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# Qualitative Proteomics Analysis of Proteins and Biomarkers of Alzheimer' Disease

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## ABSTRACT

Alzheimer'sdementia (AD) is the plague of today's world: a costly harming illness that ransacks the elder of their capacity to work and affect their recollections. Many years of research have brought about a profound comprehension of the pathological processes and a range of targets of therapy. Proteomics has contributed enormously to these advances and will keep on having a growing role in determining the nature of obsessive injuries. Moreover, proteomics (both gel-based and gel-free, mass spectrometry-based), is probably going to assume an expanding job in distinguishing biomarkers that may aid early determination and in observing movement and above all response therapy. Alzheimer's dementia is a neurodegenerative disease characterized by limited motor functions and loss of memory. Clinically it is diagnosed by accumulation of plaques in neurons and formation of NFTs by the aggregation of tau proteins. These pathological changes in the brain can be observed in preclinical stages of AD known as ASYM AD and mild cognitive dementia.



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## 1. INTRODUCTION

The brain is amongst the most disparate and basic organs in the human body. However, it is harassed by an assortment of neurodegenerative ailments explicitly connected to aging, such as Alzheimer's disease (McKetney *et al*., 2019; Serrano-Pozo, Frosch *et al*., 2011). Alzheimer's disease (AD), an age-related neurodegenerative turmoil, is the most well-known reason for dementia in aged people and one of the main sources of death in the created world (Driscoll & Troncoso, 2011). The ailment is described morphologically by a general loss of neurotransmitters and neurons and a general decrease in cerebrum volume (Desikan *et al*., 2010; Doecke *et al*., 2012). A version of Alzheimer's dementia is a noteworthy general health problem, which has shown an intimidating provocation in vast part because of the multifaceted nature of the disease. While it was for some time suspected that Alzheimer's dementia has a solitary cause, it is currently certain that it is most ordinarily the aftereffect of blended neuropathologists (Desikan *et al*., 2010; Dodge *et al*., 2017; Kapasi *et al*., 2017). Changes in protein expression are responsible for the obsessive decrease in AD, explicitly the aggregation of amyloid and tau protein into neritic plaques and neurofibrillary tangles, respectively. Current speculations show that the development of plaques is the result of excessive production of amyloid production, while abnormal phosphorylation of tau can promote aggregation. Therefore, loss of neurogenesis and synaptic pliancy occur in the hippocampus, which is mainly involved in the regulation of cognition and memory.

These effects are related to the changes in the level of neurotransmitters such as acetylcholine and glutamate, which are important in both brain and bodily function. Proteomics look set to have a critical impact in this undertaking and, and recently researchers has made advances into the comprehension of the pathophysiology of this disease. Post-translation al changes are the primary driver of such an expansion in assorted variety, and one of the principal advantages of proteomics is the capacity to pick up data relating to such occasions and, specifically, how these adjust with the beginning of illness (Xu *et al*., 2019). In another study Jingshuzu and *et al*. six regions of the brains (hippocampus, entorhinal cortex, cingulate gyrus, sensory cortex, motor cortex, cerebellum) were analyzed in isolation it reveals that temerariously influenced territories in AD(HP, ENT, CG) demonstrate the biggest number of changes in protein articulation (~30%of quantified proteins), while less influenced areas (MCx, SCx) have fewer changes (11– 13%). Strikingly, the CB, which many think to be pathologically 'unaffected', demonstrates a generous number of protein changes. Recently a group had developed economical targeted proteomics methods, which validated several biomarkers including neuroinflammation and glucose metabolism, which distinguish between AD patients and controls (Fagan & Perrin, 2012; Paterson *et al*., 2016).

The distinguishing biomarkers for the initial stage of the ailment procedure leading to a powerful and early analytic test for AD would take into consideration presymptomatic identification of sickness and would be profitable for observing the adequacy of illness mediations amid clinical preliminaries (Gibson *et al*., 1999). A superior comprehension of these modified procedures may yield knowledge into new medication targets and biomarkers for AD. Frameworks based methodologies, for example, weighted gene co-expression network analysis (WGCNA) utilized to examine biochemical and cell changes in cerebrum, and are valuable to help catch the multifaceted nature of annoyances in organic systems (Anwar, Sharf, Usman, Panday, & Asif) that identified with ailment. Proteomic technologies provide powerful means to systematically profile the peptide or protein constituents of complex mixtures and, in some instances, to use these data to impute protein identity, amount, or both. The microscopic region is as small as 3–5 m in diameter. It is possible to use LCM to

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capture neuro-pathological structures with high purity, but the number of samples collected by LCM is often limited, complicating proteomic analysis by two- dimensional gel-based methods. These limitations are alleviated by the integration of LCM with the highly sensitive LC-MS/MS technology (Liao *et al*., 2004; Soares *et al*., 2009).

