International Research Journal of Biotechnology Vol. 16(1) pp.1-4, January, 2025
Available online http://www.interesjournals.org/IRJOB


*Review Article*

# Development and Validation of a Cost-Effective ELISA for Detecting a Novel Biomarker Associated with Early-Stage Alzheimer's Disease

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**Received:** 29-Aug-2024; Manuscript No: irjob-24-146852; **Editor assigned:** 02-Sep-2024; Pre-QC No: irjob-24-146852 (PQ); **Reviewed:** 16-Sep-2024; QC No: irjob-24-146852; **Revised:** 10-Jan-2025; Manuscript No: irjob-24-146852 (R); **Published:** 17-Jan-2025, DOI: 10.14303/2141-5153.2025.103

**Abstract**

The exact cause of Alzheimer's disease is unknown, but researchers believe it's a complex interplay of genetic, environmental, and lifestyle factors. Age is the biggest risk factor, with the risk increasing after 65. Alzheimer's disease is caused by the abnormal build-up of proteins in and around brain cells. One of the proteins involved is called amyloid, deposits of which form plaques around brain cells. The other protein is called tau, deposits of which form tangles within brain cells.

Alzheimer's disease patients' neurotic plaques are mostly made up of Aβ, a 4-kDa peptide generated from the amyloid precursor protein. Evidence suggests that Aβ plays a significant role in the pathophysiology of the disease. Potential therapies that target Aβ synthesis or accumulation in the brain as β-amyloid are being vigorously researched. Quantifying the Aβ peptide is crucial for studying the disease's underlying genesis and evaluating prospective therapies. The Enzyme-Linked Immunosorbent Assay (ELISA) is a reliable and widely used method for measuring Aβ in the brains of Alzheimer's patients and transgenic mice with β-amyloid deposition.

**Keywords:** Alzheimer's disease, Genetic, Environmental, Lifestyle factors, Pathophysiology

## INTRODUCTION

Alzheimer's Disease (AD) is the most common form of dementia, accounting for two-thirds of all cases globally (Dubois B et al., 2023). It is a neurodegenerative disorder with various pathobiologic subtypes and clinical presentations, including amyloid-beta plaques and neurofibrillary tangles containing tau proteins (Popp J et al., 2017). These neuropathologic changes are associated with synapse and neuronal loss, transmitter deficiencies, neuro-inflammation, and reactive astrogliosis, leading to cognitive impairment (Chimthanawala NM et al., 2024). The prototypical clinical phenotype of AD is dementia of insidious onset and gradual progression with progressive amnestic impairment, which represents approximately 85% of cases (Sharma L et al., 2022). With increasing life expectancy across the world, the number of elderly people at risk of developing dementia is growing rapidly (Klyucherev TO et al., 2022). The dementia rises steeply with age, doubling every 4-5 years from the age of 60, so that more than one-third of individuals over 80 years of age are likely to develop a dementia (Zhang Y et al., 2024). AD remains the most common cause of dementia in all age groups. In this review, we focus on the early detection of AD, particularly in the context of subjects with be memory complaints who do not yet match criteria for AD but who are at high risk of developing a full-blown dementia syndrome in the next few years (Karki HP et al., 2021). This at-risk state is commonly referred to as Mild Cognitive Impairment (MCI). Dementia prevalence in India is 7.4% among adults over the age of 60 meaning about 8.8 million

Indians are currently living with dementia (Mehta PD et al., 2020). This is greater than the Dementia in India 2020 Report estimate of 5.8 million (Mankhong S et al., 2022).

Diagnosis of AD is challenging due to its heterogeneity in pathobiology, genetic factors, brain resilience, and distinct clinical presentation (Cutler P et al., 2008). Development of *in vivo* biomarkers has shifted the diagnosis of AD from later dementia stages of distress towards earlier stages, introducing the potential for pre-symptomatic diagnosis. Diagnosis of AD is restricted in the clinical setting to people with specific AD phenotypes and supportive biomarker findings (de Oliveira TR et al., 2020). Biomarkers are becoming increasingly available to help distinguish between different disorders and different AD phenotypes, particularly at early stages, and assist in identifying those at risk for symptomatic AD (Rani S et al., 2023).

The use of valid CSF biomarkers for the early discrimination of patients in the AD continuum from healthy controls or patients with non-Alzheimer's type dementia can be integrated into the design of therapeutic clinical trials, which will have significant value in increasing the efficiency or probability of success in the development of disease-modifying therapies (Li TR et al., 2022). In 1984–1985, Glenner, Masters and colleagues determined that neuritic plaques, one of the defining features of Alzheimer's Disease (AD), consist primarily of the small Aβ peptide. CSF Aβ was reported in 1992, identifying a vital AD biomarker, although initial reports on CSF Aβ were unclear. Subsequently, in 1993 the first CSF T-tau was measured by sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The basic CSF biomarkers, Aβ42, T-tau, and p-tau, have been examined in numerous investigations discussing fluid AD biomarker development (Shiravandi A et al., 2022).

