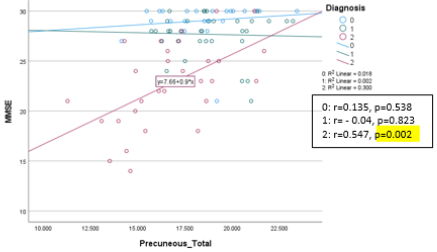
ParOper(L):



PostCingGyrus



Precuneous



Caudate(R)

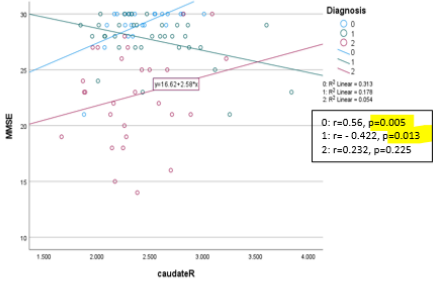


Figure 29: Scatterplot showing relation between MMSE score and gVol or relation between structural atrophy and cognitive functioning. In all the regions above, there is good correlation shown by AD patients and not by NC and MCI except the caudate(R) in which NC and MCI shows correlation with MMSE score but not AD patients and relation is also opposite which is good for classification between MCI and AD. Other regions such as SPL(R), PCG, Precuneous can be chosen for getting classification between MCI and AD, since they are showing opposite relation in these regions.

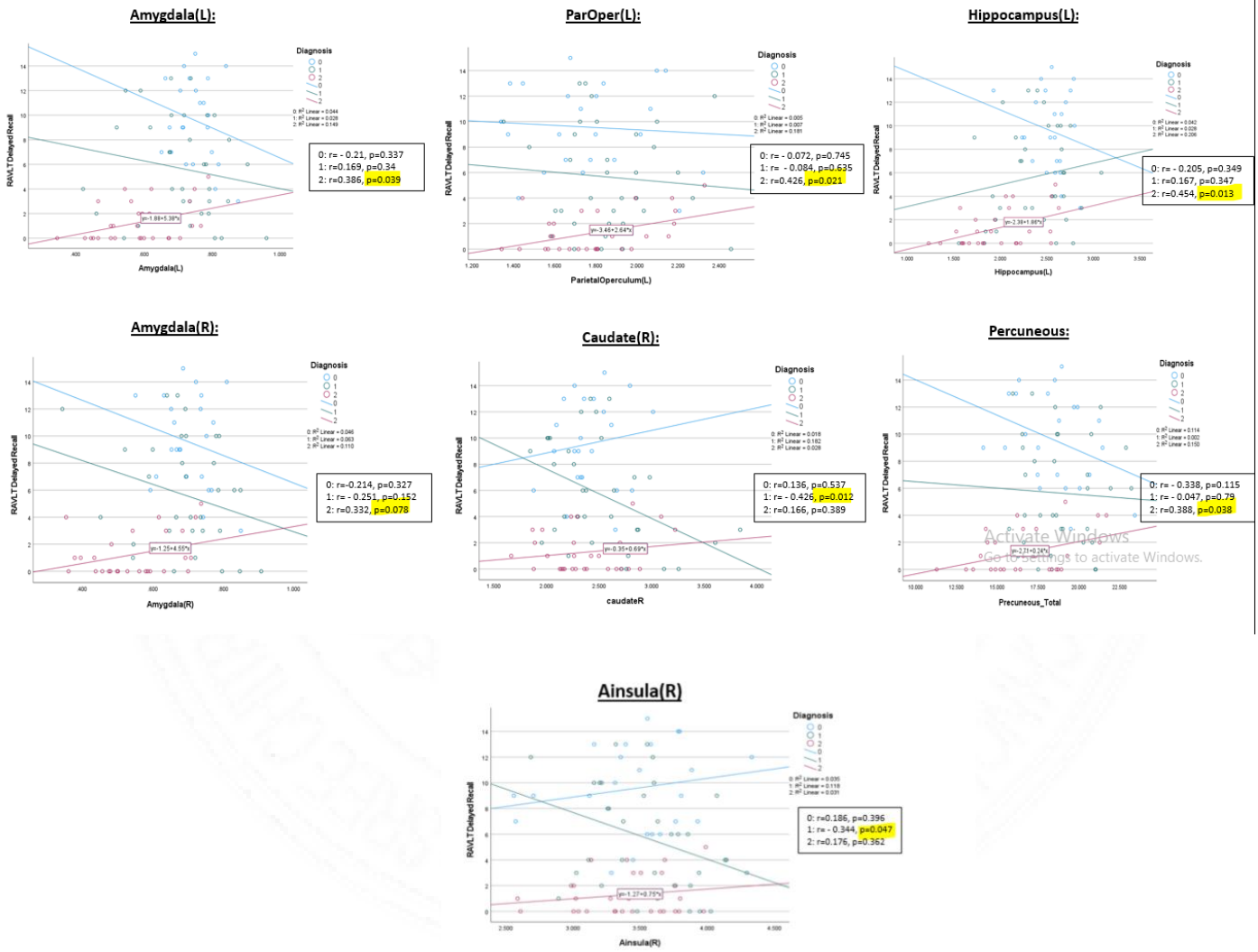


Figure 30: Scatterplot showing relation between RAVLT decay score and gVol. In the above regions, only caudate(R) and Ainsula(R) are showing significant relation in MCI but not in AD

patients and Normal. Also, all these regions showing opposite correlation value between MCI and AD patients except Amygdala(L) and Hippo(L).

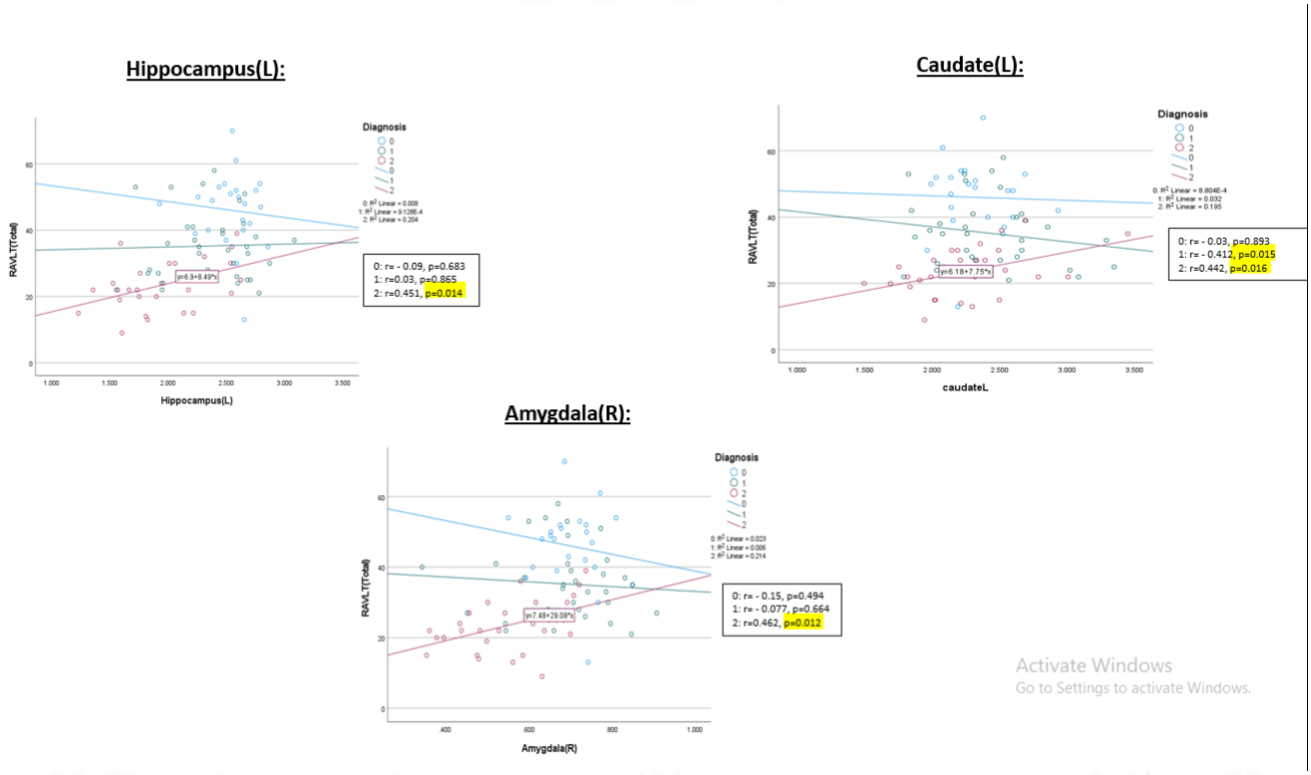


Figure 31: Scatterplot showing relation between RAVLT(total) score and gVol. AD patients showing significant relation in all the three regions but MCI shows only in caudate(L). Also, there is opposite relation between MCI and AD can be seen in caudate(L) and Amygdala(R). So perfect region for the classification between MCI and AD on the basis of these two parameters is Amygdala(R) only.

Note:

- No relation is seen between ACE(total) and gVol.
- MCI shows significant relations in caudate(R) and caudate(L) in the context of gVol with NV and GT with NV.

- When we did ROC test, gVol parameter shows max classification between NC and AD. And here also AD patients shows max correlation with NV.

Our assumptions:

- caudate can be a potential ROI for the classification between MCI and AD.
- To classify NC and AD, gVol could be a good choice.
- While to classify NC and MCI, GT parameters could be one.