The neurofibrillary tangles are argyrophilic and visualized by silver impregnation techniques, for example, the Gallyas procedure. It is essential in the field of neuropathology because of its high sensitivity for the detection of argentophilic inclusion bodies in the central nervous system. An elective technique to look at NFTs is their staining with fluorescent colors, for example, Thioflavin-S, which perceive the β-sheet creased structure of the combined helical fibers (Washburn *et al.*, 2001). Organicunostaining with antibodies can also be domelike Diego Iacono has done in his experiment while he was examining AD pathology quantitatively. This tissue examining system ensured the most elevated amount of topographic accuracy for the relationship of Aβ and tau deposition and the past observations of cortical neuronal hypertrophy in ASYMAD. He deparaffinized each segment, treat it with H2O2, and hindered it with 3% typical goat serum in Tris-supported saline for Aβ, phosphorylated- tau, and α-synuclein immune staining. All segments for Aβ immunostaining were pretreated with 99% formic corrosive for 5minutes. The following antibodies were used: 6E10 for Aβ and PHF-1 for the phosphorylated-tau protein (Du *et al.*, 2005).

## 2. METHODS

To observe the changes in protein expression during Alzheimer's dementia, protein must be separated from a brain tissue sample. In the past, high-resolution techniques were used such as 2D gel electrophoresis. It is a key technique for purifying individual proteins from complex samples based on their isoelectric points and molecular weights. It is an old and established technique, but proteins that have extraordinary size, as well as hydrophobicity cannot be from the 2-D gel method. These restrictions can be overcome by the advancement of liquid chromatography joined with tandem mass spectrometry (LC-MS/MS). The technique has been utilized to address dynamic changes in protein levels under certain conditions. Using synthetic Aβ peptide Chengan Du and *et al* used high-temperature chromatographic separation coupled with electrospray ionization mass spectrometry to quantitate Aβ in human blood serum of AD patients. Zhang also utilized multi-dimensional LC in a blend with 1D-and 2D-gels to identify serum-based biomarkers in AD. Utilizing matrix-assisted laser desorption/ionization-quadrupole-time of flight (MALDI-QTOF) and Ion-Trap- MS, they distinguished a few proteins expanded in AD (Caselli & Reiman, 2013). Whereas it may be a problem atictoiso lately aquestohomo-geneity by using traditional biochemical methods, the advent of laser capture microdissection (LCM) allows procuring a microscopic region as small as 3–5 m in diameter. It is possible to use LCM to capture neuropathological structures with high purity, but the number of samples collected by LCM is often limited (Goez *et al.*, 2018; Marcus *et al.*, 2020), complicating proteomic analysis by two- dimensional gel-based methods. These limitations can be alleviated by the integration of LCM with the highly sensitive LC-MS/MS technology (Chan *et al*., 2005; Valledor & Jorrín, 2011).

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The following antibodies were used: 6E10 for Aβ, and PHF-1 for the phosphorylated-tau protein (Lista *et al.*, 2014) (see **Figure 1**).

