

# Biomarkers of Alzheimer's Disease: A Hope for Early Diagnosis

By

Farhin Aziz  
15146007

A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

Department of Pharmacy  
Brac University  
August 2019

© 2019. Brac University  
All rights reserved.

## **Declaration**

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

---

**Farhin Aziz**

15146007

## Approval

The thesis/project titled “Emerging Biomarkers of Alzheimer’s Disease: A Hope for Early Diagnosis” submitted by

1. Farhin Aziz (15146007)

of Spring, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on [Date-of-Defense].

### Examining Committee:

Supervisor:  
(Member)

---

Dr. Raushanara Akter  
Associate Professor, Department of Pharmacy  
Brac University

Program Coordinator:  
(Member)

---

Dr. Hasina Yasmin  
Associate Professor, Department of Pharmacy  
Brac University

Departmental Head:  
(Chair)

---

Dr. Eva Rahman Kabir  
Chairperson, Department of Pharmacy  
Brac University

## **Abstract**

Alzheimer's disease is a complex neurodegenerative disease which requires very specific biomarkers for its diagnosis. This review is mainly focused on finding out the biomarkers that are currently used to diagnose Alzheimer's Disease. Biomarkers are the biological markers and help to reveal what is going on inside the brain due to Alzheimer's Disease. Thus, the biomarkers become the most important factors in early diagnosis of the disease. The literature review revealed that in most of the cases, the target biomarkers are CSF amyloid- $\beta$  and  $\tau$ -protein aggregates, although only the  $\tau$ -protein is very specific to Alzheimer's disease in comparison with any other forms of dementia. However, there are also other types of biomarkers currently being used like blood and ocular biomarkers. In the conclusion it can be inferred that, this review will help to identify the biomarkers which needs to be established for the better diagnosis and management of Alzheimer's disease.

**Keywords:** Alzheimer's Disease; Biomarkers of Alzheimer's Disease; Specific Biomarkers; Non-invasive Diagnosis; Amyloid- $\beta$ ; Tau-Protein Aggregates

## **Dedication**

Dedicated to my parents and my husband

## **Acknowledgement**

In the very beginning, I would like to show my gratitude towards Allah for allowing me to complete my work properly.

I am very grateful and obliged to my supervisor Dr. Raushanara Akter (Associate Professor of Pharmacy Department of BRAC University) who was always very supportive towards me. She guided and motivated me to do my work properly. She was very helpful to me whenever I faced any problem in my work and helped me to resolve any kind of inaccuracy in my work. Besides, I must mention Dr. Eva Rahman Kabir, Chairperson of the Department of Pharmacy, BRAC University, who gave me the opportunity to conduct my project work.

Besides, I am also thankful to all of my faculty members of the Pharmacy Department of BRAC University, who helped me to grow my interest in my studies.

In Last, I am thankful to my parents, husband and friends for being supportive towards me throughout the whole journey.

# Table of Contents

<b>Declaration.....</b>	<b>ii</b>
<b>Approval .....</b>	<b>iii</b>
<b>Abstract.....</b>	<b>iv</b>
<b>Dedication .....</b>	<b>v</b>
<b>Acknowledgement .....</b>	<b>vi</b>
<b>Table of Contents .....</b>	<b>vii</b>
<b>List of Tables .....</b>	<b>x</b>
<b>List of Figures.....</b>	<b>xi</b>
<b>List of Acronyms .....</b>	<b>xiii</b>
<b>Chapter 1 Introduction.....</b>	<b>1</b>
1.1 What is Alzheimer’s Disease? .....	1
1.2 Effects of Alzheimer’s Disease on the Brain.....	2
1.3 Types of Alzheimer’s Disease .....	4
1.4 Biomarkers of Alzheimer’s Disease .....	6
<b>1.5 Rationale of the study:.....</b>	<b>8</b>
1.6 Aim of the Study:.....	8
1.7 Objectives of the Study:.....	8
<b>Chapter 2 Different Biomarkers of Alzheimer’s Disease.....</b>	<b>9</b>
2.1 Cerebrospinal Fluid Biomarkers .....	9
2.1.1 Cerebrospinal Fluid Amyloid- $\beta$ .....	10

2.1.2. Cerebrospinal Fluid $\tau$ Proteins .....	13
<b>2.1.3 Total <math>\tau</math> Proteins .....</b>	<b>15</b>
2.1.4 Hyperphosphorylated $\tau$ Proteins .....	16
2.1.5. Combination of $A\beta$ and $\tau$ Proteins .....	17
2.2 Blood Biomarkers of the Alzheimer’s Disease.....	19
2.2.1 Plasma Amyloid- $\beta$ .....	20
2.2.2 Plasma $\tau$ Concentration.....	20
2.2.3 Plasma Neurofilament Light (NF-L) .....	21
2.2.4 Plasma Clusterin .....	22
2.3 Ocular Biomarker.....	23
2.3.1 Retinal Amyloid Beta Accumulation.....	23
2.3.2 Loss of Retinal Ganglion Cells and Nerve Fiber Layer.....	24
<b>2.3.3 Retinal Vascular Biomarkers.....</b>	<b>25</b>
2.3.4 Lens Biomarkers .....	26
2.4 Neuroimaging Techniques for the Biomarkers of Alzheimer’s disease .....	26
2.4.1 Structural Neuroimaging Techniques of Alzheimer’s Disease.....	27
2.4.1.1 Computed Tomography (CT) .....	27
2.4.1.2 Structural Magnetic Resonance Imaging (sMRI) .....	28
2.4.1.3 Diffusion Tensor Imaging (DTI) .....	29
2.4.2 Functional Neuroimaging Techniques for Alzheimer’s Disease .....	30
2.4.2.1 Functional Magnetic Resonance Imaging (fMRI) .....	31



2.4.2.2 Molecular and Beta Amyloid Imaging .....	32
2.4.2.3 Positron Emission Tomography (PET).....	32
2.4.2.4 Magnetic Resonance Spectroscopy (MRS) .....	33
2.4.2.5 Single Photon Emission Computed Tomography (SPECT) .....	34
2.4.2.6 Magnetic Encephalography .....	35
<b>Chapter 3 Discussion .....</b>	<b>36</b>
<b>Chapter 4 .....</b>	<b>38</b>
Conclusion .....	38
Future Direction .....	39
<b>References.....</b>	<b>40</b>

## List of Tables

Table 1: Biomarkers Of Alzheimer’s Disease .....	7
--	---

## List of Figures

Figure 1: 1a and 1d representing normal brains (age 74 and 104 years old), 1b representing frontoparietal temporal atrophy in Alzheimer's Disease, 1c representing 'Knife Edge' atrophy at frontotemporal region in Pick's Disease (Kövari, Hof, & Bouras, 2011) .....	3
Figure 2: Different Age Groups of people suffering from Alzheimer's Disease (Association, 2019) .....	4
Figure 3: Hypothetical Model Showing Abnormalities of the Biomarkers of Alzheimer's Disease (Selkoe, Hardy, Selkoe, & Hardy, 2016).....	6
Figure 4: The effects of amyloid- $\beta$ in the pathophysiology of Alzheimer's Disease (Morley, Farr, & Nguyen, 2018).....	11
Figure 5: Cleavage of APP and production of Amyloid- $\beta$ analogues (Weiner et al., 2015) ...	11
Figure 6: Sequence of Amyloid- $\beta$ and cleavage sites within APP. Numbers corresponding to the convention of A $\beta$ . Cleavage sites indicated for $\beta$ -, $\alpha$ -, and $\gamma$ -secretases (Findeis, 2007) .	12
Figure 7: Formation of Amyloid Plaques (Drolle, Hane, Lee, & Leonenko, 2014) .....	13
Figure 8: Disorientation of $\tau$ -proteins and its effects (Comorbidities, Zheng, Shultz, & Hovens, 2013) .....	15
Figure 9: Stages of Forming Neuro Fibrillary Tangles in the patients of Alzheimer's Disease (Šimi et al., 2016).....	16
Figure 10: A. Cellular pathology of healthy (left) and diseased (Alzheimer's Disease, right) brain; B. Brain atrophy in Alzheimer's Disease; C. time-course of progression of the Alzheimer's Disease (Pitt, 2019) .....	18
Figure 11: Laser Ophthalmoscopy and Optical Coherence Tomography detecting retinal A $\beta$ deposits in Alzheimer's Disease patients and controls (A, B). Arrowheads showing A $\beta$ deposits in the GCL, red stars indicating loss of nuclei. Nissl area loss in AD patients(C). Amyloid- $\beta$ deposition in AD (D). Laser Ophthalmoscope using Curcumin to label amyloid depositions in	

alive AD patients (E). Retinal Amyloid Index display in patients of Alzheimer’s Disease and healthy subjects on scatter bar plot using fluorescent curcumin (F). Qualitative deposition of amyloid protein in different retinal regions (G). OCT images of curcumin positive amyloid depositions as the red marks, Green lines defining the line of OCT segmentation (H). OCT revealing amyloid plaque in outer retinal layers; above retinal pigment epithelium (RPE) and along with uninterrupted RPE and Bruch’s membrane (I) (Koronyo-hamaoui, 2017). .....25

Figure 12: Brain Atrophy in Cognitively Normal Patient, in amnesic Mild Cognition Impairment Patients and in Alzheimer’s Disease Patient (Vemuri & Jack, 2010).....28

## List of Acronyms

AD	Alzheimer's disease
aMCI	Amnesic Mild Cognition Impairment
APoE	Apolipoprotein E
APP	Amyloid Precursor Protein
A $\beta$	Amyloid Beta
BOLD	Bold Oxygen Level Dependent
CMR <sub>glc</sub>	Cerebral Metabolic Glucose Utilization Rate
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DLB	Dementia with Lewy Bodies
DNA	Deoxyribonucleic Acid
DTI	Diffusion Tensor Imaging
EOAD	Early Onset Alzheimer's Disease
FDG	Fluorodeoxyglucose
FDG-PET	Fluorodeoxyglucose Positron Emission Tomography
fMRI	Functional Magnetic Resonance Imaging
FTB	Fronto Temporal Lobe Dementia
LOAD	Late Onset Alzheimer's Disease
MCI	Mild Cognition Impairment
MEG	Magnetic Encephalography
MMCI	Multi Domain Mild Cognition Impairment

MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAA	N-Acetyl Aspartate
naMCI	Non-amnesic Mild Cognition Impairment
NF-L	Neurofilament Light
NFT	Neurofibrillary Tangles
OPT	Optical Coherence Tomography
PET	Positron Emission Tomography
PHS	Paired Helical Filaments
PiB-PET	Pittsburgh Compound B Positron Emission Tomography
PSEN-1	Presenilin 1
PSEN-2	Presenilin 2
P-Tau	Phosphorylated Tau
RGCL	Retinal Ganglionic Cell Layer
RNA	Ribonucleic Acid
RPE	Retinal Pigment Epithelium
SMCI	Single Domain Mild Cognition Impairment
sMRI	Structural Magnetic Resonance Imaging
SPECT	Single Photon Emission Computed Tomography
SQUID	Superconductive Quantum Inferences Device
T-tau	Total Tau
VaD	Vascular Dementia
VBM	Voxel Based Morphometry

WHO World Health Organization

$\beta$ A Beta Amyloid

## Glossary

**Thesis:** An extended research paper that is part of the final exam process for a graduate degree. The document may also be classified as a project or collection of extended essays.

**Glossary:** An alphabetical list of key terms

This is an optional page and can be removed if not used.

Use one table row for each item to allow sorting using Word's table tools.

Apply the style **1\_Para\_NoSpace** to table rows as shown here.



# **Chapter 1**

## **Introduction**

### **1.1 What is Alzheimer's Disease?**

Alzheimer's disease is a neurodegenerative disorder that is responsible for destroying memory and cognitive abilities of a person very slowly. It was first recognized back in 1906 (Association, 2018). In 2019, the estimated total cost which will be spent on providing the care and services for the patients of Alzheimer's Disease is around \$290 billion in the United States of America (Association, 2019). It can occur to a person both at middle age and at old age. According to a study of WHO there are approximately 7 million people suffering from AD in the United States of America above the age of 65 and the study also claimed that this number will get tripled by the year of 2050 (Fiandaca, Mapstone, Cheema, & Federoff, 2014). AD is a type of dementia. Dementia can be used as a broader term and there are different types of dementia and AD is one of them. According to WHO being the most common form of dementia AD is responsible for approximately 60-70% of the total cases filed (Duthey & Ph, 2013). It destroys the brain cells in cerebral cortex. As a result, it causes the shrinkage of brain cells and restricts passage of blood, oxygen, nutrients and neurotransmitter within the brain cells.