4.6 BIVARIATE ANALYSIS:

For Bivariate analysis or between-group analysis, we went through set of tests. First, we performed ANOVA test and got the result in which graph theory feature i.e. Degree of SMG(R), Amygdala(L), Hippo(R) showed significant difference in control and MCI groups. While also seen that the p-value from ANOVA test was same for the graph features of degree= global efficiency= cost= average path length while cluster coefficient was found equal to local efficiency, this explains the multicollinearity among the variables. On the contrary, global parameter viz 'betweenness centrality' showed significant connectivity difference for the classification in the brain regions of PCC, Amy(L), Amy(R), Hippo(R), SFG(L) and Precuneus.

Thus, irrespective to other regions; Amy(L) and Hippo(R) could be use to classify MCI patients from NC but none of the parameter showed significant difference with AD patients in these regions with the value of ($p=0.25$), and ($p=0.054$) respectively.

Since we rarely achieve significance through ANOVA test due to the limitations from our dataset, we tried to check the significance with T-test (here, we did not get any parameter for comparison of NC and AD), and ROC curve (Table 10). Also, we know that the datasets we have is non-parametric (i.e not normally distributed), thus relying on the parameters which give significant classification through ROC curve was considered. During ROC-curve, we have also included gVol to see between group difference, however results are quite different from posthoc-bonferroni we did earlier during univariate analysis.

The graph theory parameter shows difference (threshold at $p<0.05$) only in AInsula(R), SMG(R), Hippocampus(R), Amydala(L), SPL(R), Caudate(R) while with gVol we found difference in more

regions. And, the regions where both the imaging modalities shows significant difference between the groups are SMG(R), Hippocampus(R), SPL(R).

Table 10: Showing variables(of structural and functional connectivity separately) having significant value through T-test and ROC curve in different combination of different diagnostic groups at selected ROIs *Detail results of T-test and ROC curve for all the three diagnostic groups.*

			T-tests(p<0.05) NC vs MCI	T-tests(p<0.05) AD vs MCI	T-tests(p<0.05) AD vs NC	ROC curve(p<0.05) NC vs MCI	ROC curve(p<0.05) AD vs MCI	ROC curve(p<0.05) AD vs NC
No alphabet code	Network							
A	networks.Salience.AInsula (L) (-44;13;1)							
B	networks.Salience.AInsula (R) (47;14;0)						GE, AvPL, degree, cost	BC
C	networks.Salience.SMG (L) (-60;-39;31)						gVol	
D	networks.Salience.SMG (R) (62;-35;32)		GE, cost, AvPL, degree	GE, AvPL, degree, cost		GE, AvPL, degree, cost	GE, AvPL, degree, cost, gVol	gVol
E	networks.DefaultMode.PCC (1;-61;38)						gVol	gVol
F	atlas.Hippocampus r		GE, cost, AvPL, degree			GE, AvPL, degree, cost, BC	gVol	GE, AvPL, cost, degree, gVol
G	atlas.Hippocampus l						gVol	
H	atlas.Amygdala r					BC	gVol	gVol
I	atlas.Amygdala l		GE, BC, cost, AvPL, degree			GE, AvPL, degree, cost, BC	gVol	
J	atlas.SPL r (Superior Parietal Lobule Right)						gVol	GE, AvPL, cost, degree, gVol
K	atlas.SPL l (Superior Parietal Lobule Left)						gVol	gVol
L	atlas.SFG r (Superior Frontal Gyrus Right)						gVol	gVol
M	atlas.SFG l (Superior Frontal Gyrus Left)					BC	gVol	
N	atlas.PO r (Parietal Operculum Cortex Right)							
O	atlas.PO l (Parietal Operculum Cortex Left)							
P	atlas.FO r (Frontal Operculum Cortex Right)						gVol	

Q	atlas.FO l (Frontal Operculum Cortex Left)						gVol	gVol
R	atlas.Caudate r					GE, AvPL, degree, cost		GE, AvPL, cost, degree
S	atlas.Caudate l							
T	atlas.MidFG r (Middle Frontal Gyrus Right)						gVol	gVol
U	atlas.MidFG l (Middle Frontal Gyrus Left)						gVol	gVol
V	atlas.Precuneous (Precuneous Cortex)		BC			BC	gVol	gVol

ROI-ROI analysis result:

To check the connectivity between the groups, we have done ROI-ROI analysis with the threshold value at $p<0.05$ with FDR corrected and two-sided, then looked for the changes in the regions having significant t-result result.

1. **MCI and NC**

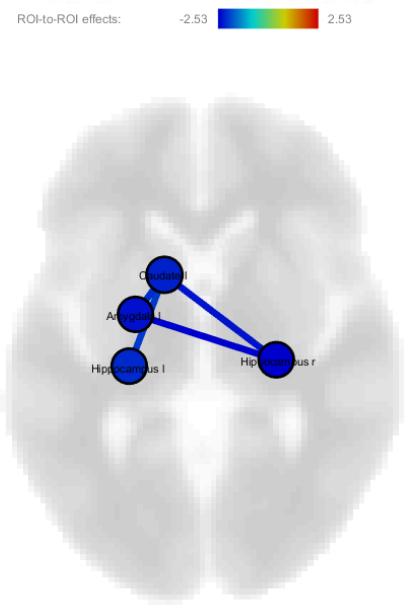


Figure 32: MCI is showing negative connectivity as compared to NC in the regions of Caudate(L), Amygdala(L), Hippo(R), Hippo(L)

In the last between-group study through ROC-curve, we also got significant difference in Hippo(R), and Amy(L) when we measured functional connectivity through graph theory.

Table 11: A comparative ROI-ROI study in MCI>NC. The T-score is negative for all ROIs.

Analysis Unit	Statistic	p-unc	p-FDR
Seed Caudate l	F(12)(44) = 2.00 Intensity = 6.50 Size = 3	0.0474	0.9786
Caudatel-Hippocampus r	T(55) = -2.34	0.0231	0.3274
Caudate l-Amygdala l	T(55) = -2.13	0.0379	0.3274
Caudatel-Hippocampus l	T(55) = -2.03	0.0468	0.3274
Seed Amygdala l	F(12)(44) = 1.55 Intensity = 4.66 Size = 2	0.1419	0.9786
Amygdalal-Hippocampus r	T(55) = -2.53	0.0142	0.2976
Amygdala l-Caudate l	T(55) = -2.13	0.0379	0.3976
Seed Hippocampus l	F(12)(44) = 1.22 Intensity = 2.03 Size = 1	0.2983	0.9786
Hippocampusl-Caudate l	T(55) = -2.03	0.0468	0.4041
Seed Hippocampus r	F(12)(44) = 1.11 Intensity = 4.87 Size = 2	0.3787	0.9786

Hippocampusr-Amygdala l	T(55) = -2.53	0.0142	0.2423
Hippocampusr-Caudate l	T(55) = -2.34	0.0231	0.2423

2. AD and MCI:

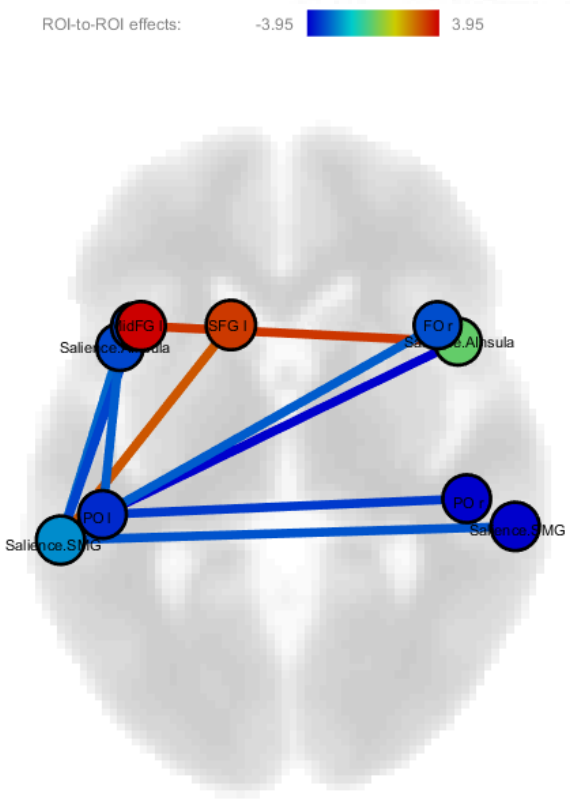


Figure 33: connectivity in AD>NC, red showed positive connectivity while blue showed negative

From graph theory connectivity analysis also, we got significant difference in regions of AInsula(R), and SMG(L). AD showed negative connectivity in most of the regions but positive connectivity in frontal cortex.

Table 12: comparative study in AD>MCI. AD has reduced connectivity in most of the node pairs except SMG(L) and SFG(L), AInsula(R) and MFG(L) showed positive connectivity in these pairs as compared to MCI.