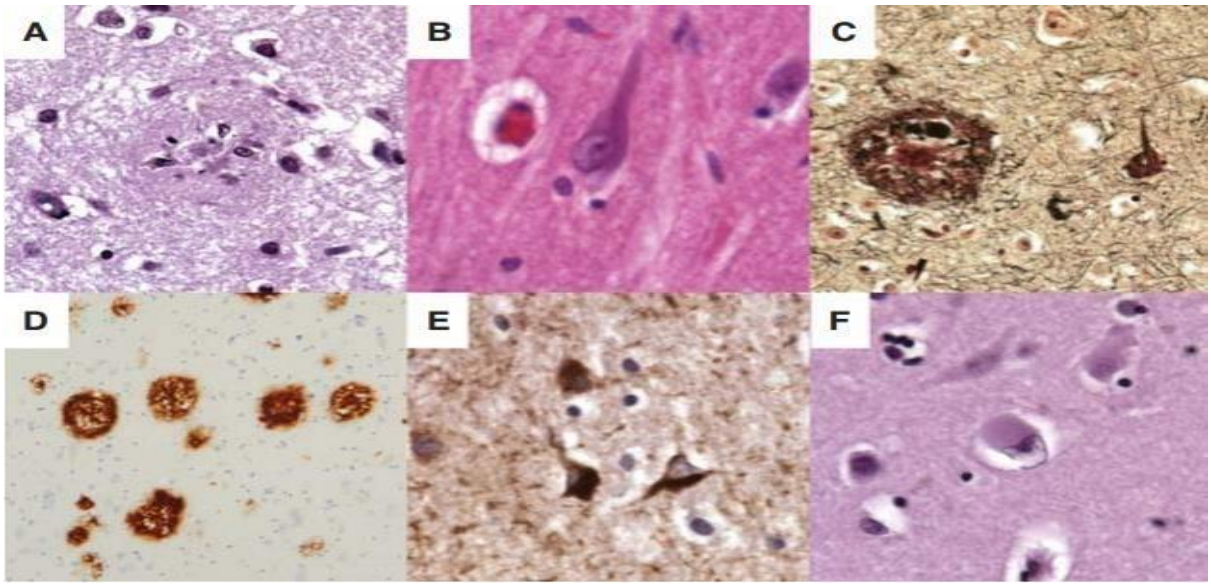


**Figure 1**. Photomicrographs of the center obsessive lesions observed in Alzheimer and Lewy body maladies.

## 3. RESULTS AND DISCUSSION

The path biological beginning of Alzheimer's disease (AD) is clinically quite known as asymptomatic AD. It takes a long time before patients start showing memory hindrance that surpasses that of their age-peers, a prognostically troubling stage named Mild Cognitive Impairment (MCI) and a few additional years before their subjective abilities decrease to a practically incapacitating degree proclaiming the clinical beginning of dementia the symptomatic AD (Asif, 2020). In a study by some scientists, they observed alterations in proteins in these three stages, the quantity of significant protein expanded in tandem with disease status in the cross-sectional examination of AsymAD (Riudavets *et al.*, 2007), MCI, and AD, showing that worldwide changes in the cleanser insoluble proteome and imperfections in proteostasis correspond with neuropathological trouble and intellectual brokenness. There was additionally a solid connection be tween's cleanser insoluble Aβ forerunner protein (APP) levels estimated by mass spectrometry with CERAD estimations, steady with the enhancement of amassed Aβ in AD (p=3.05E-10), MCI (p=0.023), and AsymAD AD (p=0.024) groups. Diego Iacano detailed serve cell body, nucleus, and neuron in these three stages by staining vertical histologic sections by cresyl violet cut according to Howard and Baddeley (see **Figure 2**).

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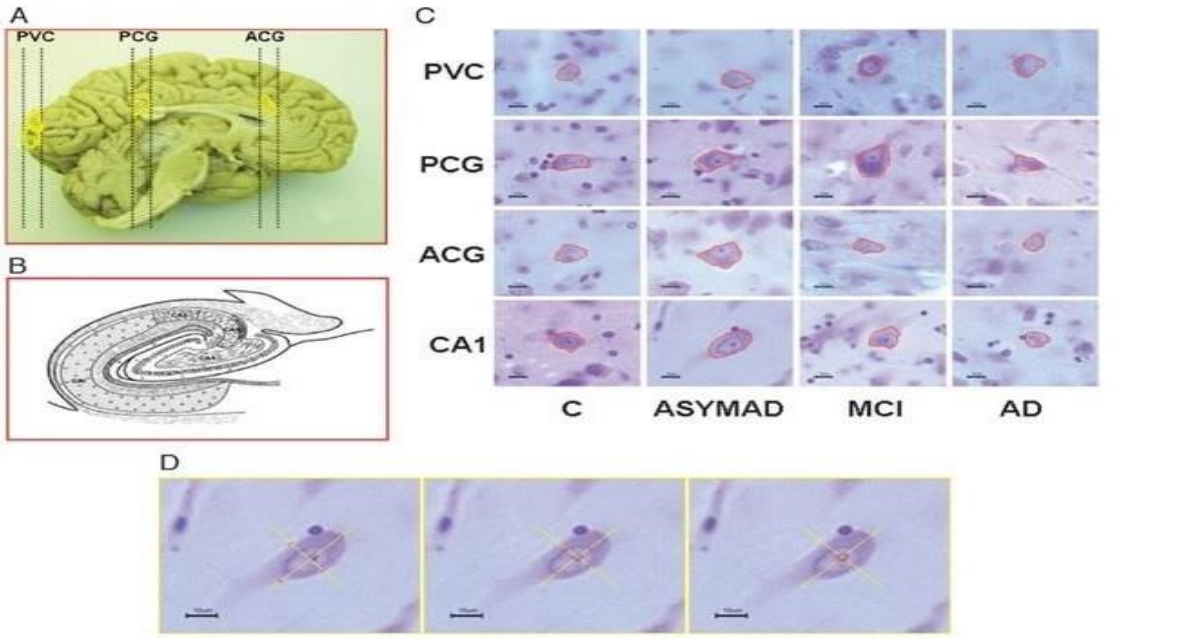


**Figure 2**. Left hemibrain from a control subject indicating where tissue punches gathered from anterior cingulate gyrus (ACG), posterior cingulate gyrus (PCG), and primary visual cortex (PVC).