Alzheimer's disease is further more damaging than all the other forms of dementia. The progression of AD cannot be reversed or stopped but with medication it can be slowed down (Duthey & Ph, 2013). The death rate due to cardiac diseases or due to cancer is gradually decreasing because of the advancements in the fields of their diagnosis, treatment and management whereas the death rate due to the Alzheimer's Disease has been increasing gradually (Fiandaca et al., 2014). The major hindrance in the treatment or management of AD is the failure in the early detection of the disease. For the early detection of this disease lies the need of definitive biomarkers. There remains a dire need of the biomarkers which will be able

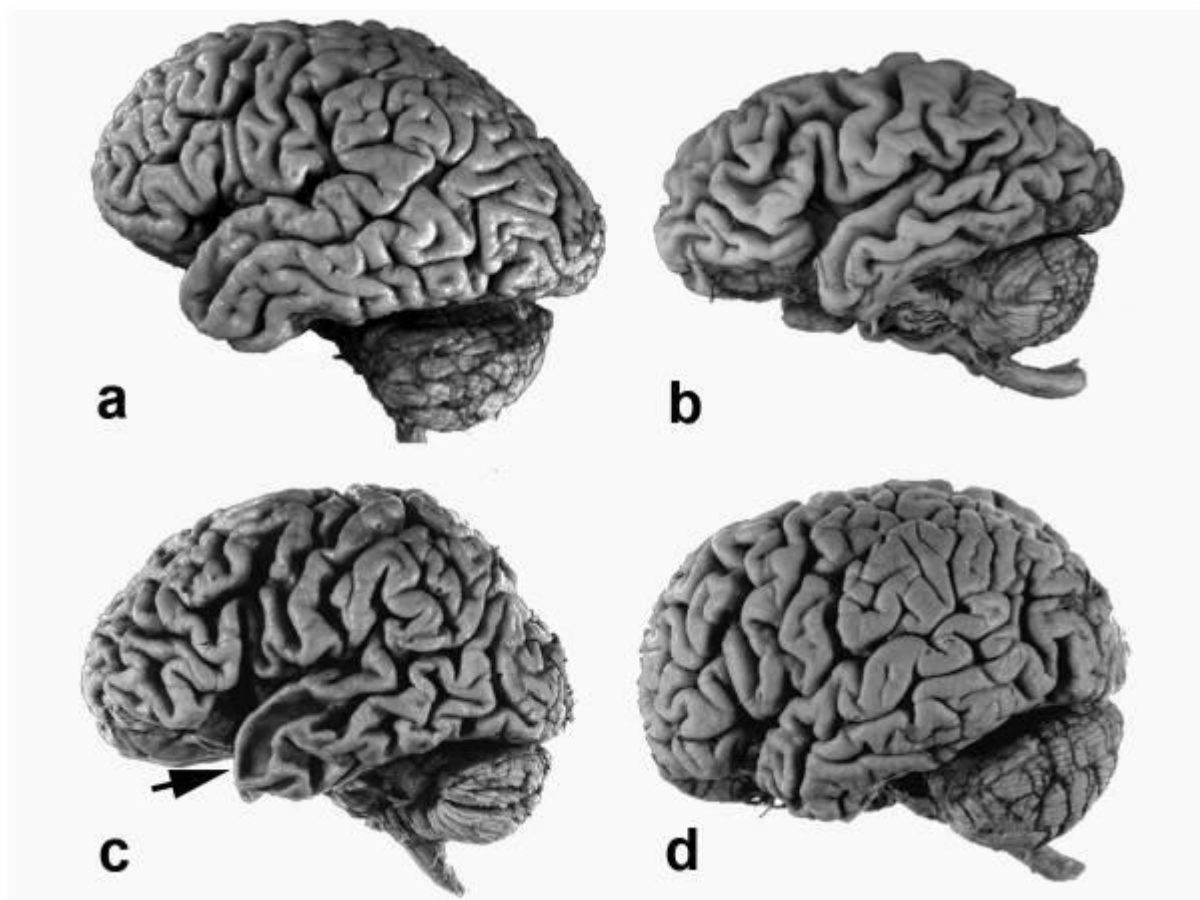
to diagnose that at which level of cognition impairment the patient is and exactly when to start the use of medications. Before the establishment of such biomarkers it cannot be promised that the disease state can be cured or improved. Because to improve the disease state towards betterment the medications need to get started before the neural fluid has already become unresponsive towards them (Fiandaca et al., 2014). Alzheimer's disease lacks of specific drug targets. To alter the disease state the drugs need to be directed towards the specific targets and without the molecular understanding of the mechanisms and biomarkers that is not possible (Jack et al., 2018).

As there is no exact signature biomarker of AD, the diagnosis of AD is a multi-step process of confirming a wide range of biomarkers. The most targeted biomarkers constitute of CSF biomarkers. Collecting the CSF fluid samples and confirming the biomarkers is time consuming and expensive. So, it should be the second line of confirming the disease whereas there should be a fast and cost effective first line of diagnosis steps to be used in large number of populations. Thus, blood-based biomarkers can be the first step of the multiple steps. The blood-based biomarkers can predict the disease and then the CSF biomarkers can contribute towards confirming the disease. Thus, the multi-step diagnosis process can detect the disease as early as possible (O'Bryanta et al., 2018).

## **1.2 Effects of Alzheimer's Disease on the Brain**

The brain consists of billions of neural cells or nerve cells which are called neurons. Each neuron is connected to the other so that they can communicate among the cells and perform their functions. Different neurons have different functions. Some stores memory, some helps to smell, aids in hearing or commands the muscles to move. For all these functions to take place properly the neurons need nutrition, energy and sufficient amount of oxygen. The Alzheimer's Disease hampers in the communication between the neurons and thus, blocks the pathways of

energy flow from neuron to neuron. This blockage happens due to two different kinds of protein depositions in the brain. AD has mainly two types of protein depositions one is  $\beta$ -amyloid plaques and tau-tangles. The  $\beta$ -amyloid plaques formed between the spaces of two adjacent neurons whereas the tau tangles are twisted tau proteins depositing inside the neurons. This is how the flow of energy, nutrition and electric impulses are blocked and due to that the cells eventually shrink and die because of AD (Alzheimer's & Dementia, 2015).



*Figure 1: 1a and 1d representing normal brains (age 74 and 104 years old), 1b representing frontoparietal temporal atrophy in Alzheimer's Disease, 1c representing 'Knife Edge' atrophy at frontotemporal region in Pick's Disease (Kövari, Hof, & Bouras, 2011)*

Specifically, where the protein accumulations first start is, still being investigated because it initially happens at a microscopic level even long before the symptoms start to emerge. But after the onset it starts to spread all over the brain. Normally there are protein depositions in the brain when a person ages but in case of AD the depositions are several folds higher (Alzheimer's & Dementia, 2015).

### 1.3 Types of Alzheimer's Disease

On the basis of age of onset and other pathological factors Alzheimer's Disease can be classified into two groups:

- Early Onset Alzheimer's Disease (EOAD)/ Familial AD
- Late Onset Alzheimer's Disease (LOAD)/ Sporadic AD (-Précoma, Rodríguez-Cruz, Berumen L, & García-Alcocer, 2016)

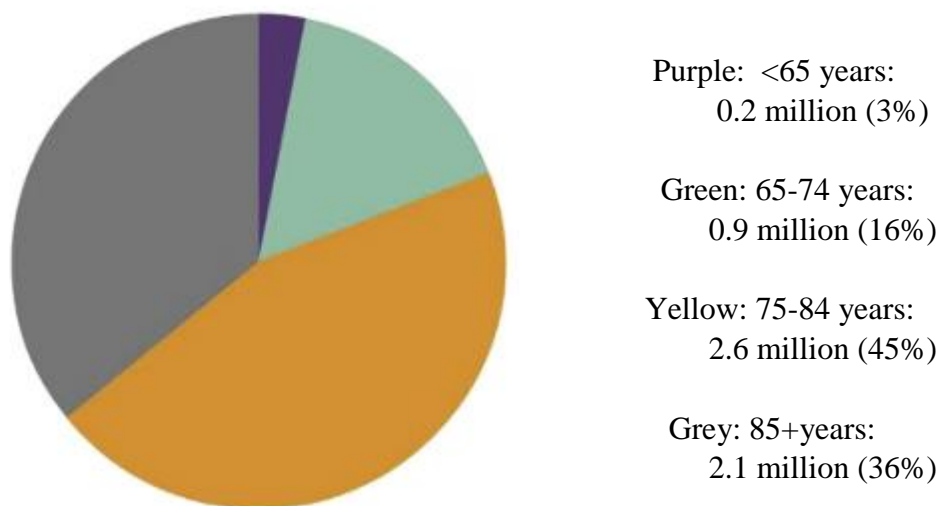


Figure 2: Different Age Groups of people suffering from Alzheimer's Disease (Association, 2019)

The EOAD appears in patients below the age of 60. Approximately 5% of total cases reported, Familial AD occurs in patients who have had family history of AD for past three generations. In most of the cases the patients carry mutations either in Presenilin-1 (PSEN-1), Presenilin-2 (PSEN-2) or in Amyloid Precursor Protein (APP). The mutations in these precursor proteins causes the production of amyloid  $\beta$ -42 which is the most neurotoxic form of amyloid fragment consisting of 42 amino acids in AD patients. Thus, leading to early onset of AD (-Précoma et al., 2016). The study of these mutations even provides scope of establishing biomarkers to diagnose at which level of cognition impairment the patient is over the different stages of EOAD. Even some findings can also be correlated with LOAD though EOAD and LOAD do not have exactly identical features, but as they carry similar clinical features these information can be useful in both of the types of AD (Yan et al., 2018).

The LOAD is reported in patients aged over 65 years. This form of AD is related to multiple factors like obesity, hypertension, hypercholesterolemia, diabetes and overall lipid metabolism (-Précoma et al., 2016). However, the main role player here is the APoE or apolipoprotein E. It is a ligand protein which carries the different lipids or lipoproteins from the circulation to different surface binding receptors and thus maintains lipid homeostasis. It is most abundant in liver and after that it is mostly abundant in brain, specifically in the CSF, hippocampus, cerebral cortex, cerebellum, medulla and as well as in some other regions with the association of lipoproteins. In the brain, APoE is secreted from the astrocytes. Moreover, when the brain cells are injured there is an overproduction of APoE to redistribute the lipids and heal the injury. When there are amyloid accumulations there are increased apolipoprotein E which binds to the  $\beta$ A and forms nondegradable complexes. Specifically, the sub type of APoE-2 which is defective in receptor binding having a cysteine but subtype E3 and E4 have arginine residue and are not defective. Thus APoE contributes in the occurrence of sporadic AD and lowers the age of the occurrence (Ueno et al., 2011).

## 1.4 Biomarkers of Alzheimer's Disease

Biomarkers are basically the substances inside the body which mark any changes that the body undergoes due to the invasion of a disease or it can be any structural changes inside or outside the body due to any disease state. So, they are also called as 'Biological Markers' (Sharma & Singh, 2016). There are wide ranges of biomarkers associated with the diagnosis of Alzheimer's disease. But two most frequently used biomarkers are amyloid beta plaques and neurofibrillary tangles which are composed of hyperphosphorylated tau. When these amyloid beta plaques aggregate and cannot get metabolized they yield to another cascade of inflammation reaction (Leuzy, Heurling, Ashton, Schöll, & Eduardo, 2018). So, there are also inflammatory biomarkers present for the diagnosis of AD. Similarly, there are researches going on urine biomarkers and ocular inflammatory biomarkers in the retina of the eye for the early detection of AD. Overall the biomarkers of this disease can be divided into few classes like CSF biomarkers, imaging biomarkers, blood biomarkers, ocular biomarkers and urine biomarkers.

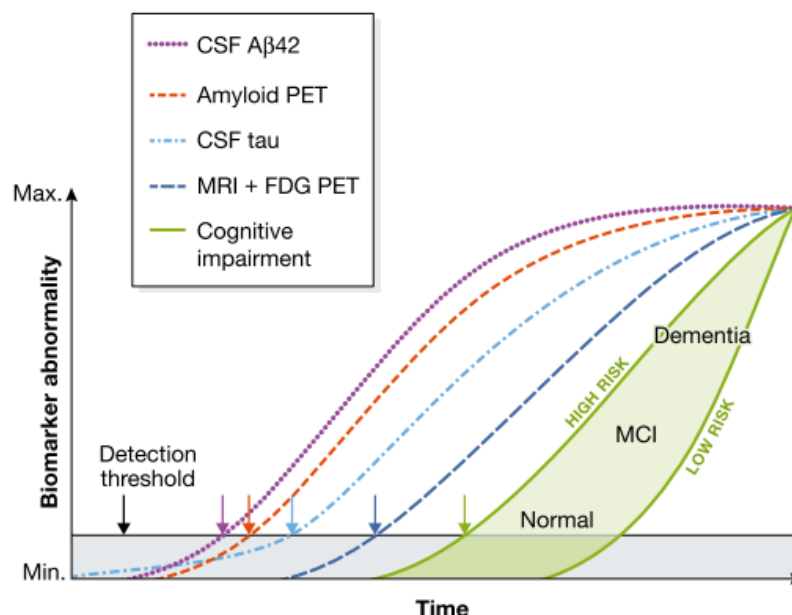


Figure 3: Hypothetical Model Showing Abnormalities of the Biomarkers of Alzheimer's Disease (Selkoe, Hardy, Selkoe, & Hardy, 2016)

<b>CSF Biomarkers</b>	<b>Blood Biomarkers</b>	<b>Ocular Biomarkers</b>
CSF Amyloid $\beta$	Plasma $\tau$ Concentration	Retinal Amyloid- $\beta$ Accumulation
CSF $\tau$ Proteins	Neurofilament Light (NF-L)	Loss of Retinal Ganglion and Nerve Fiber Layer
Total $\tau$ Proteins	Plasma Amyloid Beta	Retinal Vascular Biomarkers
Phosphorylated $\tau$ Proteins	Plasma Clusterins (O'Bryanta et al., 2018)	Lens Biomarkers ((van Wijngaarden, Hadoux, Alwan, Keel, & Dirani, 2017)
Combination of A $\beta$ -42 and $\tau$ Proteins (Holtzman, 2012)		

*Table 1: Biomarkers of Alzheimer's Disease*

## **1.5 Rationale of the study:**

Alzheimer's is such a disease which do not have an exact treatment right now. All that can be done is, to take treatment and medications as early as possible to slow down the progression as the disease cannot be cured or the course of occurrence can be reversed, rather with early diagnosis and treatment the damage can be minimized and the patient can live a better life. However, in most of the cases the disease is diagnosed long after the progression has already been started. Moreover, previously in most of the cases, the disease was confirmed at the autopsy of the patient. Therefore, for the better life and proper treatment it needs proper diagnosis and confirmation of the disease. This is why for early and correct diagnosis there lies the need of establishing biomarkers specific to the disease. This study is to compile the emerging biomarkers of Alzheimer's Disease and to correlate the biomarkers with the disease progression, how they do mechanize in normal body and how they are changed in the state of Alzheimer's Disease.