Analysis Unit	Statistic	p-unc	p-FDR
Seed PO r	F(13)(49) = 2.08 Intensity = 3.18 Size = 1	0.0333	0.1379
PO r -PO l	T(61) = -3.18	0.0023	0.0479
Seed Salience.SMG	F(13)(49) = 2.07 Intensity = 14.15 Size = 5	0.0338	0.1379
Salience.SMG -FO l	T(61) = -3.06	0.0033	0.0368
Salience.SMG - Salience.SMG	T(61) = -2.85	0.0059	0.0368
Salience.SMG -SFG l	T(61) = 2.85	0.0060	0.0368
Salience.SMG -PO l	T(61) = -2.79	0.0070	0.0368
Salience.SMG -(1)	T(61) = -2.60	0.0118	0.0496
Seed Salience.AInsula	F(13)(49) = 1.85 Intensity = 7.19 Size = 2	0.0615	0.1705
(2)-PO l	T(61) = -3.95	0.0002	0.0043
(2)-MidFG l	T(61) = 3.24	0.0019	0.0203
Seed PO l	F(13)(49) = 1.80 Intensity = 15.40 Size = 5	0.0697	0.1705
PO l -(2)	T(61) = -3.95	0.0002	0.0043

PO l -PO r	T(61) = -3.18	0.0023	0.0240
PO l -Salience.SMG	T(61) = -2.79	0.0070	0.0344
PO l -(1)	T(61) = -2.74	0.0081	0.0344
PO l -FO r	T(61) = -2.73	0.0082	0.0344
Seed MidFG l	F(13)(49) = 1.60 Intensity = 3.24 Size = 1	0.1172	0.2270
MidFG l -(2)	T(61) = 3.24	0.0019	0.0407

3. **AD and NC:**

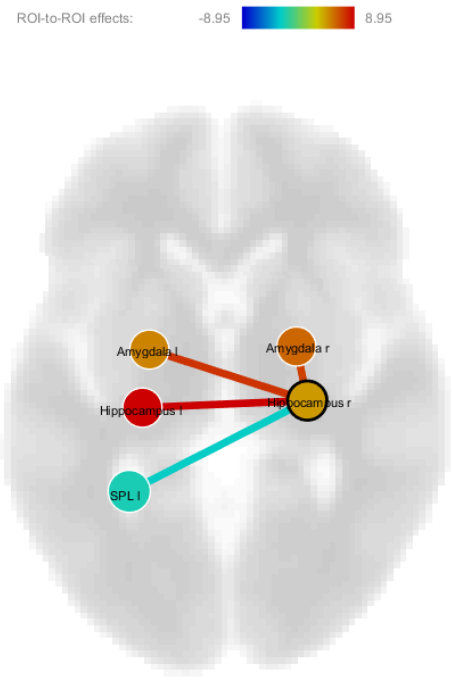


Figure 34: ROI-ROI connectivity in AD>NC

Also, graph theory gave us relevant significant between-group difference in Hippo(R). As an adapatation, AD showed positive connectivity as compared to control group.

Table 13: In AD the relevant ROI is Hippo(R) which shows positive connectivity with three regions but negative connectivity with SPL(L).

Analysis Unit	Statistic	p-unc	p-FDR
Seed Hippocampus r	F(11)(40) = 11.92 Intensity = 26.22 Size = 4	0.0000	0.0000
Hippocampus r-Hippocampus l	T(50) = 8.95	0.0000	0.0000
Hippocampus r-Amygdala l	T(50) = 7.46	0.0000	0.0000
Hippocampus r-Amygdala r	T(50) = 7.09	0.0000	0.0000
Hippocampus r-SPL l	T(50) = -2.72	0.0090	0.0470

4.7 CLASSIFICATION MODELS:

Binomial Logistic Regression:

First we binomial LR and check for those variable whose significance range meet at $p<0.1$, once we get the list of parameters, we did backward binomial LR for better filtration of parameters.

With binomial LR backward method, we tried to found the significant model ($p<0.05$) in each pair of diagnostic groups by excluding the non-significant variables step-wise, but not much significant models achieved. Only the classification model for AD vs MCI, we got parameters which also have significant impact on the model individually, those parameter are; gVol of Amygdala(R), Ainsula(R), SFG(R), SPL(R), MFG(R), and GE of SMG(R).

In other classification model for NC vs MCI, we got significant model but only parameter which has independent significance on the classification is gVol of SMG_L. While, there is significant parameter for the classification model of AD vs NC.

Also, with binomial LR, we got high beta constant value which is not statistically acceptable.

Discriminant Function Analysis:

To know better classification model, considering limitations from previous models, we chose to do analysis with this technique. After shortlisting parameters with significant impact on a model from step-wise method, we got two parameters which are Hippo(L), and SMG(R). Once we have two independent variables and a dependent variable having 3 categories. From the analysis, we got eigen value score- higher the score, better be the impact of the function on the model;

Box test score - here, we were looking for having non-significant value which indicates that we can reject the hypothesis of homogeneity in the groups thus existence of covariance satisfied.

Wilk's lambda score - gave us the chi-square value, also higher be its value better be the model working with parameters as compared to the null model (having no parameters).

Table 14: Group statistics from the descriptive analysis using Hippocampus(L) and SMG(R)

Group Statistics

Diagnosis		Mean	Std. Deviation	Valid N (listwise) Unweighted	Weighted
NC	Hippocampus(L)	2.54448617774	.212068914493	23	23.000
	SupraMarginalGyr(R)	5.83657811230	.683094567132	23	23.000
MCI	Hippocampus(L)	2.36345607876	.368002423716	34	34.000
	SupraMarginalGyr(R)	6.13616816241	.725230715211	34	34.000
AD	Hippocampus(L)	1.96555981217	.384846790367	29	29.000

	SupraMarginalGyr(R)	5.39019615414	.700429525426	29	29.000
Total	Hippocampus(L)	2.27769678278	.410423513400	86	86.000
	SupraMarginalGyr(R)	5.80449654157	.767736835223	86	86.000

Box test not significant (p=.135) – which indicates that assumption of homogeneity of observed covariance matrices was met

Variables Entered/Removed[a,b,c,d]

		Wilks' Lambda				Exact F			
Step	Entered	Statistic	df1	df2	df3	Statistic	df1	df2	Sig.
1	Hippocampus(L)	.671	1	2	83.000	20.360	2	83.000	.000
2	SupraMarginalGyr(R)	.601	2	2	83.000	11.893	4	164.000	.000

At each step, the variable that minimizes the overall Wilks' Lambda is entered.

a. Maximum number of steps is 4.

b. Minimum partial F to enter is 3.84.

c. Maximum partial F to remove is 2.71.

d. F level, tolerance, or VIN insufficient for further computation.

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	.533[a]	86.2	86.2	.590
2	.086[a]	13.8	100.0	.281

a. First 2 canonical discriminant functions were used in the analysis.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.601	42.025	4	.000

2	.921	6.770	1	.009

After the complete analysis, we also received discriminant functions which we used in forming discriminant equations, also describing the role of each independent variable in the equation defining dependent variable. Also, we got constants to complete our multivariate equation.

Canonical Discriminant Function Coefficients

	Function 1	2
Hippocampus(L)	2.569	-1.607
SupraMarginalGyr(R)	.450	1.389
(Constant)	-8.466	-4.404

Unstandardized coefficients

Two discriminant functions (two dimensions) were obtained

Discriminant function scores D1 and D2, obtained using Function 1 and Function 2

D1=(-8.466)+ [(2.569)* Hippocampus_L]+ [(0.450)*SupraMarginalGyr_R]

D2= (-4.404)+ [(-1.607* Hippocampus_L] + [(1.389)*SupraMarginalGyr_R]

While analysis, we extracted the values of D1 and D2 in respect of each patient.

Reverse-test analysis:

To check the accuracy of the classification by each equation of each diagnostic group, we first checked the distribution by ANOVA test and got significant difference among the patients

Note: D1 is better at discriminating between the three groups, than D2 – D1 accounts for 86.2% of the variance

D2 is mildly discriminates between the three groups, than D2 – D1 accounts for 13.8% of the variance. D2 is significantly higher in MCI than NC.

Table 15: ANOVA and post hoc Bonferroni with discriminant function scores

		N	Mean	Std. Deviation	ANOVA p value
Discriminant Scores from Function 1 for Analysis 1	NC	23	.6999211	.71802440	<0.001
	MCI	34	.3696855	1.06584115	
	AD	29	-.9885342	1.10468367	
Discriminant Scores from Function 2 for Analysis 1	NC	23	-.3840689	.88163495	0.033
	MCI	34	.3229815	1.05047383	
	AD	29	-.0740616	1.02616645	

Table 16: Bonferroni Results from D1 and D2 scores

Dependent Variable	(I) Diagnosis	(J) Diagnosis	Sig.
Discriminant Scores from Function 1 for Analysis 1	NC	MCI	.674
		AD	.000
	MCI	NC	.674
		AD	.000
	AD	NC	.000
		MCI	.000
Discriminant Scores from Function 2 for Analysis 1	NC	MCI	.031
		AD	.810
	MCI	NC	.031
		AD	.360
	AD	NC	.810
		MCI	.360

From the Bonferroni, we can conclude that D1 discriminates between AD and the other two groups, but does not discriminate MCI from NC.

D2 discriminates NC from MCI, but not AD from either of the other groups.

Calculation of Accuracy from the score achieved from two equations:

The scores we have attained after solving the equation D1 from each subject, we used it in ROC curve to find out the accuracy or AUC (area under curve). Also, from IBM SPSS we can have coordinates of the curve which helped us to choose specificity and sensitivity according to the purpose (screening or diagnosis), set of groups and level of accuracy required.