At the atomic dimension, the side effects of Alzheimer's disease are related with neuritic plaques and neurofibrillary tangles, identified with the collection of amyloids- beta-peptide (Aβ) (Townsend *et al.*, 2006) and to hyperphosphorylation of the microtubule-related tau protein in neurons.

Proteomics analysis of β-amyloid protein:

Deposition of amyloid-beta protein as feeble plaque increased concentration of around 4 kDa peptides in the cerebrum is one of the pathological changes in Alzheimer's disease. some species of beta-amyloid proteins are Aβ1–40 and Aβ1–42, which comprise 40 and 42 amino acids, separately are more dangerous and total more effectively than Aβ1–40 (Ondrejcak *et al*., 2018). Then neurotoxicity of Aβ is credited to Aβ oligomers, e.g., because of the restraint of LTP (Long-term potentiation) or Preps (prion protein) flagging.

Electron microscopy of negatively stained FACS-cleansed APCP demonstrated a homogeneous populace of unpredictable thick masses, estimating somewhere in the range of 2 and 14/μm, with frayed filamentous outskirts. Sometimes, the trimming fibers disengaged from the enormous thick masses. These little filamentous combinations were homogeneous, having 5.5-to 6.0-nm distances across and an uncertain length. The electron-thick centers seemed to comprise compacted arbitrary curls of filamentous structures. Treatment of APCP by performic corrosive oxidation scattered the thick centers into a meshwork of fibers estimating a normal of 10-12 nm in measurement and of variable length. The electron-thick centers seemed to comprise compacted arbitrary loops of filamentous structures. Treatment of APCP by performic corrosive oxidation scattered the thick centers into a meshwork of fibers estimating a normal of 10-12 nm in distance across and of variable length.

Neurofibrillary tangles (NFTs) are mass of filamentous tau polymers that include a part of the fibrillar pathologies in Alzheimer's dementia (AD)— different components being the neuropil strings and the dystrophic neurites that attack a subset of amyloid plaques (neuritic plaques). These structures happen in the locales of the brain in charge of the different

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subjective spaces that are undermined during AD, the thickness of tau incorporations relating admirably with the territorial and global part so psychological decay (Walji *et al*., 2016). Histochemical staining of NFTs was done by Lester. Binderandetal by a monoclonal antibody which only recognizes tau i.e. tau-C3. NFTs, neuropil threads, and dystrophic neurites in the AD brain are stained by this antibody which are the most common pathological hallmarks of in vivo accumulation (Arnold *et al.*, 1991; Rudrabhatla *et al*., 2011).

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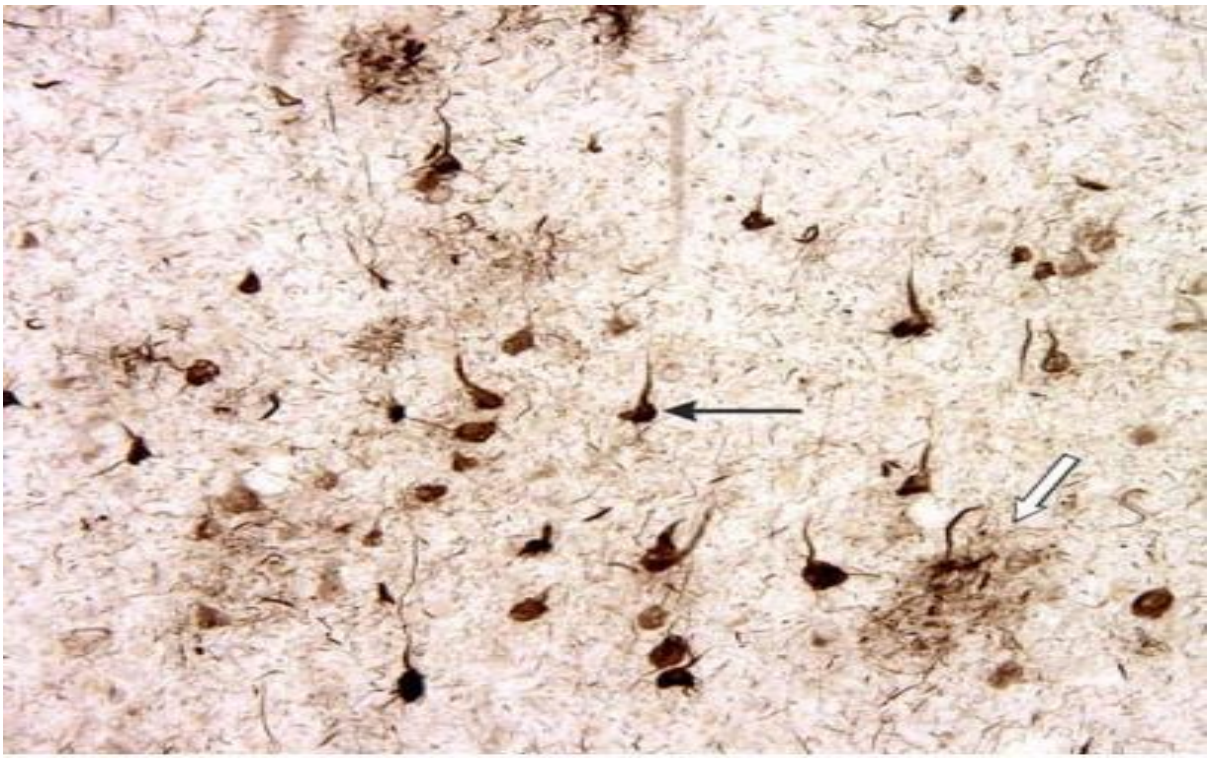