## **1.6 Aim of the Study:**

The aim of this review is to compile all of the possible biomarkers of Alzheimer's Disease which are expected to contribute towards the early diagnosis of the disease and to reveal how their pathophysiology is correlated with the Alzheimer's Disease.

## **1.7 Objectives of the Study:**

The objectives of this review are,

- To identify the emerging biomarkers of Alzheimer's Disease
- To compile the information of early diagnosis of Alzheimer's Disease
- To identify relationships among different biomarkers
- To discuss how the biomarkers show fluctuations in the AD patients



## Chapter 2

### Different Biomarkers of Alzheimer's Disease

#### 2.1 Cerebrospinal Fluid Biomarkers

Throughout the past decades many cerebrospinal fluid biomarkers of Alzheimer's Disease have been developed. Three major kinds of cerebrospinal fluid biomarkers have been developed over time which are amyloid- $\beta$  plaques (chain of long 42 amino acids), total  $\tau$  protein depositions and phosphorylated  $\tau$  proteins (Bjerke & Engelborghs, 2018). The amyloid- $\beta$  peptides and the neurofibrillary tangles form senile plaques whereas, dystrophic neurites are formed with hyperphosphorylated  $\tau$  proteins. These pathological changes or neurodegeneration starts approximately 20-30 years before the emergence of the clinical symptoms (Pawlowski, Meuth, & Duning, 2017). Cerebrospinal fluid biomarkers are more promising than blood serum or urine biomarkers. Moreover, CSF can demonstrate more biochemical changes than urine or blood serum as it is in direct contact with the brain cells. According to a number of studies which detected the 'AD Profile' in CSF in AD patients showed that there was decreased amount of A $\beta$ -42 increased amount of total  $\tau$  (T- $\tau$ ) and phosphorylated  $\tau$  (P- $\tau$ ) which eventually meant increased A $\beta$  deposition, increased T- $\tau$  and P- $\tau$  meant neuronal damage, degeneration and deposition of neurofibrillary tangles. As a result, these biomarkers have received attention for further research and have also been added in the diagnostic profile for AD by National Institute of Aging and Alzheimer's Association. Not only that, according to European Federation of Neurological Society scheduled analysis of CSF biomarkers have been recommended for differential studies of Alzheimer's Disease (Bjerke & Engelborghs, 2018). The CSF biomarkers which have been frequently studied for the diagnosis of Alzheimer's Disease are as follows:

- CSF Amyloid  $\beta$

- CSF  $\tau$  Proteins
- Total  $\tau$  Proteins
- Phosphorylate  $\tau$  Protein
- Combination of A $\beta$ 42 and  $\tau$  Protein (Holtzman, 2012).

### **2.1.1 Cerebrospinal Fluid Amyloid- $\beta$**

The major role player in the disease pathology of Alzheimer's Disease is said to be the amyloid protein and following are the effects of the amyloid protein in the occurrence and progression of Alzheimer's Disease. The most promising biomarkers of Alzheimer's Disease are cerebrospinal fluid  $\beta$ -amyloid peptides in conjunction with CSF tau-proteins because they can indicate the physiological and pathological changes due to Alzheimer's disease with most specificity and sensitivity. In AD patients the amount of CSF amyloid-beta protein level decreases gradually with the increased  $\tau$ -proteins. This condition can also be found in MCI patients who later on turn to Alzheimer's Disease patients (Tapiola et al., 2015).

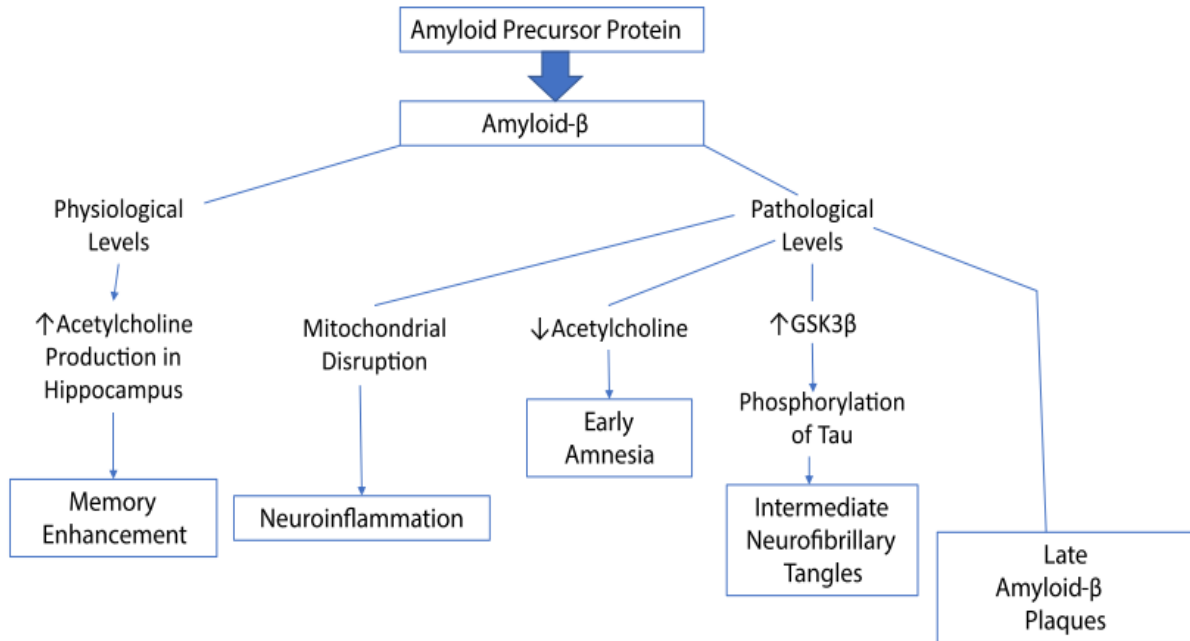


Figure 4: The effects of amyloid- $\beta$  in the pathophysiology of Alzheimer's Disease (Morley, Farr, & Nguyen, 2018).

Amyloid Protein Precursors (APP) plays the major role in depositing amyloid plaques and here, amyloid proteins are the metabolism products of the neurons which are produced through cascades of cleavage reactions where the major role players are the  $\alpha$ ,  $\beta$  and  $\gamma$  secretase (Pawlowski et al., 2017).

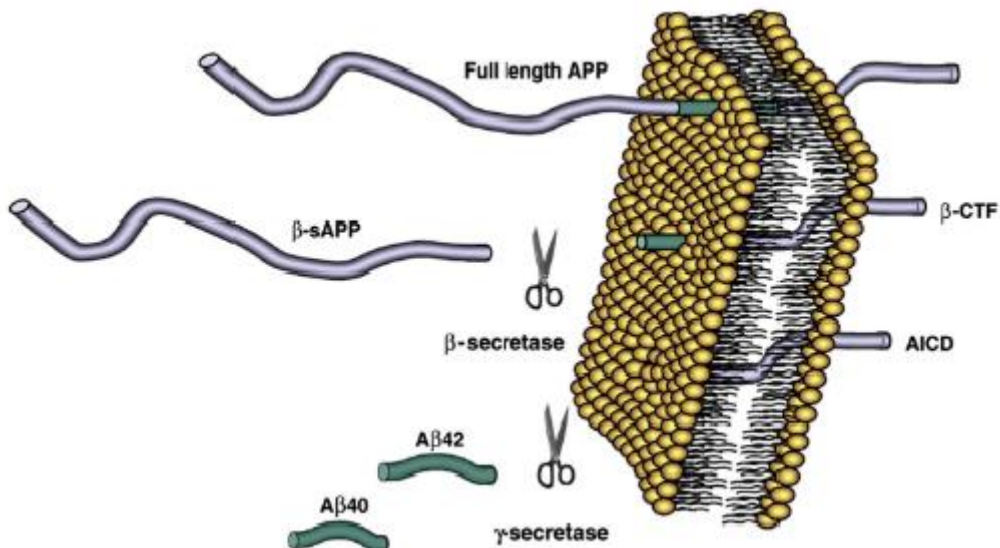


Figure 5: Cleavage of APP and production of Amyloid- $\beta$  analogues (Weiner et al., 2015)

If we look into familial AD there are three types of genes which are mutated and they are, APP (Amyloid Protein Precursor), Presenilin-1 (PS-1) and Presenilin-2 (PS-2). The mutations of these genes refer to only a few numbers of cases which are filed with AD but they are able to generalize a central idea on how the APP works on the amyloid- $\beta$  peptide formation (Findeis, 2007). APP is an integrated transmembrane protein where, there is only one transmembrane region, one big extracellular region and a small cytoplasmic tail. In humans, APP gene is encoded on the long arm of chromosome no 21 (Pawlowski et al., 2017). Mutations on the chromosome 21 of APP gene can rise the level of whole A $\beta$  (Amyloid  $\beta$ ) or only A $\beta$ -42. On the other hand, the mutation on chromosome 14 of the PS-1 gene and on chromosome 1 of PS-2 gene both can increase the level of only A $\beta$ -42 (Findeis, 2007)



Figure 6: Sequence of Amyloid- $\beta$  and cleavage sites within APP. Numbers corresponding to the convention of A $\beta$ . Cleavage sites indicated for  $\beta$ -,  $\alpha$ -, and  $\gamma$ -secretases (Findeis, 2007).

The major function of APP is to induce cellular adhesion and through this it supports the neurons in the brain. Therefore, decreased APP means decreased neural functionality, viability and outgrowth. Amyloid Precursor Proteins are located in neural bodies, dendrites and axons where, mostly their shorter (40 residues) and slightly larger (41 and 42 residues) isoforms and minimal number of other isoforms (36 or 37 residues) are produced. This production of amyloid peptides is balanced by the elimination or degradation of the peptide through different enzymatic degradation, cellular clearance, active or passive transport or even through accumulation or deposition into insoluble amyloid aggregates (Pawlowski et al., 2017).

Amyloid is composed of proteins which are insoluble in nature and it is regarded as the major hallmark of Alzheimer's Disease. According to 'Amyloid Cascade Hypothesis', the AD pathogenesis starts to take place when there is an imbalance between the production and clearance of amyloid proteins. The over production of soluble amyloid- $\beta$  isoforms means they will bind with the components of both neuronal and non-neuronal plasma membrane inside the central nervous system which will eventually cause neuronal degradation and decreased synaptic functionality (Pawlowski et al., 2017).

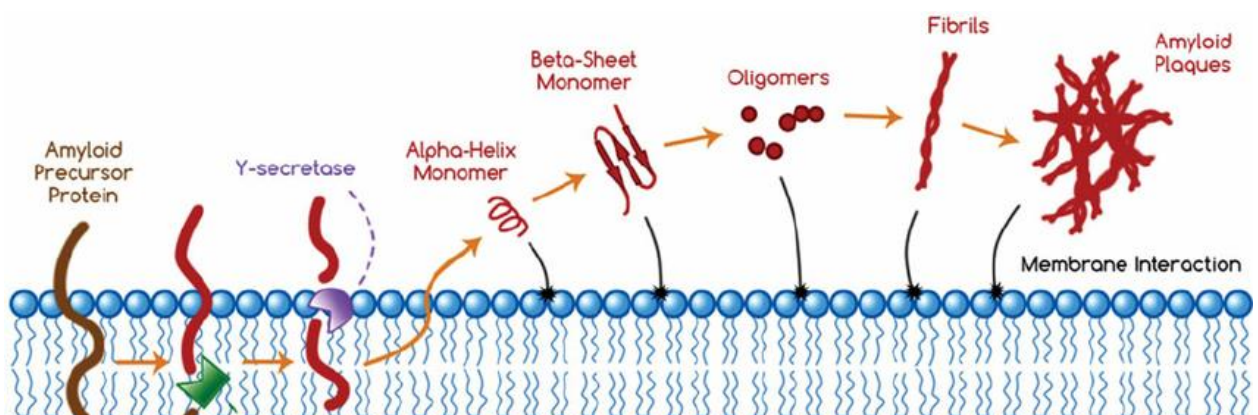


Figure 7: Formation of Amyloid Plaques (Drolle, Hane, Lee, & Leonenko, 2014)

Decreased CSF amyloid- $\beta$  protein means increased amount of amyloid aggregates. Only measuring the amount of amyloid aggregates will not be sufficient for the diagnosis of AD as with increasing age there is normally slightly increased amount of amyloid plaques or aggregates can be seen. So instead of considering A $\beta$ -42 alone it will be more efficient to consider the ratio of A $\beta$ -42 to A $\beta$ -40 where A $\beta$ -42 is more toxic in comparison to A $\beta$ -40 (Findeis, 2007).