# LITERATURE REVIEW

## Emerging biomarkers for early-stage Alzheimer's disease

**Blood-based biomarkers:** These are increasingly used to identify Alzheimer's disease pathology, including Aβ peptides, pTau, plasma pTau181, pTau217, pTau231, and blood GFAP. These biomarkers can predict cognitive decline, differentiate FTD from AD, and differentiate between primary progressive aphasia and lvPPA. Apolipoprotein E (APOE) is a genetic biomarker with a risk factor for AD, and polygenic risk scores may help identify individuals at increased risk of dementia.

**CSF based biomarkers:** A research conducted at Sahlgrenska University Hospital in Sweden measured Cerebrospinal Fluid (CSF) samples for total tau, phospho-tau, Aβ40, Aβ42, neurogranin, and neurofilament light chain. CSF SNAP-25 and synaptotagmin-1 concentrations were measured using immunoprecipitation mass spectrometry. Results showed that CSF neurogranin was higher in female participants and higher in APOE e4 carriers. Education had minimal effect on CSF synaptotagmin-1, but did not withstand multiple-comparison adjustment.

**Neuroinflammatory markers:** The V-Plex Neuroinflammation Panel 1 was used to measure paired serum and CSF samples. The panel consisted of 38 analytes and was run according to the product protocol. 96-well plates were pre-coated with capture antibodies and blocked with 5% MSD Blocker A solution. Samples and calibrators were added to the plates and incubated for 2 hours. The plates were washed three times with a home-prepared solution of PBS-Tween 20. Detection antibodies were mixed with MSD Diluents and incubated for 1-2 hours. Data were generated and interpolated using MSD discovery workbench software.

**Fluid biomarkers for synapse pathology:** Synaptopathies and synapses' role in cognition highlight the importance of biomarkers in synapse pathology. These biomarkers can link synaptic degeneration to cognitive decline, aiding diagnosis and understanding underlying pathological processes. They can also be used in drug development to monitor treatment efficacy in synaptic functioning trials. They can be implemented alongside cognitive tests to provide a more precise description of symptoms, especially at early stages.

**Amyloid-β:** β-Amyloid peptide (Aβ) is a key component of Alzheimer's disease, with three types: Diffused in gray matter, dense neuritic plaques, and blood vessels. Characterization is challenging due to solubility and heterogeneity. It is characterized by beta-amyloid accumulation and Tau protein tangles. Three core CSF biomarkers, Aβ42, T-Tau, and P-Tau, have been validated as diagnostic tools, increasing diagnostic validity to 85-90%, even in early stages.

# DISCUSSION

## Development of ELISA for Alzheimer's disease biomarkers

Advantages of ELISA over alternative methods include the following:

- High sensitivity—typically detects values in the 1-10 ng/ml range.
- Speed--results can be obtained within a few hours.
- Scale—hundreds of samples can be easily handled.
- **Versatility:** Can be used with crude or refined extracts, as well as intact or dissociated viruses.
- Specificity depends on the antibody and method.
- Antigen morphology has a limited impact.
- Quantitative precision and reproducibility.
- Possibilities for automation and "kit" development.
- Low cost and long shelf life for reagents.
- The simplicity of fundamental equipment.
- Nonhazardous reagents.
- Antigen and reagent use is cost-effective and efficient.

Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used biochemical technique for quantifying the Aβ peptide in the brain of humans and transgenic mice. It involves homogenizing the tissue and extracting Aβ from the β-

amyloid plaque. ELISA accurately measures Aβ levels, and C-terminal epitope-specific antibodies are used to differentiate Aβ40 and Aβ42, allowing for separate quantitation of these specific Aβ species. This chapter details the methodology used in our laboratory for extracting Aβ from brain tissue and quantitating Aβ40 and Aβ42 using sandwich ELISA. CSF GAP-43 was tested using ELISA. CSF Aβ42 and Aβ40 concentrations were measured using ELISA kits. By using four ELISA methods have consistently shown a moderate to marked decrease in CSF Ah42 in Alzheimer's Disease (AD). The most used ELISA, INNOTEST k h-AMYLOID (1–42), measures Ah42 in CSF. The mean sensitivity for discrimination between AD and normal aging is 86%, with a specificity of 91%. The mean decrease in AD patients compared to controls is about 50%. CSF samples using ELISA for Ab42, total tau, and phospho-tau181 after one freeze-thaw cycle and in triplicate for all other ELISAs after two freeze-thaw cycles. Samples were evaluated using commercially available ELISAs for NrCAM, YKL-40, apolipoprotein E, clusterin/apolipoprotein J, pigment epithelium-derived factor (PEDF)/serpin-F1, beta-2 microglobulin, ceruloplasmin, chromogranin A, transthyretin, and cystatin C. A sandwich ELISA was developed for carnosinase I using goat anti-human carnosinase I antibody for capture, rabbit anti-human carnosinase I antibody for detection, goat anti-rabbit: Horseradish peroxidase for reporting, and TMB Super Slow for color development. Statistical analyses were performed using SAS 9.2 for ROC/AUC calculations and logistic regression analyses, and SPSS 18 for all other analyses.