**Figure 3**. Bright field immunohistochemistry showing that Tau-C3 responds with all types of fibrillar pathologies in AD.

NFT-bearing neurons are portrayed by the loss of cytoskeletal microtubules and tubulin-related proteins. Signal transduction pathways including protein phosphorylationandde-phosphoryl ationareprobably going to assume a focal job in the development of tangles. For example, the real constituent of NFTs, the microtubule-related protein tau (MAPT) (Takashima *et al*., 2001), is hyperphosphorylated in the cerebrums of AD patients contrasted with normal persons, also, is known to underlie them is-sorting of tau from an axonal toasomatodendriticarea in neurons. This modification is thought to contribute not an axonal toasomatodendriticarea in neurons. This modification is thought to contribute not exclusively to the deregulation of microtubule elements yet in addition to tau polymerization and collection (Deng *et al*., 2009; Papasozomenos & Su, 1991) (see **Figure 4**).

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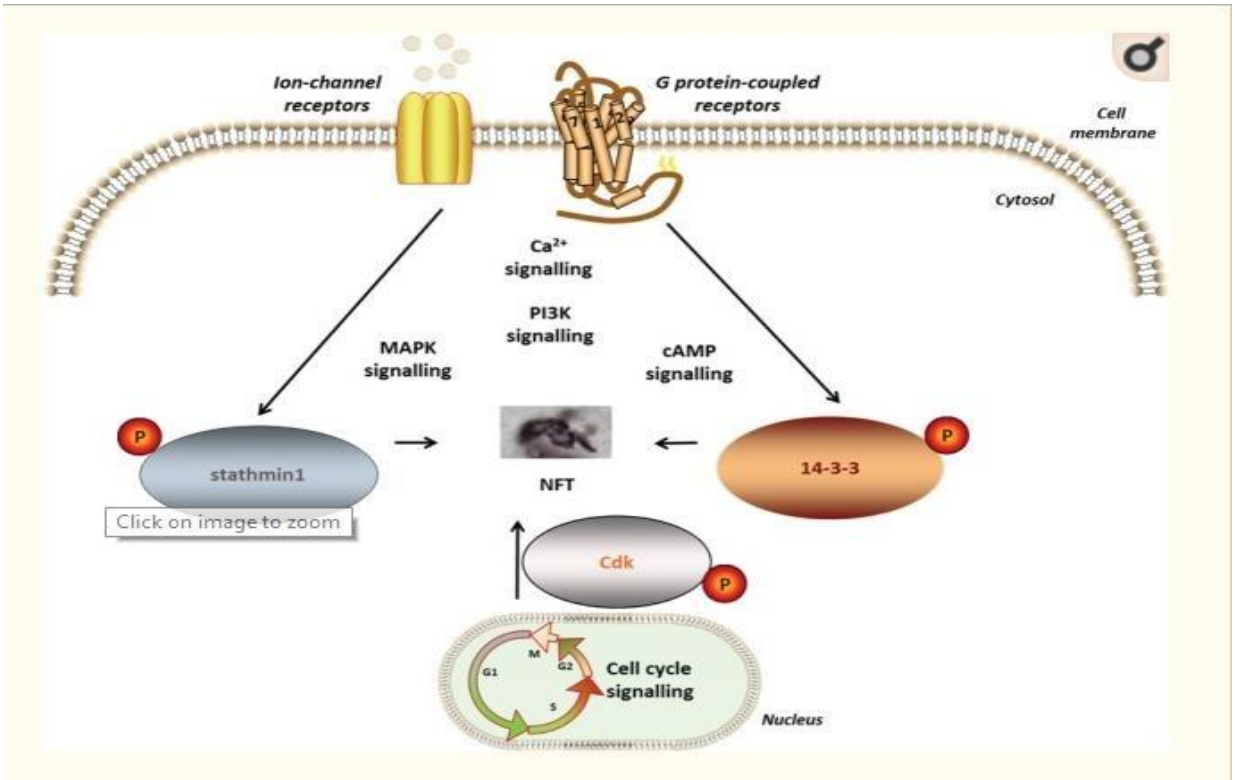