### 2.1.2. Cerebrospinal Fluid $\tau$ Proteins

$\tau$  is a protein which is highly soluble, unfolded and also heat stable which is situated mostly at the axons of the neurons.  $\tau$  proteins promote the stability and assembly of the axonal microtubules and thus assist axonal transportation and functionality (Pawlowski et al., 2017;

Ping et al., 2018). Microtubule is a part of cytoskeleton which supports the cells to take different coordinated movements and helps the cells to undergo different structural changes. Microtubules are composed of a group of proteins which are called Microtubule Associated Proteins (MAPs) and  $\tau$  protein is one of the main members of MAPs (Jouanne & Rault, 2017). Though the amount of  $\tau$  proteins increase in a person with aging, it is seen that in the case of Alzheimer's Disease the  $\tau$  proteins rise exponentially (Sharma & Singh, 2016). In the patients of Alzheimer's Disease, the  $\tau$  proteins are miss folded and they do not bind with tubulins and not even promote microtubule assembly and thus disrupt the proper organization of the microtubules due to various modifications of the  $\tau$  proteins. Specially, when they are phosphorylated this promotes disruptions in microtubule assembly and deposition into  $\tau$  aggregation. However, hyperphosphorylation is not the only modification that the  $\tau$  proteins go through rather there are many more modification processes like nitration, glycation, glycosylation, ubiquitination etc. Due to such modifications, the tau proteins transform from monomers to oligomers and then transform into helical filaments which aggregates into NTFs or Neuro Fibrillary Tangles which can be found in abundant in Alzheimer's Disease patients (Ping et al., 2018). These aggregates when are released into the extracellular fluid they can bind with the neurons and lose their ability to enter the nucleus which causes the damage of DNA structure. Moreover, it was observed that the abnormal tau-ribosomes also disturb the RNA-translation process (Pawlowski et al., 2017).

Though hyperphosphorylation of the  $\tau$  proteins play the major role in AD pathologies but there are also other important post translational modification contributing towards Alzheimer's Disease and all of these can be established as potential biomarkers. Some of the modifications can be the cleavage of the  $\tau$  aggregates by the action of proteolytic enzymes (Truncation), non-enzymatic modifications which elevates the production of aggregates in association with the

formation of glycation products (Glycation), N-glycosylation of the  $\tau$  proteins to form aggregates (Glycosylation), abnormal  $\tau$  nitration yielding more NFTs and ubiquitylated  $\tau$  proteins assisting in the formation of tangles which in turn assists neuronal death (Ubiquitination) etc (Ping et al., 2018).

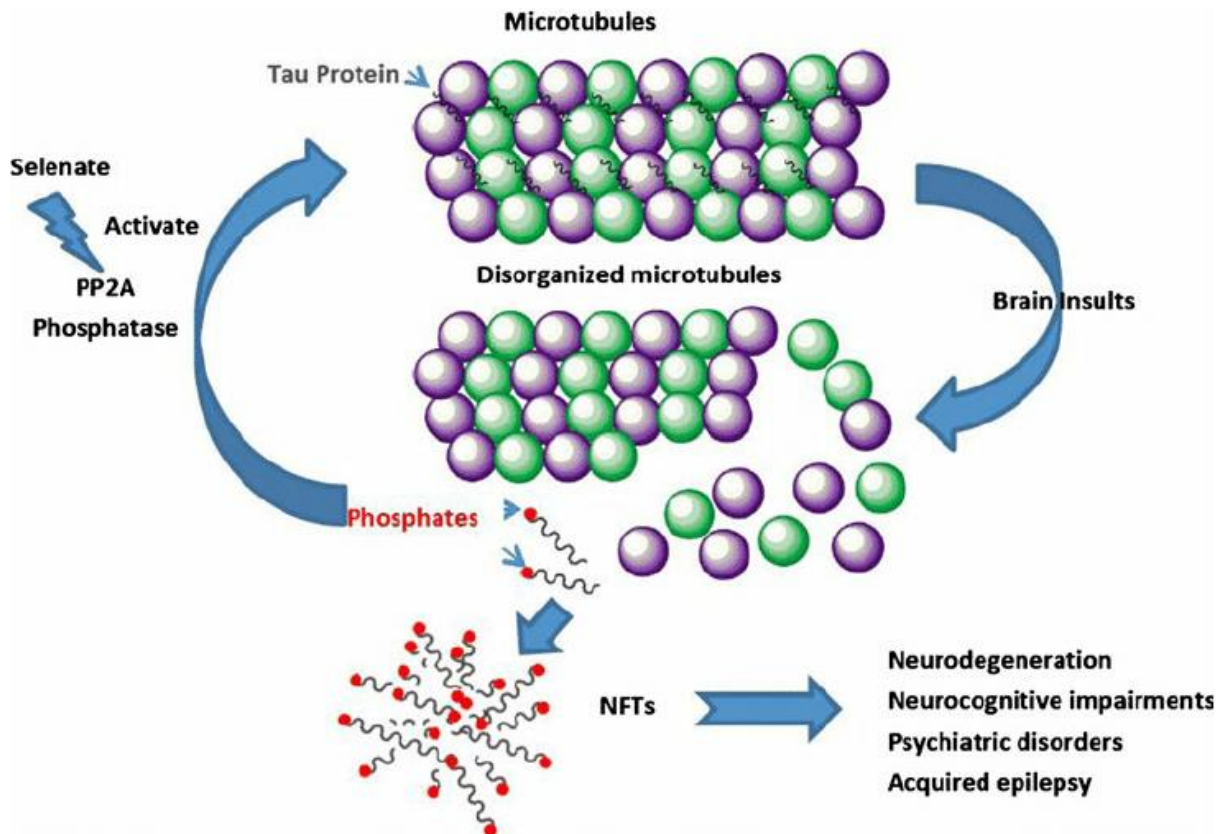


Figure 8: Disorientation of  $\tau$ -proteins and its effects (Comorbidities, Zheng, Shultz, & Hovens, 2013)

### 2.1.3 Total $\tau$ Proteins

$\tau$  proteins form NFTs or neurofibrillary tangles in AD which get attached to the neurons. Therefore, most of the amount of the  $\tau$  proteins are intra-neuronal so, the amount of circulatory or CSF  $\tau$  should decrease. However, it has been seen that in the patients of Alzheimer's Disease the amount of circulatory  $\tau$  proteins in the cerebrospinal fluid also increase markedly. So the presence of neurofibrillary tangles can mark the occurrence of the disease while the total count

of both of the intra-neuronal NFTs and CSF  $\tau$  protein can predict the extent of neurodegeneration and disease progression (Holtzman, 2012).

### 2.1.4 Hyperphosphorylated $\tau$ Proteins

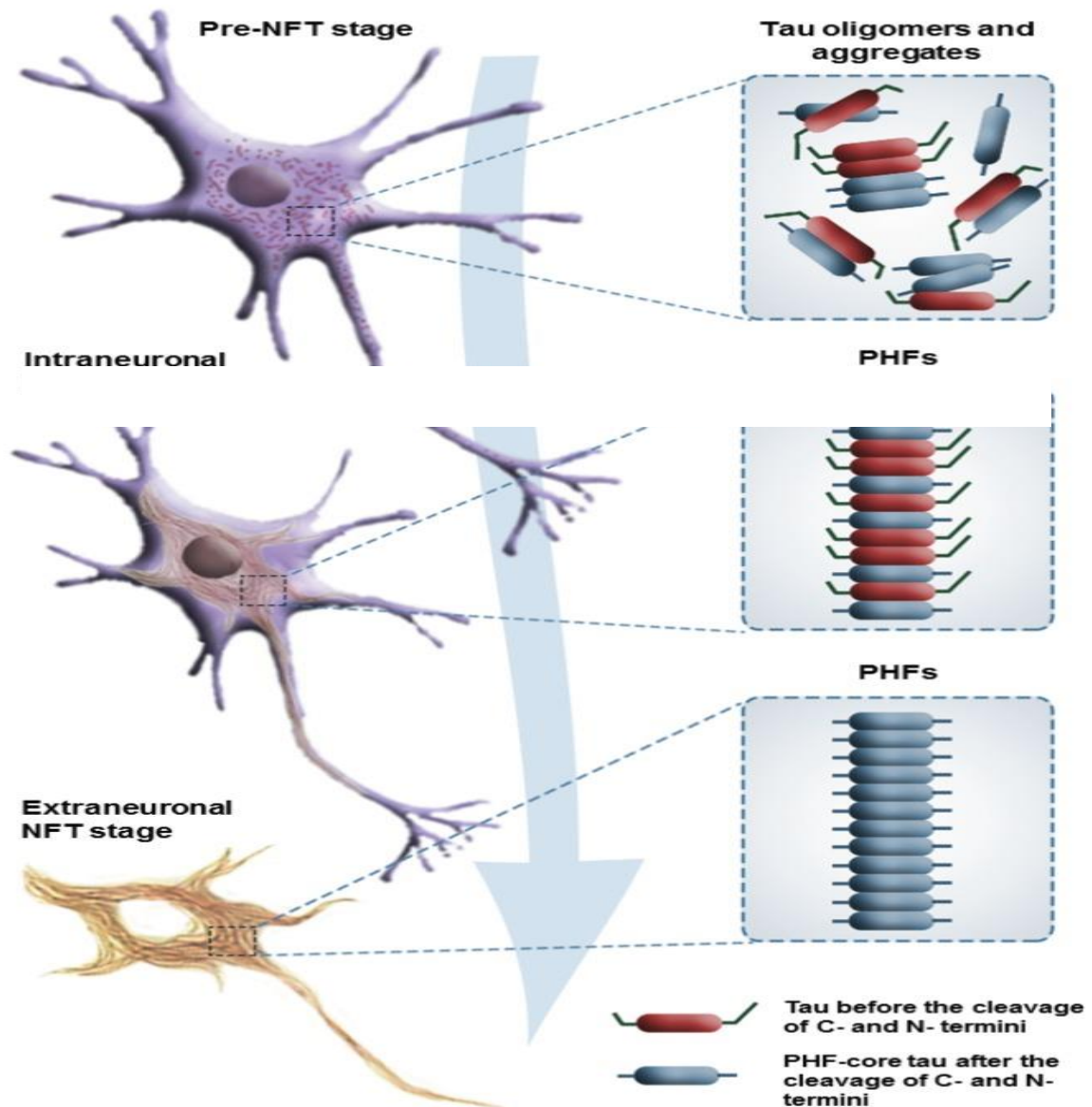


Figure 9: Stages of Forming Neuro Fibrillary Tangles in the patients of Alzheimer's Disease (Šimi et al., 2016)

It is important for the  $\tau$  proteins to undergo phosphorylation for the normal functions of the microtubules (Ping et al., 2018). It is called hyperphosphorylation when the proteins are phosphorylated to the extent which is more than the need and sometimes in the patients

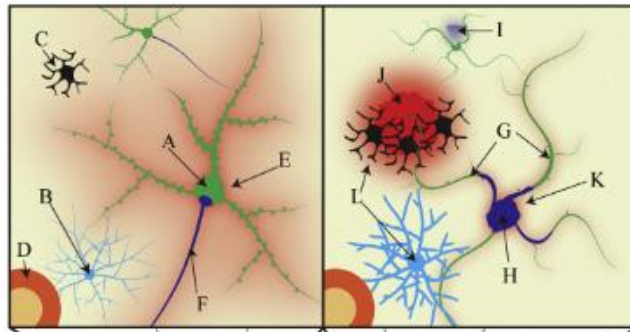


suffering from Alzheimer's Disease the extent is approximately 3 to 4 folds (Ping et al., 2018). One of the major post-translational modification of the  $\tau$  proteins is hyperphosphorylation and it is a second major hallmark in Alzheimer's Disease. It is used as both as a prognostic and diagnostic biomarker of the disease (Blennow, 2017; Jouanne & Rault, 2017; Pitt, 2019). The shape of the  $\tau$  proteins is changed when they undergo phosphorylation and this is necessary for their regular functions. There are different phosphorylation sites on the  $\tau$ -proteins but most of them are on the sequence Ser-Pro and Thr-Pro  $\tau$ -proteins which are present on the NFTs, most of them are hyper phosphorylated hence, this presence is regarded as a hallmark of the disease (Holtzman, 2012; Šimi et al., 2016). In the figure 9 it can be seen that in the pre-NFT stage there are different  $\tau$  oligomers are aggregating together. At first, there are both  $\tau$  aggregates coming from both the oligomers which have cleavage of C- and N- termini and also which do not have the cleavage of C- and N- termini and forming the paired helical filaments or PHFs which are eventually forming the intraneuronal Neurofibrillary Tangles. In the second stage, the NFTs are forms of only core oligomers which have the cleavage of C- and N- termini forming the extra-neuronal NFTs stage (Šimi et al., 2016).

### **2.1.5. Combination of A $\beta$ and $\tau$ Proteins**

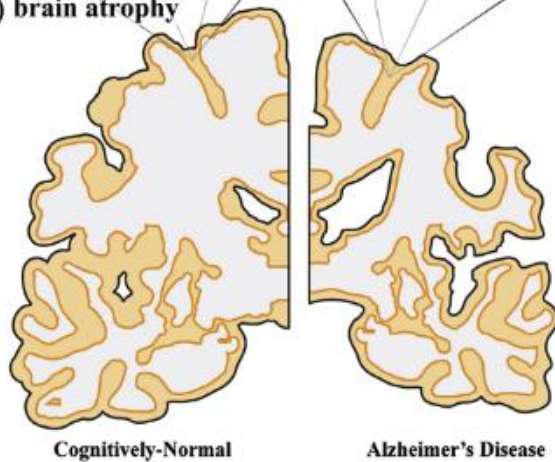
Amyloid- $\beta$  plaques and  $\tau$  proteins both are used as hallmarks of the Alzheimer's Disease and both of them give diagnostic and prognostic values. As Alzheimer's Disease does not still have any exact promising biomarker, it is better to use different biomarkers in combination. Hence, amyloid beta or A $\beta$  protein and  $\tau$  protein are better to use in combination as ratio of CSF  $\tau$  proteins to CSF A $\beta$  gives better distinguishing value of Alzheimer's disease from the rest of other forms of dementia. It can be seen that in the patients of Alzheimer's Disease the amount of CSF amyloid  $\beta$  is decreased whereas, the amount of CSF  $\tau$  protein is increased (Pitt, 2019).