For optimum application, high sensitivity and high specificity preferred

ROC analysis with D1

AD with NC

Area Under the Curve

Test Result Variable(s): Discriminant Scores from Function 1 for Analysis 1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.886	.045	.000	.798	.974

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

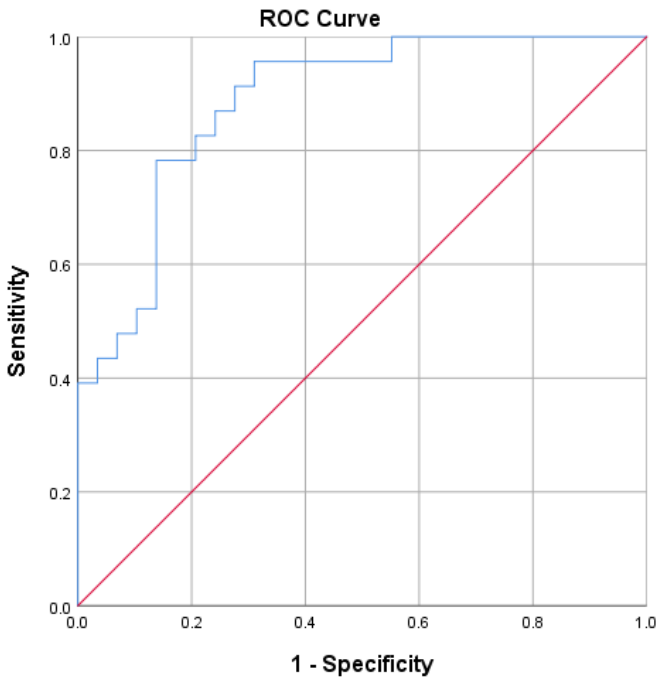


Figure 35: ROC curve representing sensitivity and (1-specificity) to classify AD from NC

ROC analysis with D1 - AD with MCI

Area Under the Curve

Test Result Variable(s): Discriminant Scores from Function 1 for Analysis 1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.812	.054	.000	.707	.918

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

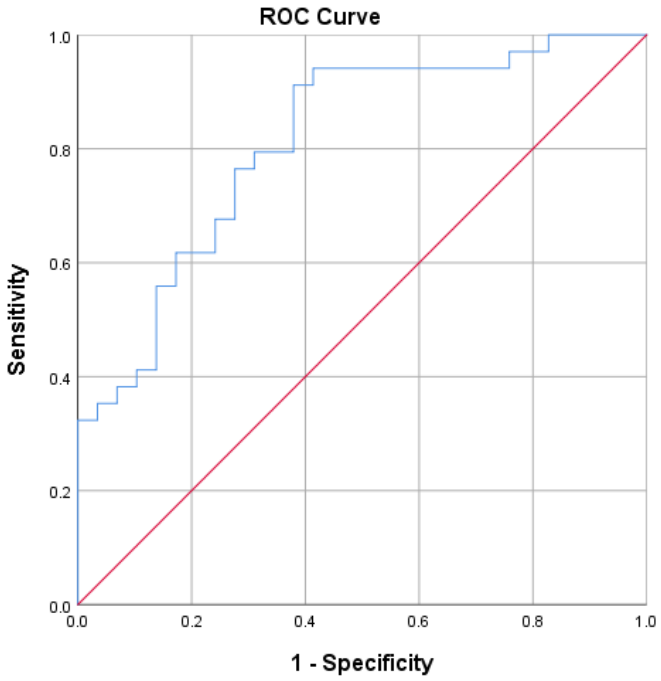


Figure 36: ROC curve representing sensitivity and (1-specificity) to classify AD from MCI

ROC analysis with D1 - AD with MCI/ NC

Area Under the Curve

Test Result Variable(s): Discriminant Scores from Function 1 for Analysis 1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.725	.055	.001	.617	.833

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

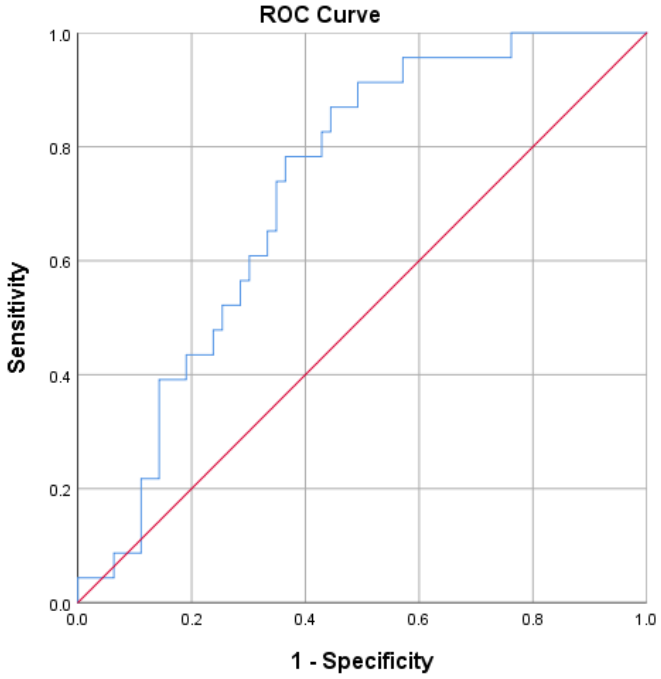


Figure 37: ROC curve representing sensitivity and (1-specificity) to classify AD from MCI+NC

Note: D1 value of NC is highest, then MCI and then AD. So, as D1 value increased, chance of being MCI or NC higher as compared to AD.

ROC analysis with D2 – MCI with NC

Area Under the Curve

Test Result Variable(s): Discriminant Scores from Function 2 for Analysis 1

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.696	.070	.013	.558	.834

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

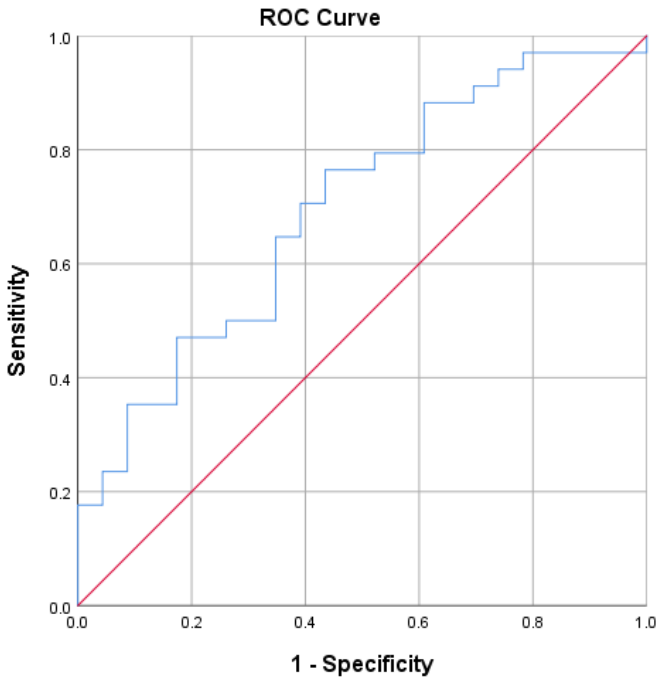


Figure 38: ROC curve representing sensitivity and (1-specificity) to classify MCI from NC

D2 value of MCI is highest, and NC is lowest. So, as D2 value increases, chance of being MCI is higher as compared to NC.

Summarized results from ROC curve analysis

- A D1 value of more than -0.5365622 has **95.7% sensitivityand 69.0% specificity** to screen in NC from AD
- A D1 value of more than -0.9970908 has **91.2% sensitivityand 62.1% specificity** to screen in MCI from AD
- A D1 value of 0.0298498 has **87.0% sensitivity and 55.6% specificity** to screen in others (MCI or NC) from AD
- A cut off of D2 value of **more than -0.4366026** screens **MCI from NC** with sensitivity of 76.5% and specificity of 56.5%

NOTE: **Coordinates of each of the ROC curve can be studied here**

As per the requirement of the clinicians, sensitivity and specificity can be decided accordingly in respect of screening or diagnosis. Higher sensitivity leads to low false negative results while Higher specificity leads to low false positive results.

CHAPTER 5

DISCUSSION:

The major objective of the study is to device multimodal quantitative diagnostic markers to classify the three diagnostic groups (NC, MCI, AD) among each other by using three modalities basic tests (viz structural atrophy, functional connectivity and cognitive ability) conducted in most of the places due to unavailability of biological tests (beta-albumin, tau protein) and other imaging techniques such as PET. The aim of the study is to know better diagnostic modality between structural and functional, which further helps in diagnosis of the progression of Alzheimer's disease. For this, we have approached univariate and multivariate analysis considering the parameters of graph theory measures explaining functional connectivity and grey-matter volume indicates structural atrophy of each selected ROI from Neurophometric atlas. Also, most of the studies are limited to DMN (Default Mode Network) (Wang J. et al, 2013) or it's sub-regions (Toussaint P.J. et al, 2014) but we have focused on 22 regions related to visual-memory, spatial-memory, working memory, cognitive ability, etc. which have shown vast variability in the connectivity in each diagnostic group and also make us feasible to conclude suitable ROI for our purpose and will be helpful in further studies. Using of graph construction method have been using widely either to find functional connectivity or structural connectivity (D.J. Philips et al., 2015). However, the results of graph theory measures are highly sensitive to the technique adopted for the construction of global graph or local graph, also to the method for edge detection (linear correlation, non linear correlation, unidirected or both side directed) (Bachman et al., 2018). We chose to follow atlas-based algorithm (number and size of cluster remains constant across all the subjects) in CONN 18.0 toolbox and for describing edge connectivity; we standardized z-score with both-sided weighted graph.

5.1 UNIVARIATE ANALYSIS

For our study, we took help of three tests which are sMRI scans, rs-fMRI scans, neuropsychological tests scores in the three diagnostic groups which are age, sex and education background controlled among each other. The selected cohort followed the same decline in

absolute grey matter volume from control to AD as seen in other studies (Guo Y. et al, 2014). To study the difference within each group with respect to graph features, we found that the close centrality can be studied among AD patients which indicates that cognitive regions in AD patients showed more involvement in close networking as compared to other groups while if we look at the networking of the whole brain then patients viz MCI & AD showed higher degree of connectivity as compared to NC group (Behfar et al, 2020). Also we found that the decrease and increase in centrality among AD patients in some of the brain regions of Amy(L), Hippo(L), Hippo(R), PCC, MFG(L), and MFG(R) is in alignment with the study of (Zhang et al, 2020) using conventional algorithm in their study. Additionally, we found increase in centrality in Amy(R) among AD patients. The finding also suggests that regional structural deterioration of the brain does not supports the loss of regional functionality.