**Figure 4**. The figure demonstrates a post-synaptic neuron with its core.

Unusually phosphorylated tau is recognizable even before NFTs in the soma and dendrites. Any way in physiological states of grown-up neurons (Malia *et al.*, 2016), Tauris is fundamentally an axonal protein however progresses toward becoming mislocalized into the somatodendritic compartment at beginning periods of pathology. Since tangle load associates with subjective decrease and neural connections misfortune in AD (Gong *et al.*, 1994; Szendrei *et al.*, 1993) (see **Figure 5**).

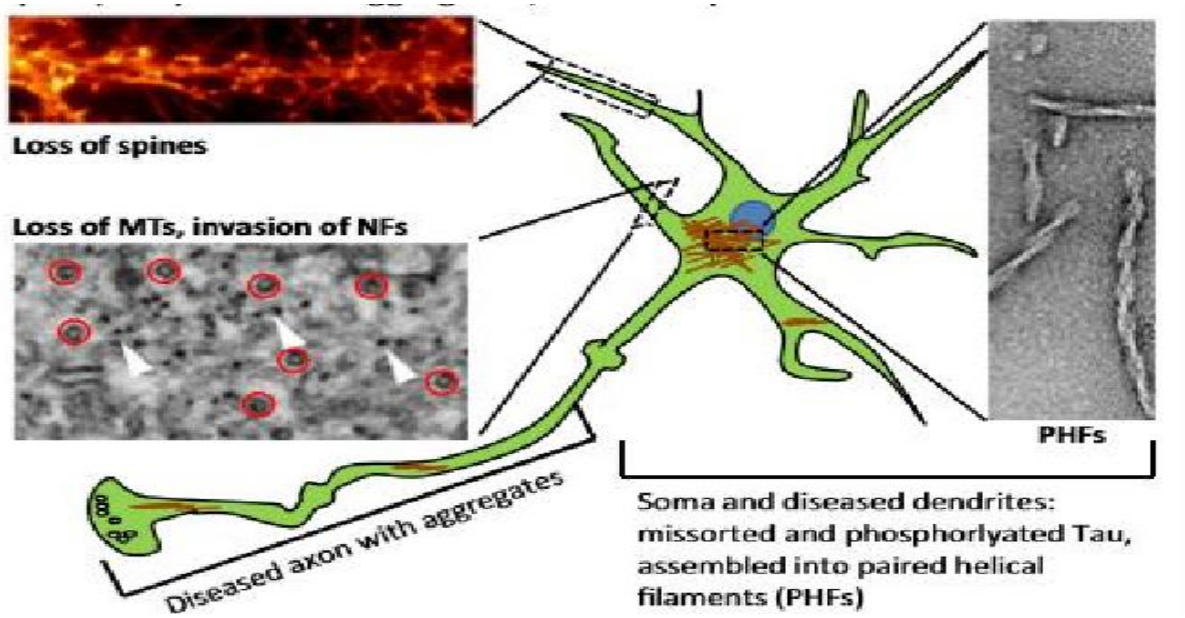


**Figure 5**. Tau arranging amid advancement also, in sickness.

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Along with other most typical types of pathological hallmarks in Alzheimer's dementia and mild cognitive impairment process of glucose metabolism is also rendered. For optimal cellular utilization of glucose-insulin is required but brain insulin resistance is present in AD and MCI and people having type 2 diabetes have a substantial risk of developing AD (Wang *et al*., 2020). Molecules face oxidative damage due to oxidative stress i.e. key grave imbalance between the production of reactive oxygen species and reactive nitrogen species and antioxidant defenses and many studies have proven that it contributes to the development of AD (see **Figure 6**).