**(A) cellular pathology**



Legend	
A-	neuron (green)
B-	astrocyte (cyan)
C-	microglia (black)
D-	blood vessel (orange)
E-	CSF Aβ42 (red glow)
F-	τ, in axon (blue)
G-	dystrophic neuritis (green)
H-	τ in soma and dendrites (blue)
I-	τ leaking from injured neuron (blue)
J-	amyloid plaque (red)
K-	reduced CSF Aβ42 (lack of red glow)
L-	glial activation (black and cyan)

**(B) brain atrophy**



**(C) time-course**

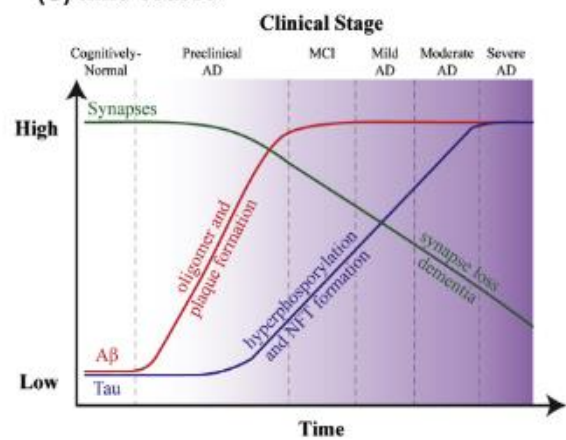


Figure 10: A. Cellular pathology of healthy (left) and diseased (Alzheimer's Disease, right) brain; B. Brain atrophy in Alzheimer's Disease; C. time-course of progression of the Alzheimer's Disease (Pitt, 2019)

Therefore, by compiling all of the Cerebrospinal Fluid Biomarkers together, a broader and generalized scenario of the progression of the Alzheimer's Disease can be obtained. For example, from the above figure different CSF biomarkers can compare between a brain which is cognitively normal and the one having Alzheimer's Disease. From part A and B different characteristics of the disease pathology can be seen. From the picture A on the left side there is section of healthy brain and on the right, there is a diseased one. On the left it can be seen, the healthy neurons are producing amyloid beta proteins which are periodically cleared out by the astrocytes and the microglia. Later on, the microglia and the astrocytes contact with the blood vessels which supplies nutrition to them for maintaining the normal functionality. Moreover, in the healthy neurons, the tau proteins are located on the axon. In contrast, on the right side, there are overproduced amyloid beta proteins which eventually forms aggregates.

Therefore, glial activation is observed surrounding the amyloid- $\beta$  aggregates which acts as sinks that soak up most of the amyloid- $\beta$  proteins from the circulatory CSF. Hence, the amount of CSF amyloid- $\beta$  protein is reduced or decreased. Moreover, the  $\tau$  proteins are relocated on the somatic body and dendrites of the neurons. This blocks the synaptic junctions between the adjacent neurons and blocks the flow of nutrients from one neuron to another, Therefore, the neurons run out of nutrition, starve and eventually cell death takes place which is called the brain atrophy decreasing the normal size of a brain. In the picture C, it shows the time course of the Alzheimer's Disease. It shows that Alzheimer's Disease pathology starts to takes place even 10-20 years before the cognitive impairment takes and with the course of time the  $\tau$  pathology also develops hampering the neural and synaptic functionality (Pitt, 2019).

## **2.2 Blood Biomarkers of the Alzheimer's Disease**

Though cerebrospinal fluid biomarkers gives more accurate diagnosis of Alzheimer's Disease, irrespective of at what stage the disease is, blood biomarkers have advantages over the cerebrospinal fluid biomarkers and thus can have very high practical value than CSF biomarkers (Leuzy et al., 2018; Zetterberg, 2018). Blood samples can be very easily collected from the patients while the collection of the cerebrospinal fluid samples need procedures that requires lumbar punctures. Moreover, there is a report of headache after the puncture and also other complications after the invasive procedure of puncture like internal bleeding, leaving the diagnostic procedure itself a burden on the patient. Therefore, it becomes a great drawback of the CSF biomarkers in comparison to the blood biomarkers (Berman et al., 2018; Bibl, Esselmann, & Wiltfang, 2012). However, there also lie problems with blood biomarkers as the proteins found in the CNS are not always found in the blood with similar concentrations. Moreover, their stability in the blood might be less than that in the CSF. For example, tau proteins have very short life in the blood in comparison with that of the CSF. Again, Amyloid- $\beta$  proteins are also found in the blood but assaying these samples become difficult when other

proteins present in the blood interferes in the procedure or even the sample can undergo photolytic reactions (Zetterberg, 2018). However, a few blood biomarkers which can be proven to be useful in the proper diagnosis of Alzheimer's Disease are as follows:

- Plasma amyloid  $\beta$
- Plasma  $\tau$  Concentration
- Neurofilament light (NF-L)
- Plasma Clustering (O'Bryanta et al., 2018)

### **2.2.1 Plasma Amyloid- $\beta$**

The expression of the protein, Amyloid  $\beta$  is higher in the brain than in the plasma but, the precursor protein APP (Amyloid Precursor Protein) is produced in almost every tissue of the body. But the test results from the blood samples show lower values as there are also different kinds of other proteins which are interfering in the assay process. But it was suggested that somehow, if the interferences could be kept low, it could correlate with the amyloid-beta pathology of Alzheimer's Disease in the brain. However, the result value could be lesser than that of CSF but, it could assist like a confirmation test of AD. The different kinds of plasma proteins which could be involved in the interference are immunoglobulin M, interleukins, pancreatic polypeptides, adhesion proteins, complement proteins etc. Therefore, the results should be interpreted with discretion as there are multiple proteins in the plasma samples (Zetterberg, 2018).

### **2.2.2 Plasma $\tau$ Concentration**

Plasma  $\tau$  concentrations have been studied for the prediction of Alzheimer's Disease but using the traditional ways  $\tau$ -proteins in the plasma or serum is almost ephemeral in AD or MCI (Henriksen et al., 2013). Therefore, plasma  $\tau$  concentration does not correlate with the CSF  $\tau$

proteins to a great extent. But in an injured brain it has been observed that the concentration of the  $\tau$  proteins in the plasma increases giving a sharp peak within the time period of initial first few hours after the injury has been occurred and then over several days the second peak becomes broader. Moreover, it has been seen that the plasma  $\tau$  concentration in the patients of Alzheimer's disease is in increased amount than a person with normal cognitive abilities and the fluctuation of the concentration is not even as clear as in the cerebrospinal fluid. It was also observed that the plasma half-life of the  $\tau$  protein is less than that of in the cerebrospinal fluid and it can be assumed that the  $\tau$  proteins in the plasma fluid are prone to enzymatic reactions. A solution to all these problems which might lead the way of implementing blood  $\tau$  proteins as a biomarker of Alzheimer's disease is to examine the neuronal exosomes to determine the  $\tau$  protein content inside them. Increased amount of total  $\tau$  and phosphorylated  $\tau$  protein was observed in exosome studies. However, more research needs to be done before marking it as a potential biomarker of Alzheimer's Disease (Zetterberg, 2018).

### **2.2.3 Plasma Neurofilament Light (NF-L)**

One presumed biomarker of Alzheimer's Disease is neurofilament light chains. Higher levels of neurofilament light chains are found in brain when there is an axonal injury. Therefore, increased amount of neurofilament light chain is found in the case of Alzheimer's Disease and also in mild cognitive impairment or MCI referring that NF-L can be proven useful in the diagnosis of Alzheimer's disease at initial stages and also it can diagnose the conversion of MCI to AD. But not necessarily it will always correlate with Alzheimer's disease because with age, the amount of CNS neurofilament light chain naturally increases (Zhou et al., 2017). Moreover, it should be considered that with along age-related increase of NFL in the CNS, the amount of NFL in the CNS is also related to other neurodegenerative diseases. Therefore, NF-L is not AD specific unlikely CSF tau proteins which is specific for Alzheimer's Disease. But,

serum NF-L levels can become useful in diagnosing familial AD (Zetterberg, 2018). In that case, blood neurofilament light chain level can be a way to correlate with the diagnosis and progression of Alzheimer's Disease. It was found that the level of NFL in the serum sample were markedly higher in the patients of Alzheimer's Disease whereas, in the serum sample of MCI patients it wasn't found so. Therefore, NFL can be regarded as a potential biomarker of Alzheimer's Disease (Zhou et al., 2017).

#### **2.2.4 Plasma Clusterin**

Plasma Clusterin which is also called Apolipoprotein-J, is mainly associated with the clearing of debris from the brain. When the clusterin gene is modified or mutated it becomes a risk factor for Alzheimer's Disease. However, on which allele the mutation is occurring it is still unclear. Clusterin exerts protective function to the neurons and it was suggested that they modify the Amyloid- $\beta$  aggregates by clearing them away. Therefore, when there is increased AD pathogenesis the amount of CSF clusterin is increased for exerting protective functions and inhibiting apoptosis. When there is increased amount of amyloid- $\beta$  aggregates in the brain there is increased amount of clusterin to clear them away and protect the cells from apoptosis. In response to the CSF clusterin there is also increased amount of plasma clusterin. As a result, with the developing of severity of the disease there is increasing amount of plasma clusterin. However, it is still not been completely determined that if plasma clusterin can diagnose the Alzheimer's Disease at the initial level. Rather it has been suggested that the clusterin starts to increase in amount when the disease has already been progressed. So, it can be used as a prognostic biomarker rather than a diagnostic biomarker. It was also found that high clusterin levels exert protective functions in the younger persons whereas, it exerts toxic functions in the older patients. Again in the younger patients increased clusterin levels exert protective functions against stroke (Weinstein et al., 2016).

## **2.3 Ocular Biomarker**

With along affecting the brain mostly, Alzheimer's Disease also affects the eyes. The retina of the eyes contains neurons that's extend to the brain and renders visual signals to the brain. As a result, the proteins responsible for AD pathology are also present in the retina. Therefore, the eye becomes easier to access than the brain imaging. Moreover, it a non-invasive process which makes the ocular biomarkers more useful for the diagnosis of the Alzheimer's Disease (Shaun Frost, Martins, & Kanagasingam, 2010). In most of the cases all the other biomarkers are very expensive to assess and for this reason research works are being carried out to find the biomarkers in other places in the body which will be very easy and less expensive in the diagnosis of Alzheimer's disease. Eye is such an organ which is just an outgrown part of the brain (S Frost et al., 2013).

Some ocular biomarkers which can be assessed for the diagnosis of the Alzheimer's Disease are,

- Retinal Amyloid Beta Accumulation
- Loss of Retinal Ganglion and Nerve Fiber Layer
- Retinal Vascular Biomarkers
- Lens Biomarkers (van Wijngaarden, Hadoux, Alwan, Keel, & Dirani, 2017)

### **2.3.1 Retinal Amyloid Beta Accumulation**

Several research works have been done on animal models where Amyloid- $\beta$  plaques were found in the retina. It was found that in both animal models and in human the retinal dysfunction was caused in Alzheimer's Disease by the accumulation of amyloid- $\beta$  proteins in the retina which alters the normal structural functionality of the retina (Anna, James, Roger, & Graham, 2016). In almost all layers of the retina the aggregation of Amyloid- $\beta$  plaques were identified. In another study, a South-American rodent showed brain Amyloid- $\beta$ , tau

accumulation, cognitive impairment due to age and also retinal amyloid- $\beta$  accumulation, tau accumulation, more specifically in the nerve fibers and ganglionic cells. Moreover, in other animal studies the amyloid- $\beta$  plaques in the case of Alzheimer's Disease, appeared in the retina even before appearing in the brain. Though these animal models have some limitations, human studies are not that much definitive. But through some post mortem histopathological studies it was found that there was amyloid- $\beta$  accumulation and on some studies, there was accumulation in the retina. Again, in some cases there were tau-protein depositions but no neurofibrillary tangle deposition in the retina. In some studies, it was possible to confirm Alzheimer's Disease using the amyloid- $\beta$  accumulation in the retina, though the number of the studies is very small. So further studies and confirmations are needed to establish retinal amyloid- $\beta$  deposition as an invasive biomarker for the detection of Alzheimer's Disease (van Wijngaarden et al., 2017).

### **2.3.2 Loss of Retinal Ganglion Cells and Nerve Fiber Layer**

Alzheimer's Disease has association with the degeneration of the retinal ganglionic cells and nerve fiber layer. A few studies showed damage of axon in the optic nerves whereas, another study showed loss of retinal ganglionic cells and nerve fiber layer. It was seen that in the patients of the Alzheimer's Disease neurons are damaged and there is an increased appearance of astrocytes throughout the retina. Moreover, retinal fiber loss was seen in the early stages of the disease in some patients using the Optical Coherence Tomography. But this change is not specific only to Alzheimer's Disease as it is also seen in other neurodegenerative diseases like Mild Cognition Impairment, Parkinson's Disease, Glaucoma and Multiple Sclerosis. Further studies need to be done to find the relevance of the specificity of these biomarkers with Alzheimer's Disease (van Wijngaarden et al., 2017).