5.2 CORRELATION ANALYSIS

Once we had an idea about the connectivity and structure decay difference between the groups, we wanted to correlate these parameters and check how cognitive functioning relate with structural atrophy as well as functional connectivity within each diagnostic group. Also, how structural atrophy correlate with functional connectivity and whether it followed the assumption made in last results. Due to multicollinearity nature of datasets, we have constricted our graph parameters only to Degree, BC, CC, and AvPL.

If we look at the Table17, results of correlation between neuropsychological tests and functional connectivity showed that maximum positive correlation is seen among MCI patients except one region i.e PCC where it is showing negative correlation, positive correlation indicates that MCI patients involve the particular region more in connectivity with other regions which helps them in increasing cognitive functioning as well while less involving of PCC helps in improving cognitive ability. This correlation was also studied by Behfar et al 2020, as compared to healthy senior adults indicating brain's mechanism to compensate cognitive decline by increasing neural connectivity. However, AD patients showed this correlation only in FO(L) and SPL(L). In SPL(L), the correlation is positive as compared to NC(r= -ve). This correlation difference clearly favors the compensatory mechanism by AD patients which was discussed earlier. Additionally, if we compare pearson coefficient(r) value in NC and MCI groups, the value for MCI was always lower than NC in the significant regions, indicating with negative connectivity. Though in the study by

(Wang J. et al, 2013) did not find any correlation of aMCI with MMSE, explaining the heterogeneity of MCI having different etiologies. In contrast, in our earlier study (Soman S.M. et al., 2020), there was no correlation found among MCI patients with cognitive functioning using different approach to study resting state networks, thus graph theory helped us in improving our earlier results. Also, it is found that there is negative correlation in FO(L) by AD patients which is in line with less cognitive ability found in AD patients, since from our within group graph theory study AD patients showed high centrality in FO(L).

Table 17: Compiled results of correlation between GT measures and cognitive functioning. *= represents r > 0.5

	NC	MCI	AD
FO(L)			P<0.05, r= -ve (RAVLT) *With BC, AvPL, Degree*
Caudate(L)		P<0.05, r=+ve* (RAVLT, delayed RAVLT) *-BC, AvPL, Degree* *-BC*	
Caudate(R)		P<0.05, r=+ve (MMSE) *AvPL, Degree*	
SPL(L)	P<0.05, r= -ve (MMSE) *AvPL, Degree*		P<0.05, r=+ve (MMSE) *AvPL, Degree*
Precuneous	P<0.05, r=+ve* (MMSE) *BC*	P<0.05, r=+ve (MMSE) *BC*	
Hippocampus(L)	P<0.05, r=+ve (ACE_Total) *AvPL, Degree*	P<0.05,r=+ve (ACE_Total), *AvPL, Degree*	

SMG(L)	P<0.05, r=-ve (ACE_Total) *CC*	P<0.05,r=+ve (ACE_Total), *CC*	
PCC		P<0.05,r=-ve (RAVLT_Total), *CC*	

Next study, correlation between structure atrophy and cognitive functioning (Table 18); which we have encountered best among AD patients (r = +ve). Here, NC group did not show any significant value except positive correlation in the region caudate (R) which means higher be the gVol of this region better be the cognitive functioning. While, MCI patients showed negative correlation in few regions such as Caudate (R), Caudate(L), PCC, Ainsula (R). These regions are also in align with our previous pair of correlation of functional connectivity and NPV, thus we can assume that due to structural atrophy they have compensated it with increasing functional connectivity or focusing towards compensatory mechanism.

Although, AD patients showed positive correlation in SMG(R), SMG(L), SPL(R), SPL(L), PO(L), Hippo(L), Precuneous, PCC, Amygdala(R), Amygdala(L), caudate(L), which means more be the structural atrophy, lower be the cognitive ability. While there is no correlation seen of grey matter volume with ACE total in any of the groups. Higher correlation in hippocampus(L)/ (R) showed role of these regions in memory.

Table 18: compile results of significant correlation between grey matter volume and neuropsychological test scores. *= r >0.5

NC (r=+ve)	MCI (r= -ve)	AD (r=+ve)
Caudate(R)	Caudate (R)	
	Caudate(L)	Caudate(L)
	PCC	PCC
	Ainsula(R)	
		Amygala(R)

		Amygdala(L)
		SMG(R)
		SMG(L)
		SPL(R)
		SPL(L)
		Precuneous*
		Hippocampus(L)*
		Hippocampus(R)*
		PO(L)

In the study of correlation between functional connectivity and structural atrophy (Table 19) , very few regions showed significant results. Among that, PO (R) could well differentiate NC and AD patients, indicates that with the decay in gVol, functional connectivity for this region increases which is opposite in NC group, this supports the Hemispheric Asymmetry mechanism observed by (Piefke et al., 2012) in parietal lobe. While in FO(R), all the three groups showed same negative correlation though AD shows maximum correlation coefficient as compared to other two groups, compensating against frontal lobe hypothesis (Cabeza and Dennis, 2012) which states that cognitive deficiencies in aging are due to structural and functional deterioration in frontal lobes. Thus, compensatory mechanism can be easily shown by AD patients which clearly depicts decrease in gVol leads to increase in functional connectivity by the particular region. (delEtoile and Adeli, 2017)

Table 19: Compiled results of correlation between GT measures and gVol reduction. *= r >0.5

	NC	MCI	AD
FO(R)	r=-ve (BC)	r=-ve (degree)	r=-ve (degree*, BC) +ve (AvPL)*
Amygdala(R)			r=-ve (CC)

PO(R)	r=+ve(CC)		r=-ve (CC)
Hippocampus(L)			r=-ve* (CC)

5.3 BIVARIATE ANALYSIS

Since to understand the connectivity measures from CONN18.0 toolbox, we needed to follow statistical computation and to check how the parameter measures differentiate between group analysis. We performed ANOVA test with posthoc Bonferroni, two-tailed T- test and ROC- curve analysis with threshold of the significance value at $P < 0.05$. Since we defined our datasets are not normally distributed or non-parametric in nature, thus we considered result from ROC-curve as relevant. Through ROC-curve we analysed parameters from graph study and grey matter volume of each region individually. We observed that graph theory parameters gave significant results in most of the regions for NC and MCI while grey matter volume gave significant classification for MCI and AD. Additionally, the regions in which both the biomarkers showed significance classification in between-group analysis are SMG(R), Hippocampus(R), and SPL(R), indicating the importance of these regions impacted among AD patients or explained the lower score of neuropsychological score (spatial memory, and navigation memory) in AD patients. Also, among graph theory measures, global parameters; GE, AvPL, degree, cost, and BC showed significant differences, in contrast to local parameters; LE, CC, which approves the assumption and importance of global parameters in showing disease progression over time than statistical changes. (Wang et al., 2014; Telesford et al., 2010)

From the above analysis results, we can also align the findings of the studies stated that before structural atrophy functional changes occured (Frisoni G.B., 2010), one of the possible reason of why MCI patients who might not have reached atrophy but due to decomposition of beta-amyloid and tau-protein, effects in function connectivity can be seen.

Functional connectivity using ROI-ROI analysis

To study the between-group difference further and check the ROIs connectivity from ROI-ROI analysis. Since from graph theory we can know the role, and strength of connectivity of each ROI but could not depict whether connectivity increase or decrease as compared to other group. For

MCI>NC, MCI patients have reduced connectivity as compared to NC and the common ROIs in both study are Hippo(R), Amy(L). Also, both of these regions showed negative connectivity with each other, this could explained the reduction in memory scores in MCI patients.

While for AD>MCI, AD also showed negative connectivity in most of the regions as compared to MCI but showed higher connectivity in the regions of MFG(L), similar to the study by (Behfar et al., 2020). Similarly, MFG(L) and SFG(L) are connected to SMG(L) and AInsula(R) respectively which also showed significance classification result in ROC-curve analysis using graph connectivity features. These regions are responsible for empathy and perceiving emotions, may be in AD groups can have higher empathy than MCI groups, while studies have shown reduced empathy in AD patients (Fischer A. et al, 2019). Probably overlapping from other regions also occurred.

For AD>NC, Hippo(R) have higher connectivity with amygdala and other side of hippocampus but Hippo(R) showed lower connectivity with SPL(L) which indicates reduction in spatial memory and navigation in AD patients.(Zhu H. et al, 2017)

Also, ROI-ROI analysis and graph theory both presents functional connectivity but in between-group analysis we found different significant regions, except some regions which are related to spatial memory, cognitive functioning, and empathy.

5.4 MULTIVARIATE ANALYSIS USING CLASSIFICATION MODELS:

After finishing up univariate& bivariate analysis, we want to check multivariate's role in defining the three groups or what impact we can have in between-group analysis. The classification models chosen for this purpose are binomial logistic regression (using backward method) and Descriminant function analysis method. Since, we did not achieve much satisfactory results from binomial LR due to several restrictive factors, some of them could be low number of subjects, non-parametric nature, multicollinearity, and high value of beta constant. However, we got a classification model for MCI and AD which includes parameters are grey matter volume of Amygdala(R), AInsula(R), SFG(R), SPL(R), and GE of SMG(R), the point of notice was that all the regions are from right hemisphere of the brain, and the same which we got from our previous analysis in between-group analysis.

Then, we tried with Descriminant function analysis classification method, which gave us two classification model includes parameters of Hippo(L), and SMG(R) grey matter volume. The role of each classification model is independent yet incomplete without each other for the complete diagnosis of each group.