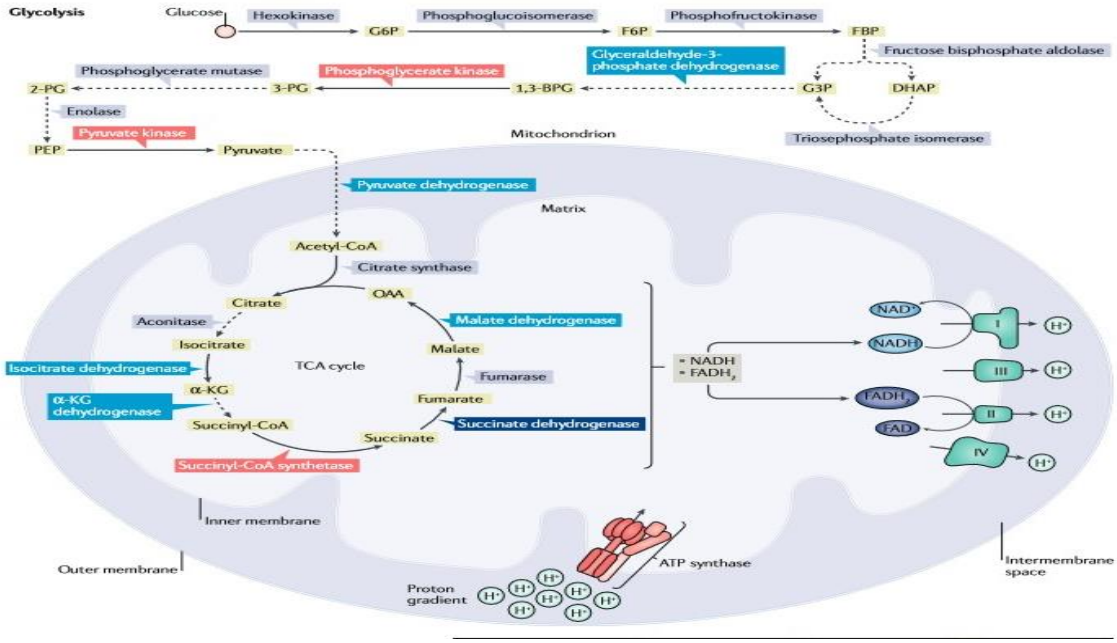


**Figure 6**. Glycolysis, the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC).

The level of oxidative damage to many molecules in the brain of AD patients is increased even in the case of MCI oxidative damage seriously occurred as the level of protein carbonyls (PCs).is increased to dangerous levels. Likewise, in the early stages of Alzheimer's, a conventional symmetric pattern of cortical atrophy is seen dominantly affecting medial temporal lobes and relatively sparing the primary motor.

ConsideredasastrongsymptomforAlzheimer'sdementia.Thiscorticalthinning results in dilation of temporal horns of lateral ventricles (Whiteman *et al*., 2005). The amyloid β is also found in the form of cerebral amyloid angiopathy (CAA) in the vessel walls. The major constituent of CAA is Aβ40. Cical capillaries, small arterioles, middle-size arteries affected by CAA. The most prominently affected areas are the posterior parietal and occipital areas (Zabel *et al*., 2018) (see **Figure 7**).