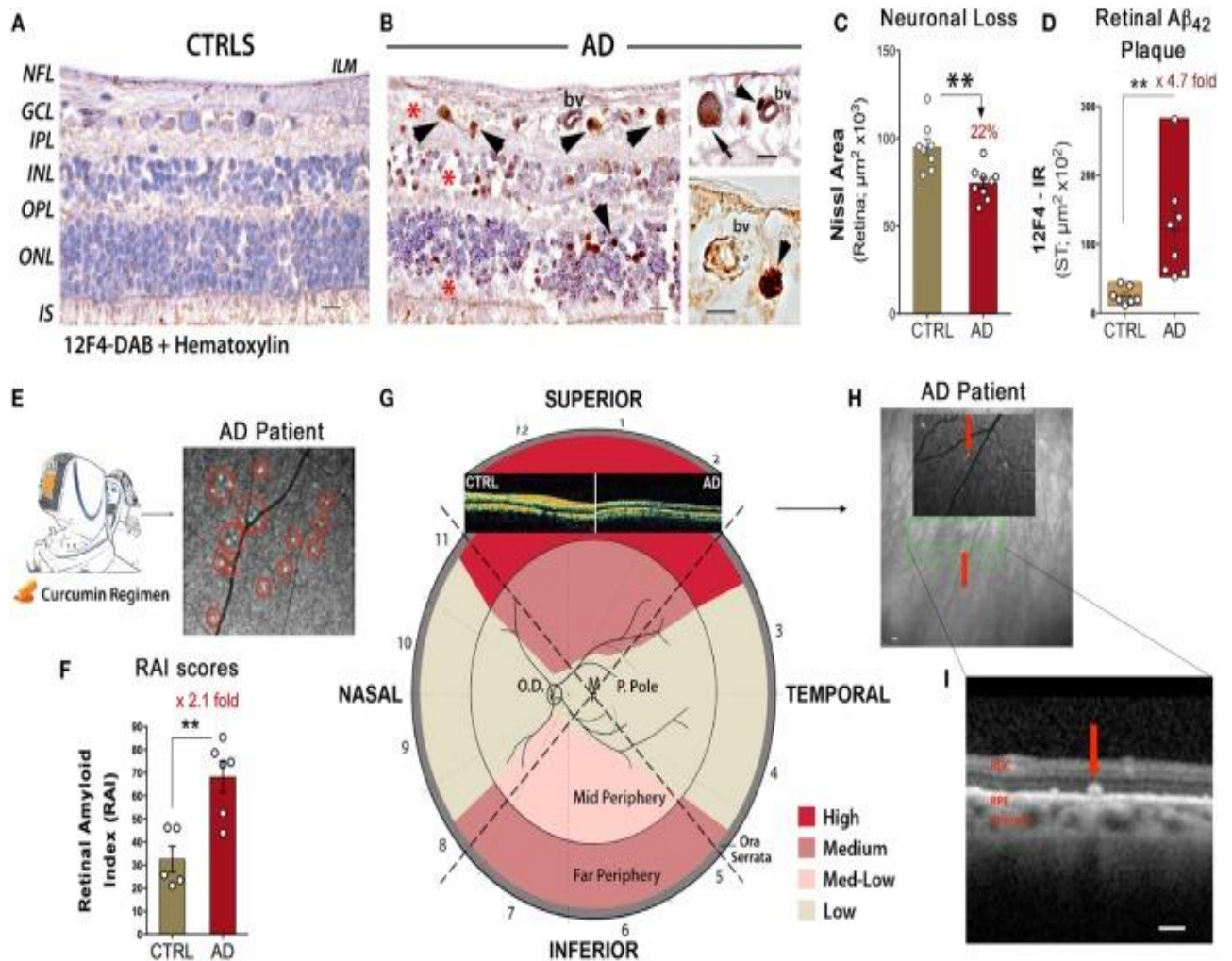


Figure 11: Laser Ophthalmoscopy and Optical Coherence Tomography detecting retinal  $A\beta$  deposits in Alzheimer's Disease patients and controls (A, B). Arrowheads showing  $A\beta$  deposits in the GCL, red stars indicating loss of nuclei. Nissl area loss in AD patients (C). Amyloid- $\beta$  deposition in AD (D). Laser Ophthalmoscope using Curcumin to label amyloid depositions in alive AD patients (E). Retinal Amyloid Index display in patients of Alzheimer's Disease and healthy subjects on scatter bar plot using fluorescent curcumin (F). Qualitative deposition of amyloid protein in different retinal regions (G). OCT images of curcumin positive amyloid depositions as the red marks, Green lines defining the line of OCT segmentation (H). OCT revealing amyloid plaque in outer retinal layers; above retinal pigment epithelium (RPE) and along with uninterrupted RPE and Bruch's membrane (I) (Koronyo-hamaoui, 2017).

### 2.3.3 Retinal Vascular Biomarkers

There are similarities between the retinal and cerebral vasculatures. In Alzheimer's Disease Amyloid- $\beta$  is accumulated in the blood vessels causing interruption in the blood flow. It also causes the narrowing of the venules and arterioles thus, leading to oxidative stress. But this is not specific to only Alzheimer's Disease. As a result, due to lack of specificity currently, retinal vascular biomarkers is not being used as a biomarker of Alzheimer's Disease (van Wijngaarden et al., 2017).

### **2.3.4 Lens Biomarkers**

The lens undergoes through degenerative changes with the normal aging where there is different protein accumulation in the lens forming cataract. In several animal studies amyloid- $\beta$  accumulation has been found in the lenses of rodents, monkey and also in the case of human studies the same is found. Therefore, amyloid- $\beta$  aggregation has been found in the lenses of the patients of Alzheimer's Disease with the appearance of the same protein accumulation in the CNS, skin and blood vessels. Amyloid- $\beta$  has been found to produce cataracts in the AD patients by binding with  $\alpha\beta$ -crystallin, which is a lens protein. The same situation is observed in Down Syndrome and early onset AD but, many studies also failed to correlate A $\beta$  accumulation in lens with Alzheimer's Disease. However, this difference in the studies might be due to the difference in the study procedures. If the extent of amyloid- $\beta$  accumulation in the lens can be related with the extent of the disease, in future it can serve as a biomarker of Alzheimer's Disease (van Wijngaarden et al., 2017).

### **2.4 Neuroimaging Techniques for the Biomarkers of Alzheimer's disease**

Neuroimaging techniques for the biomarkers have the potential of determining whether the disease is going to convert from Mild Cognition Impairment to Alzheimer's Disease or not. MCI or mild cognition impairment is a pre-dementia state where there is presence of one domain of cognition is impaired where other domains of cognition are partially preserved with normal functional capabilities and with the course of time MCI has the likeliness to get converted into actual AD. There are two types of MCI which are amnesic MCI (aMCI) and non-amnesic MCI (naMCI). In amnesic MCI the patients are affected with the impairment in memory domain whereas in non-amnesic MCI the patients are affected in other domains of cognition rather than the memory domain, like speaking, functioning, balancing etc. But in most of the cases of amnesic MCI, the patients progress towards AD (Varghese et al., 2014). Moreover, studies showed that amnesic MCI can be regarded as the transitional stage of

healthy aging and Alzheimer's Disease. However, aMCI can be of single-domain or multi-domain. Single domain amnesic MCI (SMCI) can be regarded as the earliest stage of Alzheimer's Disease whereas Multidomain MCI (MMCI) can be regarded as the later stages of AD (Fennema-notestine et al., 2010). Neuroimaging techniques are useful for both of diagnosing Alzheimer's Disease at early stage and differentiating Alzheimer's Disease from other forms of dementia (Varghese et al., 2014).

The neuroimaging techniques are broadly divided into two classes as structural neuroimaging techniques and functional neuroimaging techniques. Both of these two types of neuroimaging techniques are said to reveal the progression of MCI into AD or other types of dementias and they also differentiate AD from other types of neurodegenerative diseases

#### **2.4.1 Structural Neuroimaging Techniques of Alzheimer's Disease**

From the name it can be clearly understood that structural neuroimaging will be dealing with structure of the brain. These biomarkers are able to separate two structures within the brain from each other and in comparison, to the functional neuroimaging techniques their resolution is also higher (Varghese et al., 2014). Within the structural neuroimaging techniques there are several types as follows:

- Computed Tomography (CT)
- Structural Magnetic Resonance Imaging (sMRI)
- Diffusion Tensor Imaging (DTI)

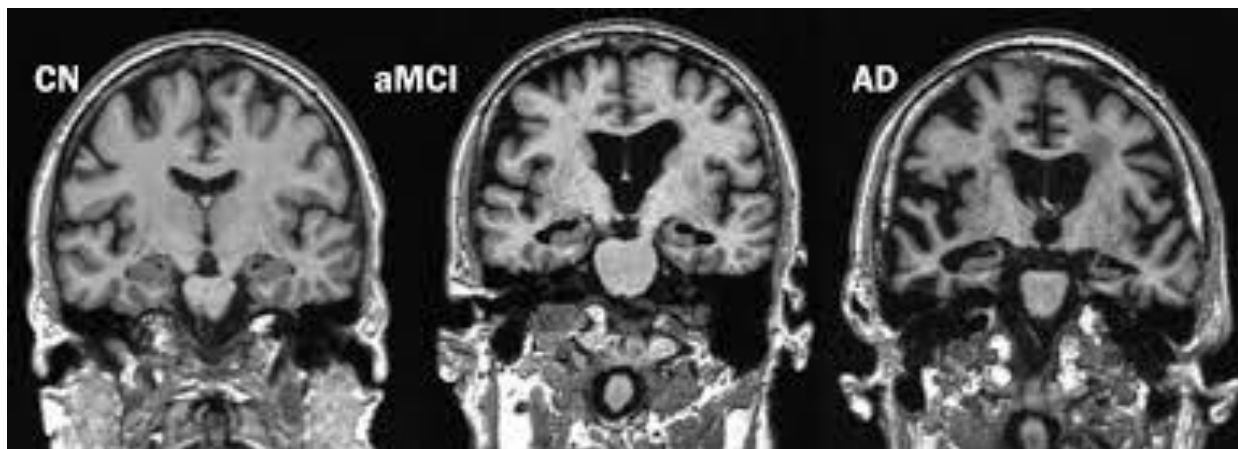
##### **2.4.1.1 Computed Tomography (CT)**

Computed tomography does not have any significance in the early detection of Alzheimer's Disease. Rather it is useful in diagnosing the later developments of the Alzheimer's Disease. It is also used to diagnose the causes of dementia which can be treated with surgery like brain

tumors. However, in many of the countries it is a very useful diagnostic technique as it is less expensive, widely available and less time consuming (Varghese et al., 2014).

#### **2.4.1.2 Structural Magnetic Resonance Imaging (sMRI)**

One of the hallmarks of Alzheimer's Disease is the cerebral atrophy (Scahill, Schott, Stevens, Rossor, & Fox, 2002). Medial Temporal atrophy is regarded as the early biomarker of the disease. However, two main medial temporal structures showing atrophy are hippocampus and entorhinal cortex and structural MRI have been successful in predicting that these atrophy has high risk of developing into Alzheimer's Disease (Varghese et al., 2014). The results of brain atrophy from the structural MRI is being correlated with tau depositions and neuropsychological changes in the patients therefore it becomes a valid biomarker of the disease (Centro, Giovanni, Fox, & Thompson, 2011).



*Figure 12: Brain Atrophy in Cognitively Normal Patient, in amnestic Mild Cognition Impairment Patients and in Alzheimer's Disease Patient (Vemuri & Jack, 2010)*

Although at all of the stages of Alzheimer's Disease the medial temporal lobe atrophy has been seen, with the progression of the disease frontal lobe has also been associated. At different stages of the disease the amount of the atrophy fluctuates significantly. Volumetric MRI has been used to determine these site-specific atrophies. In the course of disease progression there has been observed the presence of neurofibrillary tangle and amyloid plaques deposition which are also accompanied by brain atrophy at specific sites. All of these changes can be assessed

with the use of volumetric MRI. Moreover, it has been confirmed by the autopsy of the AD patients that earliest changes of the disease occur at medial temporal sites like hippocampus and entorhinal cortex. So, the volumetric MRI has been focused on these specific brain locations (Scahill et al., 2002). However, earlier manual volumetric technique was used but later on automated volumetric methods developed. Manual volumetric technique is very time consuming and it requires excellent skill at neuroanatomy (Varghese et al., 2014). Moreover, most of the manual volumetric techniques are cross-sectional. Therefore, the results might overlap between the controls and the patients due to the individuality from person to person on the volumes of different inter-compartments of the brain. But in case of the automated techniques they use longitudinal methods and use the same subject as their controls. As a result, there are less overlapping and the changes within the subject might be more accurate to differentiate the AD conditions than the healthy aging. One of the automated techniques is the Voxel based morphometry (Varghese et al., 2014).

In voxel-based morphometry repeated brain scans of the patient is being taken and then matched with the baseline scan. Thus, the overlays will create voxel patterns which will be used to quantify different volume levels of the targeted brain compartment (Scahill et al., 2002). VBM (Voxel-Based Morphometry) is mainly targeted for gray matter but anyhow it can be targeted for white matter as well. The whole process is subdivided into three steps tissue classification, spatial normalization and spatial smoothing then followed by statistical approaches (Kurth, Luders, & Angeles, 2015). Thus VBM compares gray matter loss between the AD patients and controls (Scahill et al., 2002).