Classification model 1 (D1, with function1) can help the clinicians to classify AD from other two groups with the accuracy of 72.5% having sensitivity= 87% and specificity=55.6%

While, classification model (D2, with function 2) can help to classify NC and MCI with the accuracy of 69.6% having sensitivity= 76.5%, and specificity= 56.5%

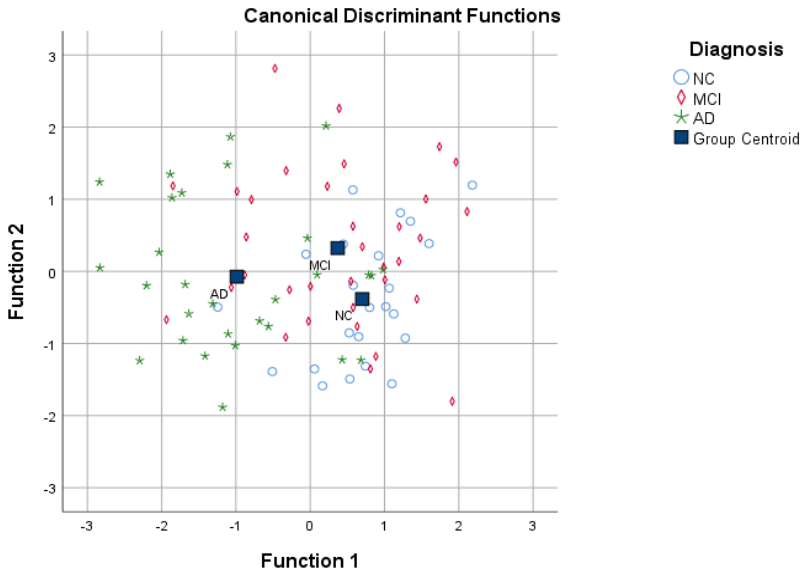


Figure 39: Classification model of three diagnostic groups on the basis of two functions derived from parameter of grey matter volume of Hippo(L), and SMG(R).

The centroids in the graph depict this – along the x axis (D1), AD is far away from MCI and NC; along the y axis, AD is in between NC and MCI, but NC and MCI are separate enough to achieve statistical significance.

The implication of these models in clinical application can be done in a way to first classify AD patients from MCI and NC, after that go for second model to classify MCI and NC. In such way, clinician can diagnose each of the group or separate out from each other.

Both of these brain regions of Hippo(L), and SMG(R) are responsible in controlling cognitive functions such as navigation memory, rhythmic memory and perceiving emotions of others. In Hippo(L) we found highest clustering in Normal group as compared to AD and MCI, though AD have high degree and centrality in this region, indicating that due to structure atrophy clusters couldn't form in MCI and AD which they tried forming connectivity with other regions, thus disturbing its original role in memory.

5.5 LIMITATIONS OF THE STUDY :

Due to non-feasibility of other tests such as CSF Aβ42, tau analysis and amyloid PET in SCTIMST hospital, Trivandrum. We were not able to stratified our NC, MCI and AD groups into high and low sub-groups, which might helped us in our analysis. Another lack was the method of finding classification model for non-parametric and multicollinear data. While doing the analysis on a group level, generally the size of cohort requirement should be quite large where our total number of subjects could not matched. Another limitation was lack of longitudinal study which would be advantage, especially while studying the heterogenous group like MCI.

5.6 FUTURE WORK:

Diagnosis of Alzheimer's disease, especially its progression has been a long lasting question for the scientists, thus a continuous research is always required to bridge the knowledge gap between diagnosis and treatment. The findings we have achieved from our study will be very helpful to take it further in order to achieve the core objective. Since, we have some limitations in our study, we want to be targeted them in future studies. Some of the valuable points to be considered are:

- Our study only includes imaging biomarkers and we know the significance of biological signal in diagnosis of AD. We can also include MR-spectrometry, though it is also imaging but provides quantitative biomolecules changes and thus will be helpful to give new insights.

- As we have randomly and on the basis of previous studies, selected our ROIs. But in further study we can also take help of ICA study; consider the whole brain and then select out significant nodes.
- Due to limitations shown by the parameters and very closed value, one can also include machine learning classification models using these biomarkers, which might give better accuracy.
- Also, we have done group-level statistical analysis, in support of personalized medicine, one should also consider testing these parameters at an individual level.
- One can also use other methods to construct graph instead of CONN toolbox.

CHAPTER 6

CONCLUSION AND SUMMARY OF THE WORK

Alzheimer's disease is one of the leading cause of Dementia, around 40% of Dementia is caused by AD. It started from an initially memory deprive to the loss in other cognitive abilities affecting millions of elderly-aged people across the world. The prime cause of AD are considered to deposition of beta-amyloid plaques and tangles formed by tau-protein, both of theses proteins play role in transferring information and nutrients from one neuron to another, so that different areas of the brain can communicate among each other. The main challenges for clinicians is not to diagnose the AD but to provide the right treatment according to the AD's continuum, and to know how much chances of a MCI to get progressed to AD.

Until now, many biomarkers have been studied either individually or in combinations, these biomarkers can be biological tests or imaging tests. But, a diagnostic tool with high sensitivity and high specificity is still under research, though each study helped us to have better insights about this disease and gave us a path to go upon. In our study, we also included two of the common imaging biomarkers; grey matter volume and functional connectivity by graph construction method which are extracted by using the modalities of structural MRI & resting state functional MRI respectively, the choice was based on the easy availability of these two techniques at most of the centers. Thus, ignoring the efficiency and complexity with other studied biomarkers.

We took three groups of control, MCI, and AD who are controlled in age, sex and educational background. The main focus contributed to the impact of each of the biomarker on each diagnostic group and how this impact creates significant difference between the groups. First, we analyze each of the biomarker (gVol and graph theory parameters) separately within the group and trace it's impact on the selected ROIs. Then, second, we trace the impact of each biomarker between the groups and what could be those ROIs, this we have done using ROC curve analysis and ROI-ROI connectivity analysis using CONN18.0 toolbox. Third, we find out the relation of each biomarker with the cognitive functioning calculated from neuropsychological tests and we found out the relation between these biomarkers in each node. Once we have completely analysed our biomarkers, we wanted to know their role in classifying each of the diagnostic groups.

For preprocessing the scans, graph construction, and functional connectivity parameters, we took help from CONN18 toolbox, and for grey matter volume extraction we used SPM12, while IBM-SPSS was used for all the statistical analysis during our study. The relevant findings we came across are:

There is continuous decline in absolute grey matter volume from control to MCI and then to AD, but this is not necessary that each selected region of the brain follow the same trend, due to the unpredictable deposition of proteins; thus leads to cell destruction and structural atrophy of different regions. Also, we found out that AD showed higher centrality as compared to other groups thus more closer connectivity can be traced. While both MCI and AD are involved in higher connectivity globally as compared to our normal group.

Since, both graph theory and ROI-ROI analysis represent functional connectivity, but with one (graph theory), we can study the strength of our network globally and locally. However, we got quite different significant regions which can be responsible for the difference in between-group except some of the common regions like Hippocampus(R), Amygdala(L), SMG(L), AInsula(R) showed difference in both of the methodologies. These regions are also responsible for spatial memory, emotions, and empathy. Also, grey matter volume showed difference when studied between MCI vs AD, while less in MCI vs NC. Though, to differentiate this group we had some of the significant graph parameters representing global network; GE, BC, Degree, and Average path length indicating a significant change in global connectivity between MCI and NC (which also support our univariate analysis).

Next, increase in connectivity in MCI patients can also be seen by correlating the graph theory parameters with cognitive functioning, we got positive relation of degree and BC with neuropsychological test score in Caudate(L), Caudate(R), Precuneous, Hippocampus(L), SMG(L) while less connectivity in PCC supports more cognitive functioning. Also, caudate(L) and caudate(R) showed greater structural atrophy leads to more cognitive functioning, thus involving other regions in connectivity to optimize the cognitive functioning. While, AD patients also showed a negative relation between grey matter volume and graph theory parameter, indicates that more is the structural atrophy more will be the connectivity in respect of the regions; FO(R), Amgdala(R), PO(R), Hippocampus(L).

With the complete analysis, combinations of parameters of both the biomarkers chosen and checked the classification accuracy. We got better classification with grey matter volume of some specific regions such as SMG(R) and Hippocampus(L). We achieved two significant classification models that can be helpful in diagnosis and screening process. With one model we can separate out AD from MCI and NC, while with other model we can separate out MCI and NC. Thus, classifying all the three diagnostic groups among each other.

Conclusion of the study:

In best of our knowledge, this is India's first study which involves these two biomarkers with the technique of graph theory; creating and studying the network organization in the brain (which is still under study). The features of studying graph in context of functional connectivity leads us to know about the strength of connectivity not only whether it is decreasing or increasing, we can state by what value it increases or decreases. Also, it classifies the whole brain into graph (considering whole-brain) and sub-graph (considering cellular-spatial) which determines the role of each region with its neighbor nodes and depicts the number of edges each node has thus, we can estimate the number of regions, a node must have connected to. Keeping this study along with grey matter volume reduction lead us to know the changes and adaptation occurred in patients as compared to normal control group. We can find out whether the changes occurred globally or locally.

As discussed, both MCI and AD patients involved in neuroplasticity or compensatory mechanism once they encounter the structural atrophy. But the connectivity in MCI patients help them to optimize cognitive functioning while AD patients couldn't do this. That's why decline in cognitive functioning is more prominent in AD patients as compared to MCI.

The reason could be MCI involves in increasing connectivity globally while AD increase connectivity with nearby regions or locally.

Also, with classification model we can say that biomarkers like grey matter volume of Hippocampus(L) and SupraMarginalGyrus(R) will be efficient but does not completely remove other biomarkers or parameters such as variation seen in Caudate(R) and Caudate(L), they could also be equally significant but due to limitations of statistical tool and datasets might not appear in this study. In the future study, we can reconsider them.

REFERENCES:

Anderson J S, Druzgal T J, Lopez-Larson M, et al., 2011. Network anticorrelations, global regression, and phase-shifted soft tissue correction. Human brain mapping 32, 919-934.