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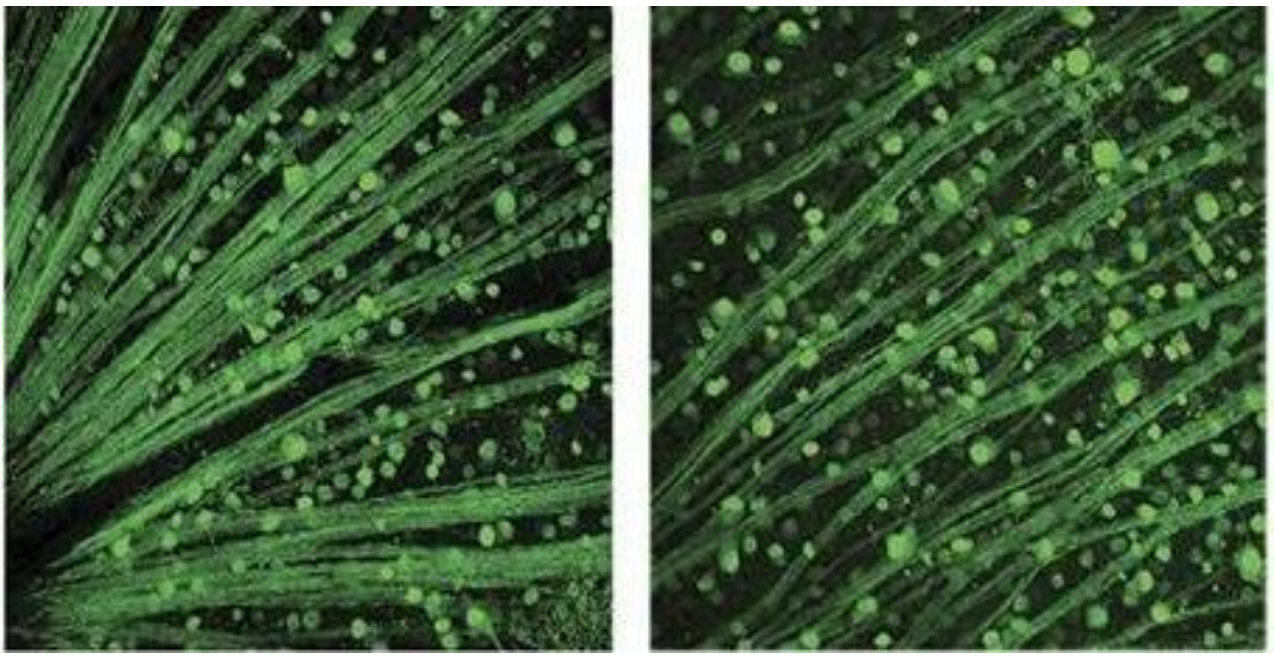

**Figure 7**. Immunohistochemistry revealed beta-amyloid deposition in the blood vessels.

Granulovacuolar degeneration (GVD). and Hirano bodies are two poorly understood lesions present in the cytoplasm of hippocampal pyramidal neurons of AD patients are present in neurons of AD patients. They cause the formation of tangles and in some cases apoptotic cell death due to their immunoreactivity (Kabir *et al*., 2021).

A wide variety of proteomics methods are used to diagnose AD in its preliminary stages, **Table 1**.

**Table 1**. Wide variety of proteomics methods used to diagnose AD in its preliminary stages.

| Methodology | Biomarkers | Samples | References |
|---|---|---|---|
| 2DE, MALDI and ESI-qTOF | Transthyretin, retinol-binding protein, β2-microglobulin, proapolipoprotein, and ApoE. | CSF | (Graves & Haystead, 2002) |
| 2DE and MALDI | ApoA1, cathepsin D, transthyretin,hemopexin, and pigment derived Epithelial factor | CSF | (Graves & Haystead, 2002) |
| 2DE and MALDI | Albumin, α1- antitrypsin, ApoE, ApoJ, complement component 3, contactin, fibrin-β, immunoglobin heavy and light Chain. | CSF | (Graves & Haystead, 2002) |
| 2DE and MALDI | α1β-glycoprotein, α1-antitrypsin, Transthyretin, ApoA1, ApoE, and ApoJ. | CSF | (Graves & Haystead, 2002) |
| 2DE and MALDI | Complement component C4b isoforms Fibronectin, | CSF | (Goedert & Spillantini, 2006) |
| 2DE and multidimensional LC, MALDI and iron trap LC/MS/MS | α1- acid glycoprotein, α1- acid glycoprotein, ApoB100, complement factor H, and histidine-rich Glycoprotein. | Serum | |
| 2DE and LC/MS/MS | Complement factor H, α2- macroglobulin, immunoglobulin heavy and light chain, desmoplakin, albumin | Plasma | (Khachaturian, 1985) |

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**Table 1 (continue)**. Wide variety of proteomics methods used to diagnose AD in its preliminary stages.

| Methodology | Biomarkers | Samples | References |
|---|---|---|---|
| SELDI | Cystatin C, β2- macroglobulin, and VGF precursor, | CSF | (Mucke, 2009) |
| ICAT | 83 differentially expressed proteins in two separate cohorts of AD cases and controls. | CSF | (Wenk, 2003) |

## 4. CONCLUSION

Alzheimer's dementia is a neurodegenerative disease characterized by limited motor functions and loss of memory. Clinically it is diagnosed by accumulation of plaques in neurons and formation of NFTs by the aggregation of tau proteins. These pathological changes in the brain can be observed in preclinical stages of AD known as ASYM AD and mild cognitive dementia. Proteomics analysis of these symptoms can act as biomarkers for the early diagnosis of this disease. Various proteomics techniques are being used for decades for analysis, with time those techniques are improved for a better and clear analysis.

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## 6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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