### Validation of ELISA assays

Currently, there is no sensitive and cost-effective method for quantifying proteins, despite various methods like capillary electrophoresis, resonance light scattering, surface plasmon resonance, ELISA, and PCR. This presents a quick, cost-effective, adaptable, and ultrasensitive detection method for quantifying Ab42, tau, and p-tau181 proteins in various bodily fluids such as cerebrospinal fluid, saliva, serum, and urine. The newly created assay costs 1-2 orders of magnitude less than the commercially available ELISA kit. The purpose of this is to identify such CSF protein biomarkers for AD using Enzyme-Linked Immunosorbent Assays (ELISA). The ELISA sandwich colorimetric assay can accurately detect low quantities of Aβ peptides in blood, despite the challenges of the low amounts and high hydrophobic nature of these peptides. To ensure consistent and reproducible results, high affinity antibodies and standardization of the Aβ blood test protocol are crucial. The ELISA kits were used to quantify CSF quantities of Aβ1-42, total tau (tau), and tau phosphorylated at threonine 181 (ptau181).

### Comparative analysis with other diagnostic techniques

In BioFINDER-1, a 3T MRI scanner was used for anatomical T1-weighted imaging, using Magnetization-Prepared Rapid Gradient-Echo (MP-RAGE) images and the free surfer image analysis pipeline v.6.0 for anatomical segmentation and cortical thickness calculations. Cortical thickness was calculated from a temporal meta-region of interest, including the entorhinal, fusiform, inferior temporal, and middle temporal cortex. Aβ imaging was performed at baseline, and centiloids were derived using the Computational Analysis of PET from AIBL pipeline. In WRAP, participants underwent T1-weighted MRI and amyloid-Pittsburgh Compound B (PiB) imaging, determining cortical thickness in typical AD signature regions. Aβ burden was assessed using global cortical average distribution volume ratios. Accurate diagnosis of neurodegenerative diseases like Alzheimer's Disease (AD) has been a challenging task, but with the advent of disease-modifying treatments, there is a growing urgency to improve this area. The AT(N) classification of biomarkers used for a biological definition of AD is discussed, along with the biomarkers most likely to be adopted for clinical use. Some biomarkers, such as the Aβ42/40 ratio and phospho tau, are already ready for clinical use. Real-world considerations like co-morbidities and ethnicity are also considered in studies. This area of research is focused on providing a robust, equitable diagnosis for all communities, ensuring access to new therapeutics. As novel imaging techniques and biomarkers emerge, a more accurate, early, and less invasive detection and diagnosis of AD may be closer than ever. The candidate biomarkers for Alzheimer's Disease (AD) using Genome-Wide Association Studies (GWAS), traditional statistical studies, network analysis of gene interactions, and machine learning algorithms. These studies have identified genes related to immunity, microglia, and blood, and identified risk factors for AD. The integration of gene expression data with GWAS data has led to the development of Transcriptome-Wide Association Studies (TWAS), which identify genes with significant associations between their expression in specific tissues and the disease.

## CONCLUSION

Alzheimer's Disease (AD) is a neurodegenerative disorder causing cognitive impairment, with the most common form accounting for two-thirds of all cases globally. The disease is caused by abnormal build-up of proteins in and around brain cells, including amyloid and tau proteins. Aß, a 4-kDa peptide, plays a significant role in the pathophysiology of AD.

Potential therapies targeting Aß synthesis or accumulation in the brain as B-amyloid are being researched. Quantifying the Aß peptide is crucial for studying the disease's underlying genesis and evaluating prospective therapies. The Enzyme-Linked Immunosorbent Assay (ELISA) is a widely used method for measuring Aß in the brains of Alzheimer's patients and transgenic mice with B-amyloid deposition. The development of *in vivo* biomarkers has shifted the diagnosis of AD from later dementia stages of distress towards earlier stages, introducing the potential for pre-symptomatic diagnosis. The use of valid CSF biomarkers for early discrimination of patients in the AD continuum from healthy controls or patients with non- Alzheimer's type dementia can be integrated into the design of therapeutic clinical trials, increasing the efficiency or probability of success in the development of disease-

modifying therapies.

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