Ashraf Ghulam M., Baeesa Saleh S, 2018. Investigation of Gal-3 Expression Pattern in Serum and Cerebrospinal Fluid of Patients Suffering From Neurodegenerative Disorders. Frontiers in Neuroscience12. DOI=10.3389/fnins.2018.00430

Bachmann Claudia, Jacobs Heidi I. L., Porta Mana PierGianLuca, Dillen Kim, Richter Nils, von Reutern Boris, Dronse Julian, OnurOezguer A., Langen Karl-Josef, Fink Gereon R., KukoljaJuraj, Morrison Abigail, 2018. On the Extraction and Analysis of Graphs From Resting-State fMRI to Support a Correct and Robust Diagnostic Tool for Alzheimer's Disease. Frontiers in Neuroscience12. DOI=10.3389/fnins.2018.00528

Behfar Q., BehfarSk, ReuternB.v., et al, 2020.Graph Theory Analysis Reveals Resting-State Compensatory Mechanisms in Healthy Aging and Prodromal Alzheimer's Disease. Front. Aging Neurosci. 12. https://doi.org/10.3389/fnagi.2020.576627

Binnewijzend M A, Schoonheim M M, SanzArigita E, et al., 2012. Resting-state fMRI changes in Alzheimer's disease and mild cognitive impairment. Neurobiology of aging 33, 2018-2028.

Bi Xia-an, Jiang Qin, Sun Qi, Shu Qing, Liu Yingchao, 2018. Analysis of Alzheimer's Disease Based on the Random Neural Network Cluster in fMRI. Frontiers in Neuroinformatics 12, 60. DOI=10.3389/fninf.2018.00060

Braak H, Braak E, 1996. Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. Acta Neuropathol.92, 197–201.

Bruno, D., &SchurmannVignaga, S., 2019. Addenbrooke's cognitive examination III in the diagnosis of dementia: a critical review. Neuropsychiatric disease and treatment, 15, 441–447. https://doi.org/10.2147/NDT.S151253

Castellazzi G, Palesi F, Casali S, et al., 2014. A comprehensive assessment of resting state networks: bidirectional modification of functional integrity in cerebro-cerebellar networks in dementia. Frontiers in neuroscience 8, 223.

Chapman RM, Mapstone M, Porsteinsson AP, et al, 2010. Diagnosis of Alzheimer's disease using neuropsychological testing improved by multivariate analyses. J ClinExpNeuropsychol., 32(8), 793-808. doi:10.1080/13803390903540315

Chen, J., Duan, X., Shu, H. et al., 2016. Differential contributions of subregions of medial temporal lobe to memory system in amnestic mild cognitive impairment: insights from fMRI study. Sci Rep 6. https://doi.org/10.1038/srep26148

Cheng Zaohuo, Yin Jiajun, Yuan Hongwei, Jin Chunhui, Zhang Fuquan, Wang Zhiqiang, Liu Xiaowei, Wu Yue, Wang Tao, Xiao Shifu, 2018. Blood-Derived Plasma Protein Biomarkers for Alzheimer's Disease in Han Chinese. Frontiers in Aging Neuroscience10. DOI=10.3389/fnagi.2018.00414

Damoiseaux JS, 2012. Resting-state fMRI as a biomarker for Alzheimer's disease?Alzheimers Res Ther4(2), 8. doi:10.1186/alzrt106

Dai Z, Yan C, Li K, et al., 2015. Identifying and Mapping Connectivity Patterns of Brain Network Hubs in Alzheimer's Disease. Cerebral cortex. New York, N.Y. : 1991, 25, 3723-3742.

delEtoile, J., and Adeli, H. (2017). Graph theory and brain connectivity in Alzheimer's disease. Neuroscientist 23, 616–626. doi: 10.1177/ 1073858417702621

El-Gamal Fatma E. A., Elmogy Mohammed M., Ghazal Mohammed, Atwan Ahmed, Casanova Manuel F., Barnes Gregory N., Keynton Robert, El-Baz Ayman S., Khalil Ashraf, 2018. A Novel Early Diagnosis System for Mild Cognitive Impairment Based on Local Region Analysis: A Pilot Study. Frontiers in Human Neuroscience11. DOI=10.3389/fnhum.2017.00643

Esposito F, Aragri A, Pesaresi I, et al., 2008. Independent component model of the defaultmode brain function: combining individuallevel and population-level analyses in restingstate fMRI. Magnetic resonance imaging 26, 905-913.

Farahani Farzad V., Karwowski Waldemar, Lighthall Nichole R., 2019. Application of Graph Theory for Identifying Connectivity Patterns in Human Brain Networks: A Systematic Review. Frontiers in Neuroscience. 13. DOI=10.3389/fnins.2019.00585

Fischer A, Landeira-Fernandez J, Sollero de Campos F, Mograbi DC, 2019. Empathy in Alzheimer's Disease: Review of Findings and Proposed Model. J Alzheimers Dis. 69(4), 921-933. doi: 10.3233/JAD-180730.

Fox NC, Freeborough PA, 1997. Brain atrophy progression measured from registered serial MRI: validation and application to Alzheimer's disease. J MagnReson Imaging7, 1069-1075. 10.1002/jmri.1880070620.

Frisoni G.B., Fox N.C., Jack C.R. Jr., Scheltens P., Thompson P.M., 2010. The clinical use of structural MRI in Alzheimer disease, Nat. Rev. Neurol. 6 (2), 67–77.

Guo Y, Zhang Z, Zhou B, Wang P, Yao H, Yuan M, An N, Dai H, Wang L, Zhang X, Liu Y. 2014. Grey-matter volume as a potential feature for the classification of Alzheimer's disease and mild cognitive impairment: an exploratory study. Neurosci Bull30(3), 477-89. doi: 10.1007/s12264-013-1432-x.

India KSD. 2015. http://dementiacarenotes.in/dementia/dementia-india-2015-info/.

Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ, 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol9, 119-128. 10.1016/S1474-4422(09)70299-6.

Jinhui Wang, XinianZuo, Zhengjia Dai, Mingrui Xia, Zhilian Zhao, Xiaoling Zhao, Jianping Jia, Ying Han, Yong He, 2013. Disrupted Functional Brain Connectome in Individuals at Risk for Alzheimer's Disease. Biological Psychiatry, 73(5), 472-481. https://doi.org/10.1016/j.biopsych.2012.03.026.

Khan Tapan K. 2018. An Algorithm for Preclinical Diagnosis of Alzheimer's Disease. Frontiers in Neuroscience12. DOI=10.3389/fnins.2018.00275

Lama R. K., Kwon G. R., 2021. Diagnosis of Alzheimer’s disease using Brain Network. Frontiers in Neuroscience15. https://doi.org/10.3389/fnins.2021.605115

Laptinskaya Daria, ThurmFranka, Küster Olivia C., Fissler Patrick, Schlee Winfried, Kolassa Stephan, von Arnim Christine A. F., Kolassa Iris-Tatjana, 2018. Auditory Memory Decay as

Reflected by a New Mismatch Negativity Score Is Associated with Episodic Memory in Older Adults at Risk of Dementia. Frontiers in Aging Neuroscience10. DOI=10.3389/fnagi.2018.00005

Leech R, Sharp D J, 2014. The role of the posterior cingulate cortex in cognition and disease. Brain 137, 12-32.

Liu, J., Tan, G., Lan, W. et al., 2020. Identification of early mild cognitive impairment using multi-modal data and graph convolutional networks. BMC Bioinformatics 21. https://doi.org/10.1186/s12859-020-3437-6

Liu Z, Zhang Y, Yan H, Bai L, Dai R, Wei W, Zhong C, Xue T, Wang H, Feng Y, You Y, Zhang X, Tian J., 2012. Altered topological patterns of brain networks in mild cognitive impairment and Alzheimer's disease: a resting-state fMRI study. Psychiatry Res., 202(2), 118-25. doi: 10.1016/j.pscychresns.2012.03.002.

Long Zhuqing, Jing Bin, Guo Ru, Li Bo, Cui Feiyi, Wang Tingting, Chen Hongwen., 2018. A Brainnetome Atlas Based Mild Cognitive Impairment Identification Using Hurst Exponent. Frontiers in Aging Neuroscience10. DOI=10.3389/fnagi.2018.00103

Luo Xiao, Li Kaicheng, Zeng Qingze, Huang Peiyu, JiaerkenYeerfan, QiuTiantian, Xu Xiaojun, Zhou Jiong, Xu Jingjing, Zhang Minming, 2018. Decreased Bilateral FDG-PET Uptake and Inter-Hemispheric Connectivity in Multi-Domain Amnestic Mild Cognitive Impairment Patients: A Preliminary Study. Frontiers in Aging Neuroscience10. DOI=10.3389/fnagi.2018.00161

Malaak N. Moussa, Matthew R. Steen, Paul J. Laurienti, and Satoru Hayasaka - PLoS One. 2012; 7(8): e44428. Published online 2012

Mason S.E., McShane R., Ritchie C.W., 2010. Diagnostic Tests for Alzheimer’s Disease: Rationale, Methodology, and Challenges. International Journal of Alzheimer’s Disease. https://doi.org/10.4061/2010/972685

McKeown M J, Makeig S, Brown G G, et al., 1998. Analysis of fMRI data by blind separation into independent spatial components. Human brain mapping 6, 160-188.

Mehta S., 2019 Apr 22. Mild Cognitive Impairment. Medscape.