#### **2.4.1.3 Diffusion Tensor Imaging (DTI)**

Diffusion Tensor Imaging is another advanced form of Magnetic Resonance Imaging (Varghese et al., 2014). Where voxel-based morphometry is mainly targeted for gray matter,

the diffusion tensor imaging can be targeted for the white matter (Kurth et al., 2015; Oishia, Mielke, Albert, & Moria, 2012). In this technique the property of thermal motion of water is being used. Water molecules can move freely within the brain if it is not being encountered with any restrictions. For example, water molecules can move freely along the uninterrupted axons. But there are certain structures in the brain which disrupts the movement of water like tightly packed axons. Alongside, water molecules are easily diffused through the white matter but in case of grey matter the movement is not that much aligned as through the white matter. So in case of white matter the movement of water molecule is anisotropic whereas in the case of grey matter it is isotropic (Oishia et al., 2012). Here, anisotropic means that non-random movement of water molecules are greater along the length of the axons than the movement along the width. When there is any disturbances in the axons water molecules move more freely or more randomly through the axons reducing, the value of anisotropy of water which is a hallmark of Alzheimer's Disease. Thus it tracks the fibers within the brain (Varghese et al., 2014). However, there are a few drawbacks associated with diffusion tensor imaging. First of all, only due to the water movement the neural anatomy cannot be determined completely as there are many more microscopic structures coming in the pathway of water movement. Another problem is, the diffusion readings are averaged over a large volume of voxels leading the voxel measurements to macroscopic. Therefore, it becomes difficult to assume whether the anatomical changes are due to internal changes of the somatic structures or only due to the different reorganization of the fiber bundles. But, this problem can be minimized to an extent if the number of voxels are increased or different parameters are derived from each voxels (Oishia et al., 2012).

## **2.4.2 Functional Neuroimaging Techniques for Alzheimer's Disease**

Functional Neuroimaging techniques reveal the structure and functions of different parts of the brain. Functional Neuroimaging Techniques are mainly focusing on the functions rather than

the structural features and even if does their spatial resolution is lesser than structural neuroimaging techniques (Varghese et al., 2014). Different functional neuroimaging techniques available currently for Alzheimer's Disease may include:

- Functional MRI (fMRI)
- Molecular and Beta Amyloid Imaging
- Positron Emission Tomography (PET)
- Magnetic Resonance Spectroscopy (MRS)
- Single Photon Emission Computed Tomography (SPECT)
- Magnetic Encephalography (MEG)

#### **2.4.2.1 Functional Magnetic Resonance Imaging (fMRI)**

Functional Magnetic Resonance Imaging is not exactly used for the early detection of Alzheimer's Disease but sMRI is used for this purpose and the results derived from sMRI are being matched with fMRI to get a more confirmed diagnosis. Whereas, fMRI is used for the treatment or management of the disease and is used for determining functional abnormalities in the patients. In this technique the concentration of different chemicals is measured at different locations of the brain with the help of neuro imaging when the person is stimulated to do something, in particular when the person is doing any cognitive task and compared to the neuro imaging at a resting state. For example, it might measure the amount of oxygen concentration at different specific regions of the brain while there is any cognitive stimulation. Therefore, from the resulted activity the resting state activity is subtracted to yield the blood oxygen level dependent response or to yield the amount of increased blood flow level due to the stimulation. In this way it is correlated with the Alzheimer's Disease patient that how much his BOLD ( Blood Oxygen Level Dependent) response has decreased which actually means the decrease in cognitive stimuli or decrease in cognitive performance (Varghese et al., 2014).

### **2.4.2.2 Molecular and Beta Amyloid Imaging**

Beta Amyloid plaques are one of the hallmarks of Alzheimer's Disease. These protein depositions start to accumulate in the brain long before expressing the early symptoms of the disease. Therefore, imaging and quantifying these protein plaques can lead the way of early detection that a person is going to develop dementia and specifically AD as this technique is useful in differentiating AD from other forms of dementia. Moreover, it is used for the management of AD by monitoring the efficacy of the treatment. For example, when a patient is receiving anti-amyloid treatment with the vaccines or antibodies such as, bapineuzumab (antibody), using Pittsburgh compound B in PET the amount of protein accumulation and effectiveness of the treatment is determined. During the imaging of amyloid beta plaques in the brain different markers are used and Pittsburgh Compound B is one of them and chemically it is neutrally charged, fluorescent derivative of thioflavin-T which was developed in the University of Pittsburgh School of Medicine located in Pennsylvania, United States of America (Barber, 2010; Varghese et al., 2014).

However, there are many more imaging compounds which are being used in PET for the quantification of beta amyloid plaques in the brain such as,

- Florbetapir F 18
- Florbetaben
- 18F Flutemetamol (Varghese et al., 2014)

### **2.4.2.3 Positron Emission Tomography (PET)**

Positron Emission Tomography is a technique which can render high resolution three dimensional images of the neural activity and used both in healthy and ill subjects to explore the functional activity alterations within the brain (Varghese et al., 2014). PET normally investigates the functional activities or pathophysiological changes of different



neurodegenerative diseases with the help of different imaging compounds and can differentiate different forms of dementia. Moreover, it can predict the transformation from MCI to Alzheimer's Disease (S Shokouhi, D Claassen, 2014; Varghese et al., 2014). However, the most used imaging compound is 18-fluorodeoxyglucose or FDG and it is used in measuring cerebral metabolic glucose utilization rate (CMR<sub>glc</sub>) at different neural regions which shows the decreased glucose usage at various important brain regions like memory or learning skills and the technique is called FDG-PET. According to a report, around 93% of average accuracy can differentiate AD patients from healthy persons where sensitivity is 96% and specificity is 90%. PET can also differentiate other forms of neurodegenerative diseases like dementia from Lewy Bodies. In all of these cases hypometabolism is observed in patients and by assessing the abnormal metabolic activities the disease is diagnosed and the accuracy depends on the skill and training of the observer. However, the complete confirmation is only possible after the autopsy of the brain (S Shokouhi, D Claassen, 2014).

#### **2.4.2.4 Magnetic Resonance Spectroscopy (MRS)**

Magnetic Resonance Spectroscopy produces spectrum depending on the chemical composition of the metabolites and two chemical correspondents can be separated from each other depending on the chemical shifts produce on the spectrum. Thus, it correlates dementia with the metabolites and at the same time it predicts future disease progression, tracks down the present disease progression and also can differentiate different dementias from each other depending on the characteristic metabolite formation. H1 MRS can be useful in future to early detection of dementia and differential study of dementia and can also be useful in determining the transformation of MCI to actual AD. Whereas, Proton MRS can render metabolic patterns in both of the patients with mild cognitive impairment and also with extreme neural abnormalities. Proton MRS is also capable of differentiating different form of dementia and hippocampal MRS also diagnoses the conversion from aMCI to actual AD (Varghese et al.,

2014). The main form of dementias which are differentiated with this technique are mainly Dementia with Lewy Bodies (DLB), Frontotemporal Lobe Dementia (FTD) and Vascular Dementia (VaD). In many cases the patients have overlapping pathological changes. For an example, in case of differentiating VaD from AD. One important difference is the location of metabolites however, they can have other changes which are similar to each other. In case of both AD and VaD the amount of N-acetyl aspartate (NAA) is decreased in a similar manner but in case of VaD the amount is greater in the white matter. Again, in VaD the choline myo-inositol (MI) level is normal and the amount is greater than the amount in AD. So to differentiate VaD from AD two important markers can be MI and grey matter. In this way MRS associates in distinguishing different form of dementias from each other by analyzing the metabolite levels and chemical shifts of the spectrum (Kantarci, 2013).

#### **2.4.2.5 Single Photon Emission Computed Tomography (SPECT)**

Single Photon Emission Computed Tomography imaging technique uses radioactive agents specific for targeted brain cells to identify their cellular or chemical changes and relate them to corresponding diseases. This technique also differentiates between different forms of dementia and also detects the progression of the disease. This can determine the amount of amyloid plaques and the amount of plaque accumulation is directly involved with AD. However, computing the exact amount of amyloid plaque deposition in a living brain is not an easy task therefore, SPECT has been used in combination with either PiB-PET (Pittsburgh Compound B- Positron Emission Tomography) or FDG-PET (Fluorodeoxyglucose- Positron Emission Tomography). SPECT can become useful in early detection of AD and in comparison, with PET it is less expensive but PET is more specific (Varghese et al., 2014). Also, SPECT can give values regarding regional cerebral blood flow and a reason of their less specification is that, many a times it gives false positive results (Scheltens, 2009).

#### **2.4.2.6 Magnetic Encephalography**

This one is also an imaging technique and it utilizes the oscillations created by the brain on the application of a magnetic field. These neuromagnetic fields are recorded with sensors called SQUID (Superconductive Quantum Interference Device). SQUIDs are composed of two types of sensors, one is magnetometer and another is gradiometer. Magnetometer and gradiometer are used as a pair and set at a little distance from one another where magnetometer measures magnetic field and gradiometer measures the difference of magnetic fields between their two locations. Thus, it correlates between the functionality and cognitive activities in the AD patients. Moreover, in future it can even diagnose the conversion from normal cognition to MCI. From the imaging, it is seen that the AD patients show higher MEG patterns, increased slow rhythms and decreased fast activity than in a normal subject and it has been suggested that the low activity is related to AD. It can also predict the earliest conversion of MCI to AD and can detect changes in functional organization in CNS (Mandal, Banerjee, Tripathi, & Sharma, 2018; Varghese et al., 2014).

## **Chapter 3**

### **Discussion**

Disease specific biomarkers for the diagnostic and prognostic studies of Alzheimer's Disease is very crucial. Alzheimer's Disease is such a neurodegenerative disease that is associated with different kinds of biomarkers. Moreover, the exact cause of the disease, risk factors and molecular understanding of the disease is still very unclear. On the other hand, more population are becoming old and with increasing age there lies an inherent risk factor for this disease development. Therefore, the proper diagnosis plays the major role in the treatment of the disease. There are many biomarkers which refer to Alzheimer's Disease in different ways. But in some cases, those are not very specific all the time and can be seen to occur in the case of other neurodegenerative diseases as well. However, their amount or concentration at different sites can be a tool for differentiative studies. The most frequently used and most useful biomarkers for the disease detection are the imaging biomarkers. They have been proved to refer to the occurrence of the disease most efficiently. Then the second type of biomarkers which are used both as prognostic and diagnostic biomarker for the Alzheimer's Disease are the Cerebrospinal Fluid Biomarkers. As the disease is mainly neural related so the CSF biomarkers are the closest ones to the disease site. The major CSF biomarkers of the disease are the amyloid- $\beta$  aggregates and the tau protein deposition. The amyloid- $\beta$  aggregates can also be found in other types of dementias as well but the amount might vary whereas, the tau protein deposition is very specific towards the Alzheimer's Disease and is very useful in differentiating Alzheimer's Disease from the rest of the kinds of the dementias. But collecting these biomarkers are not easy as well, they are both time consuming and expert dependent procedures. Therefore, the blood biomarkers can serve as a very useful and quick form to

diagnose the disease and also the procedure is not that much required of expert personnel. Even collecting the blood samples is very easy and less painful. Once these biomarkers are accurately correlated with the disease, they will be the easiest accessible biomarkers for the diagnosis of the Alzheimer's Disease. Other types of the biomarkers are ocular biomarkers. These have the direct connection to the CNS, therefore accessing the ocular biomarkers will be helpful to easily access the CNS biomarkers. As, there are different kinds of neurodegenerative disease showing similar kinds of biomarkers, the AD biomarkers need to be correlated with the disease very specifically. The neuroimaging techniques can provide with differentiating studies of Alzheimer's Disease from rest of the neurodegenerative diseases. These techniques can detect the pathophysiological changes that the brain goes through and also gives very specific brain imaging results of the Alzheimer's Disease. Moreover, these imaging results are also found to be matched with the brain autopsy results of the patients after their death. There are many biomarkers which are expected to correlate with AD pathology but those are still being questioned as their exact relation with the Alzheimer's Disease is still not being cleared out. So, there should be more confirmatory studies to establish the biomarkers and to make the diagnostic procedures more efficient.

## **Chapter 4**

### **Conclusion**

Alzheimer's Disease is a complex neurodegenerative disease associated with different forms of pathologies. As there is no cure of the disease but there can be a prevention against the progression of the disease and diagnosing the disease at the earliest stage is the most necessary. It can be said that the most important role is being played by the proper biomarkers in the management of Alzheimer's Disease. Therefore, discovering the biomarkers very specific to the disease is very important and for diagnosing such a complex disease, it is not possible to depend on only one form of biomarkers. Rather there should be a complete framework with established correlations between the different biomarkers where each one of them will cross check the results of the other diagnostic procedures and come to a specific and accurate diagnostic result for Alzheimer's Disease. Moreover, there should be more emphasis given on discovering the correlations between the disease and non-invasive biomarkers and blood biomarkers as in many of the countries the imaging biomarkers and CSF biomarkers are still not easily accessible, costly and most importantly very time consuming. In this situation, with annually increased number of Alzheimer's Patient establishing non-invasive and circulatory biomarkers will improve the situation towards a betterment for the Alzheimer's Disease.

## **Future Direction**

- To identify differentiative studies of the biomarkers to characterize Alzheimer's Disease from other different forms of neurodegenerative diseases.
- To identify the specific correlations between the circulatory biomarkers and Alzheimer's Disease, as circulatory samples are the most convenient to collect from a patient.
- To identify specificity of ocular biomarkers towards Alzheimer's Disease, as ocular nerves are in direct contact with the central nervous system and can serve as a non-invasive biomarker of the disease.

## References

- Précoma, M., Rodríguez-Cruz, A., Berumen L, & García-Alcocer. (2016). The Etiology of Alzheimer's Disease, 1–12. <https://doi.org/10.1016/j.bbr.2014.12.012>
- Alzheimer's & Dementia. (2015). Basics of Alzheimer ' S Disease. *Alzheimer's Association Report, 11*, 1–26. <https://doi.org/10.3233/JAD-142658>.Heritability
- Anna, G., James, E. K., Roger, C. V., & Graham, S. (2016). Amyloid  $\beta$  accumulation and Inner retinal degenerative changes in Alzheimer's disease transgenic mouse. *Neuroscience Letters*. <https://doi.org/10.1016/j.neulet.2016.04.059>
- Association, A. (2018). 2018 Alzheimer ' s disease facts and figures. *Alzheimer's & Dementia, 14*(3), 367–429. <https://doi.org/10.1016/j.jalz.2018.02.001>
- Association, A. (2019). 2019 Alzheimer ' s disease facts and figures. *Alzheimer's & Dementia, 15*(3), 321–387. <https://doi.org/10.1016/j.jalz.2019.01.010>
- Barber, R. C. (2010). Biomarkers for Early Detection of Alzheimer Disease. *Journal of American Osteopath Association, 110*(9), 10–15.
- Berman, S. E., Clark, L. R., Rivera-rivera, L. A., Racine, A. M., Rowley, H. A., Bendlin, B. B., ... Sahlgrenska, T. (2018). Intracranial arterial 4D-flow in individuals with Mild Cognitive Impairment is associated with cognitive performance and amyloid positivity. *Journal of Alzheimer's Disease 60*(1), 243–252. <https://doi.org/10.3233/JAD-170402>.
- Bibl, M., Esselmann, H., & Wiltfang, J. (2012). Neurochemical biomarkers in Alzheimer ' s disease and related disorders. *Therapeutic Advances in Neurological Disorders, 335–348*. <https://doi.org/10.1177/1756285612455367>
- Bjerke, M., & Engelborghs, S. (2018). Cerebrospinal Fluid Biomarkers for Early and Differential Alzheimer ' s Disease Diagnosis. *Journal of Alzheimer's Disease, 62*, 1199–



1209. <https://doi.org/10.3233/JAD-170680>

Blennow, K. (2017). A Review of Fluid Biomarkers for Alzheimer ' s Disease : Moving from CSF to Blood THE CORE CSF BIOMARKERS. *Neurology and Therapy*, 6(s1), 15–24.

<https://doi.org/10.1007/s40120-017-0073-9>

Centro, I., Giovanni, S., Fox, N. C., & Thompson, P. M. (2011). The clinical use of structural MRI in Alzheimer disease. *Nature Reviews Neurology*, 6(2), 67–77.

<https://doi.org/10.1038/nrneurol.2009.215>.

Drolle, E., Hane, F., Lee, B., & Leonenko, Z. (2014). Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer ' s disease.

*Drug Metabolism Reviews*. <https://doi.org/10.3109/03602532.2014.882354>

Duthey, B. B., & Ph, D. (2013). Priority Medicines for Europe and the World " A Public Health Approach to Innovation " Update on 2004 Background Paper Written by Saloni Tanna Background Paper 6 . 11 Alzheimer Disease and other Dementias, (February).

Fiandaca, M. S., Mapstone, M. E., Cheema, A. K., & Federoff, H. J. (2014). The critical need for defining preclinical biomarkers in Alzheimer ' s disease. *Alzheimer's & Dementia*,

10(3), S196–S212. <https://doi.org/10.1016/j.jalz.2014.04.015>

Findeis, M. A. (2007). The role of amyloid  $\beta$  peptide 42 in Alzheimer ' s disease.

*Pharmacology And Therapeutics*, 116, 266–286.

<https://doi.org/10.1016/j.pharmthera.2007.06.006>

Frost, S., Kanagasingam, Y., Sohrabi, H., Vignarajan, J., Bourgeat, P., Salvado, O., ... Rowe, C. C. (2013). Retinal vascular biomarkers for early detection and monitoring of Alzheimer

' s Retinal vascular biomarkers for early detection and monitoring of Alzheimer ' s disease. *Translational Psychiatry*, 3(2), e233-8. <https://doi.org/10.1038/tp.2012.150>

- Frost, S., Martins, R. N., & Kanagasigam, Y. (2010). Ocular Biomarkers for Early Detection of Alzheimer ' s Disease. *Journal of Alzheimer's Disease*, (61 8), 1–39.
- Henriksen, K., Bryant, S. E. O., Hampel, H., Trojanowski, J. Q., Montine, T. J., Jeromin, A., ... Interest, B. B. (2013). The future of blood-based biomarkers for Alzheimer ' s disease. *Alzheimer's and Dementia*, 1–17. <https://doi.org/10.1016/j.jalz.2013.01.013>
- Holtzman, D. M. (2012). CSF biomarkers for Alzheimer's disease: Current utility and potential future use. *Neurobiol Aging*, 32(Suppl 1), 1–9. <https://doi.org/10.1016/j.neurobiolaging.2011.09.003>.CSF
- Jack, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Budd, S., ... Silverberg, N. (2018). NIA-AA Research Framework : Toward a biological definition of Alzheimer ' s disease. *Alzheimer's & Dementia*, 14(4), 535–562. <https://doi.org/10.1016/j.jalz.2018.02.018>
- Jouanne, M., & Rault, S. (2017). SC. *European Journal of Medicinal Chemistry*. <https://doi.org/10.1016/j.ejmech.2017.07.070>
- Kantarci, G. J. and K. (2013). Magnetic resonance spectroscopy in Alzheimer ' s disease. *Neuroshyciatric Disease and Treatment*, 687–696.
- Koronyo-hamaoui, M. (2017). Optical Coherence Tomography in Alzheimer ' s Disease and Other Neurodegenerative Diseases. *Frontiers in Neurology*, 1–13. <https://doi.org/10.3389/fneur.2017.00701>
- Kövari, E., Hof, P. R., & Bouras, C. (2011). The Geneva brain collection. *Annals of the New York Academy of Sciences*, 1225(SUPPL. 1), 131–147. <https://doi.org/10.1111/j.1749-6632.2011.06008.x>
- Kurth, F., Luders, E., & Angeles, L. (2015). Voxel-Based Morphometry, Brain Mapping: An

Encyclopedia Reference, *1*, 345–349.

Leuzy, A., Heurling, K., Ashton, N. J., Schöll, M., & Eduardo, R. (2018). In vivo Detection of Alzheimer ' s Disease. *Yale Journal of Biology and Medicine*, *91*, 291–300.

Mandal, P. K., Banerjee, A., Tripathi, M., & Sharma, A. (2018). A Comprehensive Review of Magnetoencephalography ( MEG ) Studies for Brain Functionality in Healthy Aging and Alzheimer ' s Disease ( AD ), *Frontiers in Computational Neuroscience*, *12*(August).  
<https://doi.org/10.3389/fncom.2018.00060>

Morley, J. E., Farr, S. A., & Nguyen, A. D. (2018). Alzheimer Disease. *Clinics in Geriatric Medicine*. <https://doi.org/10.1016/j.cger.2018.06.006>

O'Bryanta, S. E., Mielke, M. M., Rissman, R. A., Lista, S., Vanderstichele, H., Zetterberg, H., ... Area, B. B. B. P. I. (2018). The Future of Blood Based Biomarkers of Alzheimer's Disease. *Alzheimer's And Dementia*, *13*(1), 45–58.  
<https://doi.org/10.1016/j.jalz.2016.09.014.Blood>

Oishia, K., Mielkeb, M. M., Albertc, M., & Moria, C. G. L. S. (2012). DTI Analyses and Clinical Applications in Alzheimer's Disease Kenichi. *Journal of Alzheimer's Disease*, *26*(Suppl 3), 287–296. <https://doi.org/10.3233/JAD-2011-0007.DTI>

Pawlowski, M., Meuth, S. G., & Duning, T. (2017). Cerebrospinal Fluid Biomarkers in Alzheimer ' s Disease — From Brain Starch to Bench and Bedside. *Diagnostics*.  
<https://doi.org/10.3390/diagnostics7030042>

Ping, F., Khuen, C., Ng, Y., Yian, R., Soi, K., & Chye, M. (2018). Tau Proteins and Tauopathies in Alzheimer ' s Disease. *Cellular and Molecular Neurobiology*, (0123456789). <https://doi.org/10.1007/s10571-017-0574-1>

Pitt, J. (2019). Biomarkers of Alzheimer ' s Disease, 885–894. <https://doi.org/10.1016/B978->

- S Shokouhi, D Claassen, and W. R. (2014). Imaging Brain Metabolism and Pathology in Alzheimer's Disease with Positron Emission Tomography. *Journal of Alzheimer's Disease And Parkinsonism*, 4(2). <https://doi.org/10.4172/2161-0460.1000143>.Imaging
- Scahill, R. I., Schott, J. M., Stevens, J. M., Rossor, M. N., & Fox, N. C. (2002). Mapping the evolution of regional atrophy in Alzheimer ' s disease : Unbiased analysis of fluid-registered serial MRI. *Proceedings of the National Academy of Sciences of the United States of America*, 99(7), 1–5.
- Scheltens, P. (2009). Imaging in Alzheimer's Disease. *Dialogues in Clinical Neuroscience*, 191–199.
- Selkoe, D. J., Hardy, J., Selkoe, D., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer ' s disease at 25 years. *EMBO Molecular Medicine*, 8(6), 595–608.
- Sharma, N., & Singh, A. (2016). Exploring Biomarkers for Alzheimer ' s Disease. *Journal of Clinical and Diagnostic Research*, 10(1967), 1–6. <https://doi.org/10.7860/JCDR/2016/18828.8166>
- Šimi, G., Leko, M. B., Wray, S., Harrington, C., Delalle, I., Jovanov-Milošević, N., ... Hof, P. R. (2016). Tau Protein Hyperphosphorylation and Aggregation in Alzheimer ' s Disease and Other Tauopathies , and Possible Neuroprotective Strategies. *Biomolecules*. <https://doi.org/10.3390/biom6010006>
- Tapiola, T., Alafuzoff, I., Herukka, S., Parikkinen, L., Hartikainen, P., Soininen, H., & Pirttilä, T. (2015). Cerebrospinal Fluid  $\beta$ -Amyloid 42 and Tau Proteins as Biomarkers of Alzheimer-Type Pathologic Changes in the Brain. *Arch Neurol*, 66(3), 382–389.
- Ueno, K., Nakamura, S., Shimotani, H., Yuan, H. T., Kimura, N., Nojima, T., ... Kawasaki,

- M. (2011). Apolipoprotein E: Structure and Function in Lipid Metabolism, Neurobiology, and Alzheimer's Diseases. *Nature Nanotechnology*, 6(7), 408–412. <https://doi.org/10.1016/j.nbd.2014.08.025>. Apolipoprotein
- van Wijngaarden, P., Hadoux, X., Alwan, M., Keel, S., & Dirani, M. (2017). Emerging ocular biomarkers of Alzheimer disease. *Clinical and Experimental Ophthalmology*, 45(1), 54–61. <https://doi.org/10.1111/ceo.12872>
- Varghese, T., Sheelakumari, R., James, J. S., & Mathuranath, P. (2014). A review of neuroimaging biomarkers of Alzheimer's disease. *Neurol Asia*, 18(3), 239–248.
- Vemuri, P., & Jack, C. R. (2010). Role of structural MRI in Alzheimer's disease. *Alzheimer's Research and Therapy*, 2(4). <https://doi.org/10.1186/alzrt47>
- Weiner, M. W., Veitch, D. P., Aisen, P. S., Beckett, L. A., Cairns, N. J., Cedarbaum, J., ... Saykin, A. J. (2015). 2014 Update of the Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception. *Alzheimer's & Dementia*, 11(6), e1–e120. <https://doi.org/10.1016/j.jalz.2014.11.001>
- Weinstein, G., Beiser, A. S., Preis, S. R., Courchesne, P., Chouraki, V., Levy, D., & Seshadri, S. (2016). Plasma clusterin levels and risk of dementia, Alzheimer's disease, and stroke. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*, 3, 103–109. <https://doi.org/10.1016/j.dadm.2016.06.005>
- Yan, L., Liu, C. Y., Wong, K., Huang, S., Mack, W. J., Jann, K., ... Wang, D. J. J. (2018). NeuroImage: Clinical Regional association of pCASL-MRI with FDG-PET and PiB-PET in people at risk for autosomal dominant Alzheimer's disease. *NeuroImage: Clinical*, 17(December 2017), 751–760. <https://doi.org/10.1016/j.nicl.2017.12.003>
- Zetterberg, H. (2018). Blood-based biomarkers for Alzheimer's disease — An update. *Journal*

*of Neuroscience Methods*, (October), 0–1.

<https://doi.org/10.1016/j.jneumeth.2018.10.025>

Zhou, W., Zhang, J., Ye, F., Xu, G., Su, H., Su, Y., ... Neuroimaging, D. (2017). Neuroscience

Letters Plasma neurofilament light chain levels in Alzheimer ' s disease & *Neuroscience*

*Letters*, 650, 60–64. <https://doi.org/10.1016/j.neulet.2017.04.027>



## Appendix A.

### An Example of an Appendix

Appendices should be used for supplemental information that does not form part of the main research. Remember that figures and tables in appendices should not be listed in the List of Figures or List of Tables. Refer to the Thesis Template Instructions for more information.