Mistur R, Mosconi L, Santi SD, et al., 2009. Current Challenges for the Early Detection of Alzheimer's Disease: Brain Imaging and CSF Studies. J Clin Neuro, 5(4), 153-166. doi:10.3988/jcn.2009.5.4.153

Moretti, D.V., 2015. Conversion of mild cognitive impairment patients in Alzheimer's disease: prognostic value of Alpha3/Alpha2 electroencephalographic rhythms power ratio. Alz Res Therapy 7, 80. https://doi.org/10.1186/s13195-015-0162-x

Pasquini L, Scherr M, Tahmasian M, et al., 2015. Link between hippocampus' raised local and eased global intrinsic connectivity in AD. Alzheimer's & dementia : the journal of the Alzheimer's Association 11, 475-484.

Paule-Joanne Toussaint, Sofiane Maiz, David Coynel, Julien Doyon, Arnaud Messé, Leonardo Cruz de Souza, Marie Sarazin, Vincent Perlbarg, Marie-Odile Habert, Habib Benali, 2014. Characteristics of the default mode functional connectivity in normal ageing and Alzheimer's disease using resting state fMRI with a combined approach of entropy-based and graph theoretical measurements,NeuroImage,Volume101,778-786. https://doi.org/10.1016/j.neuroimage.2014.08.003.

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E., 1999. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol.56, 303–308

Phillips DJ, McGlaughlin A, Ruth D, et al, 2015. Graph theoretic analysis of structural connectivity across the spectrum ofAlzheimer3s disease: The importance of graph creation methods. Neuroimage: Clinical 7, 377-390. http://dx.doi.org/10.1016/j.nicl.2015.01.007 2213-1582

Power J D, Barnes K A, Snyder A Z, et al., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. NeuroImage 59, 2142-2154.

Public health and aging: Trends in aging--United States and worldwide. JAMA. 2003; 289(11):1371-3. doi:10.1001/jama.289.11.1371

Ranjith N, Mathuranath PS, Sharma G, Alexander A., 2010. Qualitative aspects of learning, recall, and recognition in dementia. Ann Indian Acad Neurol.13(2), 117-122. doi:10.4103/0972-2327.64639

RajamanickamK., 2020.A Mini Review on Different Methods of Functional-MRI Data Analysis. Fortune Journal3(1), 044-060. DOI: 10.26502/aimr.0022

Rodakowski J, Schulz R, Gentry A, Garand L, Lingler JH. 2014. Attribution of mild cognitive impairment etiology in patients and their care partners. Int J Geriatr Psychiatry 29(5), 464-469. doi:10.1002/gps.4028

Rosazza C, Minati L, Ghielmetti F, et al., 2012. Functional connectivity during resting-state functional MR imaging: study of the correspondence between independent component analysis and region-of-interestbased methods. AJNR. American journal of neuroradiology 33, 180-187.

Sanz-Arigita E J, Schoonheim M M, Damoiseaux J S, et al., 2010. Loss of 'small-world' networks in Alzheimer's disease: graph analysis of FMRI resting-state functional connectivity. PloS one 5.

Schwindt G C, Chaudhary S, Crane D, et al., 2013. Modulation of the default-mode network between rest and task in Alzheimer's Disease. Cerebral cortex (New York, N.Y. : 1991) 23, 1685-1694.

Shaji K, Jotheeswaran ANG, Girish N, Bharath S, Dias A, Pattabiraman M, et al., 2010. The Dementia India Report 2010 [Internet. Alzheimer’s and related disorders society of India. http://ardsi.org/downloads/ExecutiveSummary.pdf.

Shi F, Liu B, Zhou Y, Yu C, Jiang T, 2009. Hippocampal volume and asymmetry in mild cognitive impairment and Alzheimer's disease: meta-analyses of MRI studies. Hippocampus19, 1055-1064. 10.1002/hipo.20573.

Soman S.M., Raghavan S., Rajesh P.G., Mohanan N., Thomas B., Kesavadas C., Menon R.N., 2020. Does resting state functional connectivity differ between mild cognitive impairment and early Alzheimer's dementia?. Journal of the Neurological Sciences418. https://doi.org/10.1016/j.jns.2020.117093.

Son, S. J., Kim, J., & Park, H. (2017). Structural and functional connectional fingerprints in mild cognitive impairment and Alzheimer's disease patients. PLoS One, 12(3), e0173426. https://doi.org/10.1371/journal.pone.0173426

Supekar K, Menon V, Rubin D, et al., 2008. Network Analysis of Intrinsic Functional Brain Connectivity in Alzheimer's Disease. PLoS Computational Biology 4, e1000100.

Tabei Ken-ichi, Satoh Masayuki, Ogawa Jun-ichi, Tokita Tomoko, Nakaguchi Noriko, Nakao Koji, Kida Hirotaka, TomimotoHidekazu, 2018. Cognitive Function and Brain Atrophy Predict Non-pharmacological Efficacy in Dementia: The Mihama-Kiho Scan Project2. Frontiers in Aging Neuroscience10, 87. DOI=10.3389/fnagi.2018.00087

Telesford, Q. K., Morgan, A. R., Hayasaka, S., Simpson, S. L., Barret, W., Kraft, R. A., et al. (2010). Reproducibility of graph metrics in fMRI networks. Front. Neuroinformat. 4, 117. doi: 10.3389/fninf.2010.00117

The World Bank, Data for India. 2017. https://data.worldbank.org/?locations=IN-XN.

Toussaint P.J., Maiz S., Coynel D., Doyon J. et al., 2014. Characteristics of the default mode functional connectivity in normal ageing and Alzheimer's disease using resting state fMRI with a combined approach of entropy-based and graph theoretical measurements. NeuroImage101, 778-786. https://doi.org/10.1016/j.neuroimage.2014.08.003.

United Nations Organization. World population ageing: 1950-2050. New York: U. N. P. o. Ageing, United Nations; 2001. www.un.org/esa/population/publications/worldageing19502050/. Accessed March 22, 2009

Van Dijk K R, Sabuncu M R, Buckner R L. 2012. The influence of head motion on intrinsic functional connectivity MRI. NeuroImage 59, 431-438.

Vemuri, P., Jack, C.R., 2010. Role of structural MRI in Alzheimer's disease. Alz Res Therapy 2, 23. https://doi.org/10.1186/alzrt47

Vemuri P, Jones DT, Jack CR Jr. Resting state functional MRI in Alzheimer's Disease, 2012. Alzheimers Res Ther4(1), 2. doi:10.1186/alzrt100

XueJ., Guo H., Gao Y., et al., 2019.Altered Directed Functional Connectivity of the Hippocampus in Mild Cognitive Impairment and Alzheimer's Disease: A Resting-State fMRI Study. Front. Aging Neurosci. 11.https://doi.org/10.3389/fnagi.2019.00326

Wang J., Zuo X., Dai Z., et al., 2013.Disrupted Functional brain connectome in individuals at risk for Alzheimer's Disease. Biol Psychiatry 73, 472-481. https://doi.org/10.1016/j.biopsych.2012.03.026

Wang L, Zang Y, He Y, et al., 2006 Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. NeuroImage 31, 496-504.

Wang, H. E., Bénar, C. G., Quilichini, P. P., Friston, K. J., Jirsa, V. K., and Bernard, C. (2014). A systematic framework for functional connectivity measures. Front. Neurosci. 8, 405. doi: 10.3389/fnins.2014.00405

Wee CY, Yap PT, Zhang D, et al., 2012. Identification of MCI Individuals Using Structural and Functional Connectivity Networks. Neuroimage59(3), 2045–2056. doi:10.1016/j.neuroimage.2011.10.015.

Wolf H, Hensel A, Kruggel F, et al., 2004. Structural correlates of mild cognitive impairment. Neurobiol Aging25, 913–924.

Wu Z, Xu D, Potter T, Zhang Y, 2019. Alzheimer's Disease Neuroimaging Initiative. Effects of Brain Parcellation on the Characterization of Topological Deterioration in Alzheimer's Disease. Front Aging Neurosci. 11, 113. doi: 10.3389/fnagi.2019.00113.

Yang H, Xu H, Li Q, et al. 2019. Study of brain morphology change in Alzheimer's disease and amnestic mild cognitive impairment compared with normal controls. Gen Psychiatr., 32(2):e100005. doi:10.1136/gpsych-2018-100005

Yuan Y, Gu ZX, Wei WS, 2009. Fluorodeoxyglucose-positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: a meta-analysis. AJNR Am J Neuroradiol30, 404-410. 10.3174/ajnr.A1357.

Zarei M, Beckmann C F, Binnewijzend M A, et al., 2013. Functional segmentation of thehippocampus in the healthy human brain and in Alzheimer's disease. NeuroImage 66, 28-35.

Zhang H Y, Wang S J, Xing J, et al. 2009. Detection of PCC functional connectivity characteristics in resting-state fMRI in mild Alzheimer's disease. Behavioural brain research 197, 103-108.

Zhang X., Liu J., Chen Y. et al. 2021. Brain network construction and analysis for patients with mild cognitive impairment and Alzheimer's disease based on a highly-available nodes approach. Brain and Behavior11, e02027. https://doi.org/10.1002/brb3.202

Zhao Q, Lv Y, Zhou Y, Hong Z, Guo Q, 2012. Short-term delayed recall of auditory verbal learning test is equivalent to long-term delayed recall for identifying amnestic mild cognitive impairment. PLoS One7(12), e51157. doi:10.1371/journal.pone.0051157

Zhou J, Greicius M D, Gennatas E D, et al, 2010. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain : a journal of neurology 133, 1352-1367.

Zhou J, Liu S, Ng KK, Wang J., 2017. Applications of Resting-State Functional Connectivity to Neurodegenerative Disease. Neuroimaging Clin N Am.27(4), 663-683. doi: 10.1016/j.nic.2017.06.007.

Zhu H, Yan H, Tang N, et al. 2017. Impairments of spatial memory in an Alzheimer's disease model via degeneration of hippocampal cholinergic synapses. Nat Commun. 8(1), 1676. doi:10.1038/s41467-017-01943-0